Short Communication

Comparison between the Widal test and culturing technique in the diagnosis of enteric fever in Khartoum State, Sudan

Yagoub Hamadt Allah Elhaj Abd Elseed[1, 2]

[1]Department of Microbiology, Faculty of Medical Laboratory Sciences, Omdurman Islamic University, Khartoum, Sudan.
[2]Department of Laboratory Medicine, Faculty of Applied Medical Sciences, Al Baha university, Al Baha, Saudi Arabia.

Salmonella infection is a common bacteria disease that causes enteric fever in humans. Presently, Widal test, stool, blood and urine cultures are the most diagnostic means of confirming *Salmonella* infection in humans since they are based on the isolation, identification and demonstration of the presence of antibodies in the serum. However, the stool and serum sample of an infected patient against the (O) and (H) antigens of the bacteria requires thorough laboratory analysis. The aim of this study was to assess the comparison between the Widal test and culturing technique in diagnosis of salmonellosis. Both tests were done in this study in different age and sex group. Samples (blood, urine and feces) were collected from 60 patients suspected to have enteric fever with Widal test results. Among those patients, the Widal test results were positive while the culture results provided mostly no growth, n=37 (92.5%); whereby 3 (7.5%) of cultures provided positive growth of *Salmonella*. The bacteria isolated were identified by Gram's reaction and biochemical characteristics including triple sugar iron agar, urease, citrate utilization test, indole and oxidase test. Most patients, 70% (n:32) suspected to have enteric fever were female, however only 30% (n:15) of total cases were males. Also, there were 55% cases in the range of age 20-40 years old and 27% of cases in the range of age 15-20 years old with 18% in the range of age 40-60 years.

Key words: Widal test, culture technique, *Salmonella*, enteric fever.

INTRODUCTION

Typhoid fever is a systemic disease caused by *Salmonella typhi* and is the major cause of morbidity and mortality worldwide (Shrivastava et al., 2011). Typhoid fever is a major health problem in developing countries and its diagnosis on clinical ground is difficult. Diagnosis in developing countries including Sudan is mostly done by Widal test. However, the value of the test has been debated. Hence, evaluating the result of this test is necessary for correct interpretation of the result. Options for the diagnosis of typhoid fever are clinical signs and

symptoms, serological markers, bacterial culture, antigen detection and DNA amplification (Wain and Hosoglu, 2008). Moreover WHO estimate for annual global incidence of salmonella infection are about 20 million cases with greater than six hundred thousand (> 600, 000) deaths. It is encountered in tropical countries including India, South and Central America and Africa, where they constitute serious source of morbidity and mortality with rapid population growth, increased urbanization, limited safe water and infrastructure and health problems (Hohman, 2011).

Blood, bone marrow and stool culture are the most reliable diagnostic methods but they are expensive techniques and some bacterial culture facilities are often unavailable (Wain et al., 2008). 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 equip-ment (Beyene et al., 2008; Ley et al., 2010).

Salmonella Typhi, the causative agent, is most frequently isolated from blood during the first week of illness but can also be isolated during the second or third week of illness, during the first week of antimicrobial therapy and during clinical relapse (Baker et al., 2010). Isolation of *Salmonella* Typhi from bone marrow is the current gold standard method for confirming a case of typhoid fever. However, this requires equipment, supplies and trained laboratory personnel seldom found in primary health-care facilities in the developing world (Wain and Hosoglu, 2008). Blood culture is a more practical albeit less sensitive alternative to bone marrow culture. However, it is not always available and, when it is, it takes 2 to 3 days. As a result, diagnosis may be delayed or overlooked and patients without typhoid fever may receive unnecessary and inappropriate antimicrobial treatment. For this reason, in developing countries, typhoid rapid antibody tests can facilitate diagnosis and disease management.

MATERIALS AND METHODS

Study area and period

The study was conducted in Omdwanban, Asalam and Salamat hospitals (Khartoum, Sudan) from December 2012 to March 2013. These hospitals are some of the largest public hospitals in Sudan, located in Khartoum.

Study design and patient population

A comparative study on febrile patients was conducted in which patients were screened for typhoid fever and suspected patients were enrolled in the study, then blood sample were collected and tested for confirmation of the disease. Patients were screened by their physician for the clinical symptom of typhoid fever which is fever of two or more days before admission accompanied by other clinical symptoms of typhoid fever in the absence of any other known febrile illnesses. Febrile patients whose presumptive clinical diagnosis were typhoid fever and sent to the laboratory by their

physician for Widal test were included in the study. However, those febrile patients who had received antibiotic treatment for their symptom within two weeks before coming to the hospital and those who are diagnosed for other known febrile illness were not included in this study. By using these inclusion and exclusion criteria, about 60 suspected febrile patients were recruited for this study then data and blood, urine and feces samples were collected from these 60 patients for Widal test and culture. Feces were collected at the second week of fever, while urine sample was collected at third week of fever. The samples were transported immediately to the laboratory for bacteriological investigation.

Cultivation

Bacteriological tests were done according to Barrow and Feltham, 1993. All specimens were inoculated on culture media including, Mac Conkey agar, XLD, DCA and KIA, and incubated in aerobic atmosphere at optimum temperature 35-37°C and pH range from 7 - 7.2.

Blood culture

The organism may be recovered from the bloodstream at any stage of the illness but are most commonly found during the first 7-10 days and during relapses. The organism can also be recovered from the blood clot from a sample taken for serological test. The clot is digested with streptokinase or minced, and incubated in broth.

Subculturing and biochemical identification

After 24 h incubation, sub-culturing was performed from the Typtic Soya broth on XLD agar (OXOID, England). After overnight incubation, positive cultures were used further while negative broth cultures were incubated for seven days and sub cultured before reported negative. Suspected colonies obtained on the above media were screened by biochemical tests using triple sugar iron agar (TSI) (BBL™), citrate utilization test, motility (Difco™), urease test (Himedia ltd. India) and lysine decarboxylation (LDC) [Difco™] test.

Stool and urine culture

Specimens of stool and urine were also submitted for examination, although the isolation of *Salmonella* from either of these specimens may indicate merely that the patient is a carrier. In typhoid fever, stool may contain *Salmonella* from the second week and urine culture from the third week of the infection. In paratyphoid B infections, the clinical course may be much shorter than in typhoid; diarrhea may occur early and stool cultures are often positive in the first week of the illness.

Isolation and purification of bacteria

All isolates were identified by using bacteriological tests such as Gram stain, motility test sugar fermentation and KIA, indole test, urease test, citrate utilization test and oxidase test which was explained above.

Widal test

Blood samples collection

Sixty (60) venous blood samples were collected from individuals

Table 1. Age and sex of patients included in the study.

Rang of age	Male		Female		Total
	n	%	n	%	%
15-20 years	3	30	7	70	100
25-40 years	4	16.67	20	83.33	100
40-60 years	8	61.54	5	38.46	100

Table 2. Isolation of *Salmonella* from patients whose blood samples showed significant Widal test.

Parameter	Widal test	Culture result	D.F	Ratio of isolate	Level of significant
S. typhi	40	2	118	5%	***
S. paratyphi	40	1	188	2.5%	***

Table 3. Medical history and other disorders observed among the patients.

Other disorders	Number of patients	Percentage
Previous infection by *Salmonella*	21	65.63%
Rheumatoid arthritis	8	25%
Malaria	3	9.37%
Total	32	100%

suspected to have *Salmonella* infection. Then blood specimens were centrifuged at 4000r.p.m for 5 min. The Widal test was done to detect the presence of *Salmonella* antibodies in patient's serum. It is an agglutination test, use for detection of specific O and H antigens.

Salmonella antigens suspensions are commercially available in 5 ml amounts from Wellcome Reagent Ltd which was used. The reagents are suitable for rapid slide (screen) testing and tube testing. Qualitative slide agglutination and semi quantitative tube agglutination (titration) were performed using febrile antigen kits of *Salmonella* Typhi (Wellcome Reagent, Ltd). The slide agglutination test is used as a screening test for the presence of anti TO and anti TH antibodies in the patient's serum. For the slide agglutination test, a drop of *Salmonella* Typhi O and H antigens was added on a drop of serum on card and rotated at 100 rpm for one minute and reported as reactive or non-reactive. For those slide agglutinations whose results are reactive and weakly reactive, titer was determined. In the tube agglutination test (titration), serum sample was serially diluted by using fresh 0.95% saline preparation from 1:20 to 1:640 for anti TO and anti TH separately in 12 test tubes. Then a drop of O antigens and H antigens were added in the test tubes, equal amount in all. Based on the manufacturer manual, an antibody titer of 1:80 and higher for anti TO and 1:160 and higher for anti TH antibodies were taken as a cut of value to indicate recent infection of typhoid fever.

RESULTS

Sixty (60) symptomatic patients suspected to have typhoid fever were included in this study and had been selected randomly from the out patients in Omdwanban, Alsalam and Salamat hospitals (Khartoum, Sudan). Total cases investigated in this study were 60 patients including six normal healthy individuals as control negative selected from hospital laboratory staff in the range of age 20-30 years old, majority of them had been complaining of enteric fever sometimes in their lives. 40 patients of total cases were found with significant Widal test for enteric fever while 14 of them were negative. The highest antibody titer was 1\320 and the titer 1\20 used as control negative.

Most suspected enteric fever patients 70% were female, however only 30% of total cases were males. Also there were 55% of cases in the range of age 20-40 years old and 27% of cases in range of age 15-20 years old while 18% in the range of age 40-60 years (Table 1). The culture of samples provided mostly no growth (95%) whereby 5% of cases reflected positive growth for *Salmonella* species (Table 2). On other hand, previous infection by *Salmonella* and complaining from rheumatoid arthritis were the commonest post medical history condition observed among patients, previous infection by *Salmonella* was 35% of total cases and rheumatoid arthritis was 14% of them, while 3% of patients have malaria (Table 3).

Interpretation of results by statistical analysis show that (Table 1) detection of *S. typhi* and *S. paratyphi* isolation by culture method have high significance as compared to Widal test (t. cal >0.01). Percentage of *S. typhi* was 5% and *S. paratyphi* was 2.5% isolate as compared to the positive Widal test application results.

DISCUSSION

Typhoid fever has an important socio-economic impact, so accurate diagnosis of the disease at an early stage is important not for etiological diagnosis but also for identifying individuals that may serve as potential carrier who may be responsible for acute typhoid fever outbreak (Charles et al., 2012).

This study was carried out in Khartoum. The patients were randomly selected from the out-patients clinic of Omdwanban, Asalam and Salamat hospitals (Khartoum, Sudan). Sixty patients suspected of having enteric fever with Widal test application result were used. The Widal test was done to those patients and results which had been obtained show that there were 40 patients significant for enteric fever while the culture results of those patients were mostly negative for *Salmonella* (95% no *S. typhi* or *S. paratyphi* isolation). Depending on this results, we suggest that significant titer of antibodies detected in patient serum by Widal test is not specific for having typhoid fever, our findings were similar to the findings from study conducted by Salih and John in 2008 which found that the Widal test and other serological diagnostic tools have limitations because of their low sensitivity and non-specificity (Wain et al., 2008). Moreover, although Widal test has been in use for more than a century, the value of the test to diagnose typhoid fever has been debated for as many years as it has been available. However, the definitive diagnosis of typhoid fever depends on the isolation of *Salmonella* Typhi from blood, stool, urine and other body fluids (Shrivastava et al., 2011).

Among the patients included in this study, 55% are in the range of age 20-40 years, this may indicate that most *Salmonella* infections occur mainly in adults, which is partially similar to the study conducted in India to detect that the incidence of enteric fever increase with increasing age reaching its peak between 15-25 years old (Wain and Hosoglu, 2008), and disagree with the study done by Gizachew (2011) which stated that infection occurs in all age group with higher incidence and more variable clinical presentation in children.

False positive results of Widal titer were so high in this study (92.5%). These false positive results may be associated with cross reacting antibodies from serum of febrile patient other than typhoid fever. Our finding is supported by study conducted in Cameroon to show the prevalence of typhoid fever of febrile patients with clinically compatible symptom of typhoid fever, in 45% of the patients, there was true diagnosis of malaria but only 2.5% of the patients had culture proven typhoid fever (Nsutebu et al., 2003).

Conclusion

Widal test has a low sensitivity, specificity and positive predictive value, but it has good negative predictive value which indicates that negative Widal test result have a good indication for the absence of the disease. Nevertheless, using Widal test as the only laboratory test for the diagnosis of typhoid fever will result in misleading diagnosis. Therefore, it is very essential to use culture technique to diagnose enteric fever.

Conflict of interests

The authors did not declare any conflict of interest.

REFERENCES

Baker S, Favorov MO, Dougan G (2010). Searching for the elusive typhoid diagnostic. BMC Infect. Dis. 10:45.

Barrow GI, Feltham RK (1993). Cowan and Steel's manual for the identification of medical bacteria, 3rd Ed, Cambridge University Press.

Beyene G, Asrat D, Mengistu Y, Aseffa A, Wain J (2008). Typhoid fever in Ethiopia. J. Infect. Dev. Ctries. 2(6):448-453.

Charles AM, Adam MH, Gad, El Rab MO, Morshed MG, Shakoor Z (2012). Detection of salmonella typhi agglutinatins in sera of patients with other febrile illnesses and healthy individuals. J. Afr. Med. 10(1):41-44.

Gizachew A (2011). A comparative study of blood culture and Widal test in the diagnosis of typhoid fever in febrile patients, Department of Microbiology, Immunology and Parasitology. J. Med. Microbiol. 1(1):9-22.

Hohman EL (2011). Approach to patient with non-typhoid salmonella in a stool culture: http://www.uptoday.com/home/index.html. Accessed 03-02-2013.

Ley B, Mtove G, Thriemer K, Thriemer K, Amos B, Seidlein LV, Hendriksen I, Mwambuli A, Shoo A, Malahiyo R, Ame SM, Kim DR, Ochiai LR, Clemens JD, Reyburn H, Wilfing H, Magesa S, Deen JL (2010). Evaluation of the Widal tube agglutination test for the diagnosis of typhoid fever among children admitted to a rural hospital in Tanzania and a comparison with previous studies. BMC Infect. Dis. 10:180.

Nsutebu EF, Martins P, Adiogo D (2003). Prevalence of typhoid fever in febrile patients with symptoms clinically compatible with typhoid fever in Cameroon. Trop. Med. Int. Health 8(6):575-578

Shrivastava B, Shrivastava V, Shrivastava A (2011). Comparative study of the diagnostic procedures in salmonella infection, causative agent of typhoid fever. An overview study. Int. Res. J. Pharm. 2(9):127-129.

Wain J, Diep TS, Be Bay PV, Walsh AL, Vinh H, Duong NM, Ho VA, Hien TT, Farrar J, White NJ, Parry CM, Day NPJ (2008). Specimens and culture media for the laboratory diagnosis of typhoid fever. J. Infect. Dev. Ctries. 2(6):469-474.

Wain J, Hosoglu S (2008). The laboratory diagnosis of enteric fever. J. Infect. Dev. Ctries. 2:421-425.