ISSN: 2279 - 0594

Available Online at www.jbpr.in

Journal of Biomedical and Pharmaceutical Research, Volume 3, Issue 5, 2014, 18-20

RESEARCH ARTICLE

COMPARISON OF TYPHIDOT-EIA AND WIDAL TEST IN RESPECT TO POLYMERASE CHAIN REACTION AS DIAGNOSTIC PROCEDURES FOR EARLY DIAGNOSIS OF TYPHOID FEVER

Hajir Abd-Alhafeez Abd-Alrahman and Mohammed Nafi

AL-Neelain University, Faculty of Medical Laboratory Science – Khartoum - Sudan

Received 20 August 2014; Accepted 06 September 2014

ABSTRACT

Background: The public health burden of typhoid fever can be substantially reduced by early diagnosis and appropriate antibiotic therapy.

Aim: This is a descriptive cross-sectional study; aimed to compare Typhidot-EIA test and Widal test in respect to polymerase chain reaction (PCR) for the detection of the early typhoid fever.

Methods: A total of 80 suspected cases of typhoid fever attending Khartoum Hospitals between August and October 2013, were included in this study. Blood samples were collected from all cases during the first week of illness. Typhidot-EIA test, Widal test and PCR were performed for all samples.

The Result: Out of 80 patients, 35 (43.8%) were found to have rising titer and considered as Widal positive patients and 21 (26.3%) were positive using Typhidot-EIA test; While only 10 (12.5%) were positive by PCR. The sensitivity and specificity of Widal test and Typhidot-EIA test against PCR were 60%, 59% and 80%, 81% respectively

Conclusion: The DOT EIA IgM seems to be a practical alternative to Widal test for early diagnosis of typhoid fever

Key words: Salmonella, Typhidot-EIA, Typhoid fever, Widal test

INTRODUCTION:

Typhoid fever is still a significant public health burden in many developing countries and the incidence has been estimated as approximately 22 million cases with at least 200,000 deaths occurring worldwide annually [1]. The disease is endemic in Africa, Asia, the Middle East and Central and South America [2]. The definitive diagnosis of typhoid fever depends on the isolation and identification of *S. typhi* from blood, bone marrow, urine or stool [3]. In developed countries, the value and clinical application of the Widal test has diminished in recent years. Unfortunately, in many developing countries, the test is still widely used, though the test has many limitations and suboptimal sensitivity and specificity [4]. Suboptimal sensitivity results from negativity in early infection, prior antibiotic therapy and failure to mount an immune response by certain individuals [5]. Poor specificity, an even greater problem and is a consequence of pre-existing baseline antibodies in endemic areas, cross reactivity with other Gram-negative infections and oral typhoid vaccination. The purity and standardization of antigens used for the Widal test is a major problem and often results in poor specificity and poor reproducibility of test results [5]. The test becomes positive only in the second week of illness, so its value for early diagnosis of the diseases is limited [6]. Therefore, the limitations of the above traditional methods have prompted other novel tests to be developed such as Enzyme linked immunosorbent assay and the polymerase chain reaction [7]. The dot-enzyme immunoassay (DOT-EIA) is a newer serologic test based upon the presence of specific IgM antibodies to a specific 50-KDa outer membrane protein (OMP) antigen on *S. typhi* strains and has been commercially marketed as a Typhidot-EIA. This test also can detect IgG antibodies in serum [8]. The sensitivity and specificity of the DOT-EIA test has been reported to vary from 70 - 100% and 43 - 90% respectively [9]. The detection of IgM reveals the early phase of infection, while the detection of both IgM and IgG suggests the middle phase of infection. In areas of high endemicity IgG can persist for more than 2 years after typhoid infection [10]. Polymerase chain reaction (PCR) based methods have been exploited recently because they can theoretically amplify DNA only from *Salmonella typhi* (specificity) and should detect even

*Corresponding author: Hajir Abd-Alhafeez | E-mail: hajirhafeez188@gmail.com

low numbers of live or dead bacterial cells (sensitivity) [11]. The aim of the current study is to compare Typhidot-EIA and Widal test for the detection of the early typhoid fever.

METHODS:

This is a descriptive, cross-sectional study was carried out between August and October 2013 in the Microbiology Department of Medical Laboratory College, Al-Neelain University. Approval was taken by the ethical review board of the Faculty of Medical Laboratory Sciences Al-Neelain University. Verbal consent was taken from each study unit before collecting the demographical and clinical data.

Study Population:

Eighty (80) clinically suspected cases of typhoid fever patients attending different hospitals at Khartoum-Sudan between August and October 2013 were included in this study. The patients were selected according to clinical features, which include fever, chills, rigor, altered bowel habit, raised spot on the trunk, bradycardia, headache, myalgia. Cases having fever with at least one of the above clinical features within the first week of illness were considered as typhoid suspects.

LABORATORY PROCEDURE:

Specimen collection: Five ml venous blood sample was collected from each suspected case at the first week of illness and divided into two containers, 2.5 ml in EDTA for PCR, and other 2.5 ml in a plain container for serological tests, all specimens were stored at -20^{O}C prior to testing..

Widal test: This test was performed using Murex Biotech limited, UK agglutination kit and the manufacturer's instructions were followed.

Typhi-DOT-EIA test: This test was performed using Reszon Diagnostics International Sdn. Jaya, Selangor, Malaysia kit and the manufacturer's instructions were followed.

Detection of Salmonella using PCR:

DNA extraction: One mL of blood containing 20 mM potassium EDTA as anticoagulant was centrifuged at 10,000 rpm for 5 minutes. One mL of lysis buffer (0.2% Triton X100 in Tris HCl, pH 8.0) was added to the pellet. The mixture was gently aspirated several times to effect hemolysis. The tube was centrifuged at 12,000 rpm for 6 minutes, the supernatant was discarded, and the procedure was repeated once. The pellet was washed once with distilled water. After the removal of the supernatant, the pellet was resuspended in 20-30 µL of distilled water. The tubes were sealed, kept in boiling water for 20 minutes, and brought back to room temperature before being used as a template for PCR.

PCR Procedure: The flic-d gene sequence of salmonella serovar typhi was detected by PCR,. The primer sequences were as follows: forward primer ACTCAGGCTTCCCGTAACGC; reverse primer GGCTAGTATTGTCCTTATCGG. The reaction was performed in a 50 µL volume using Jena Bioscience, Germany master mix of thermostable DNA polymerase for PCR. Thermo-cycling conditions in a Techne thermocycler *(Bibby Scientific Limited, Beacon Road, Stone, Staffordshire, ST15 0SA, UK)* were as follows: 95°C for 5 min, followed by 35 cycles of 93°C for 30 Sec, 55°C for 30 Sec and 72°C for 40 Sec, with a final extension at 72°C for 5 min. The amplified products (5 µl) were separated by electrophoresis on 1% agarose gel and visualized by staining with ethidium bromide using UV gel documentation system. A163-bp PCR product was amplified with the above flic-d gene specific primers.

Ethical Clearance:

Approval was taken by the ethical review board of the Faculty of Medical Laboratory Sciences Al-Neelain University. Verbal consent was taken from each study unit.

RESULT:

A total of 80 clinically suspected cases of typhoid fever were studied, Widal test, Typhidot-EIA test and PCR were performed for all 80 typhoid suspected cases during the first week of illness. Out of 80 patients, 35 (43.8%) were found to have rising titer and considered as Widal positive patients and 21 (26.3%) were positive using Typhidot-EIA test; While only 10 (12.5%) were positive by PCR (Table 1 and 2).

The sensitivity and specificity of Widal test and Typhidot-EIA test were evaluated against PCR as standard test. The sensitivity and specificity of Widal test were 60% and 59% respectively; while the sensitivity and specificity of Typhidot-EIA test were 80% and 81% respectively

Comparison between

Table 1: Association between PCR and Widal test:

		PCR		Total
		Positive	Negative	
Widal	Positive	**06**	**29**	**35**
	Negative	**04**	**41**	**45**
Total		**10**	**70**	**80**

Table 1: Association between PCR and Typhidot-EIA test:

		PCR		Total
		Positive	Negative	
Typhidot-EIA	Positive	**08**	**13**	**21**
	Negative	**02**	**57**	**59**
Total		**10**	**70**	**80**

DISCUSSION:

Typhoid fever still remains a major endemic public health problem in Sudan especially in areas where healthcare facilities being limited and peoples are illiterate, living in unhygienic surroundings, drink raw-water from tube wells

and not habitual of hand-washing from toilet by soap. Isolation of causative agent via culture has remained the gold standard test for diagnosis but culture facilities are often limited or even unavailable especially in our country, where disease is more prevalent. In addition, culture method is expensive time-consuming and usually negative due to prior usage of antibiotics. Widal test is still widely used in our country, though the test has poor sensitivity and specificity. The current study tries to introduce Typhidot-EIA test for the detection of the early typhoid fever in our country due to its acceptable sensitivity and specificity comparing with Widal test.

Our study showed that the sensitivity of Widal test was 60%, while the sensitivity of Typhidot-EIA test was 80%. 35 out of 80 (43.8%) patients were positive by Widal test, 21 out of 80 (26.3%) patients were positive by Typhidot-EIA, while only 10 (12.5%) were positive by PCR. In those 10 patients positive by PCR 6 of them were positive by Widal test and 8 were positive by Typhidot-EIA test. This indicates that the Widal test failed to detect 40% of typhoid patients this may be due to early infection (IgG not produced). In other hand, Typhidot-EIA test failed to detect 20% of typhoid patients this may be due to masking of the IgM antibodies by the IgG or due to decreased levels of IgM.

In addition to that, our study showed the specificity of Widal test was 59%, while the specificity of Typhidot-EIA test was 81%. 29 out of 35 (83%) patients were false positive by Widal test, this variation may be pre-existing antibodies (IgG) in endemic areas, cross reactivity with other Gram-negative infections and oral typhoid vaccination; While, 13 out of 21 (62%) patients were false positive by Typhidot-EIA test, this may be due to cross reactivity with other Gram-negative infections.

In this context, Typhidot-EIA proves to be a reliable alternative serological test in endemic areas where studies have shown acceptable sensitivity and specificity for this test.

CONCLUSION:

Typhidot-EIA is a reliable alternative serological test to diagnose typhoid fever than the widely used Widal test.

REFERENCES:

1. Crump, A; Fouad, G; Youssef, A; Luby, P; Wasfy, O. and Rangel, M. Estimating the Incidence of Typhoid Fever and Other Febrile Illnesses in Developing Countries. CDC 2003; 9.
2. Gillespie, S. Salmonella infection in Manson's Tropical Diseases. 21st ed. Cook GC, Zumla A. Saunders, Elsevier science, Health science Division, London 2003:937-47.
3. Wain, J; Bay, B; Vinh,, H; Nguyen, N; Diep, S; and Walsh, L. Quantitation of Bacteria in bone marrow from patients with typhoid fever. Relationship between count and clinical features. *Journal of clinical microbiology* 2001;39:1571-1576.
4. Rodrigues, C. The Widal test more than 100 yrs old: Abused but still used. *J Assoc physicians India* 2003;51:7-8.
5. Olopoenia, A; and King, A. Widal agglutination test -100 yrs later: Still plagued by control–verse. *Postgrad Med J* 2000; 76:80-84.
6. Parry, M; Hoa, N; Diep, S; Wain, J; Chinh, T; and Vinh, H. Value of single tube Widal test in diagnosis of typhoid fever in Vietnam. *J Clin Microbiol* 1999; 37:2882-2886.
7. Haque, A; Ahmed, J; and Qureshi, J. Early detection of Salmonella by polymerase chain reaction. *A nn Saudi Med* 1999;19:337-340.
8. Choo, K; Oppenheimer, J; Ismail B; and Ong, H. Rapid sero-diagnosis of typhoid fever by dot enzyme immunoassay in an endemic area. *Clin Infect Dis* 1994;19:172-176.
9. Khan, E; Azam, I; Ahmed, S; and Hassan, R. Diagnosis of typhoid fever by DOT enzyme immunoassay in an endemic region. *J Pak Med Assoc* 2002; 52:415-417.
10. Saha, K; Talukdar, Y; Islam, M; and Saha, S. A highly ceftriaxione resistant *Salmonella typhi* in Bangladesh. *The Ped Infect Dis J* 1999; 18:297-303.
11. Levy, H; Diallo, S; Tennant, M; Livio, S; Sow, O; Tapia, M; Fields, I; Mikoleit, M; Tamboura, B; Kotloff, L; Lagos, R; Nataro, P; Galen, E; and Levine, M. PCR method to identify Salmonella enteric servers typhi, paratyphi A, and paratyphi B among salmonella Isolates from the blood of patients with clinical enteric fever. *J Clin Microbiol* 2008, 46:1861-1866.
12. Kundu, R; Ganguly, N; Gosh, K; Yewale, N; Shah, C; and Shah, K. IAP Task Force Report: Diagnosis of enteric fever in children. *Ind Paediatr* 2006;43:875-83.

