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TYPHOID FEVER
EVALUATION OF TYPHIDOT (IGM) IN EARLY AND RAPID DIAGNOSIS OF TYPHOID FEVER

DR. ATIF SITWAT HAYAT
MBBS, M.D (Medicine)
Consultant Physician
Assistant Professor of Medicine,
Northern Institute of Medical
Sciences (NIMS) Abbottabad,
NWFP, Pakistan.

DR. NAILA SHAIKH
MBBS, DCP
Senior Lecturer,
Liaquat University of Medical and
Health Sciences Jamshoro, Sind, Pakistan.

DR. SYED IQBAL AHMED SHAH
MBBS, FCPS (Medicine)
Assistant Professor of Medicine,
Liaquat University of Medical and
Health Sciences Jamshoro, Sind, Pakistan.

ABSTRACT... Background: Typhoid fever is widely recognized as a major public health problem in developing countries. A simple, reliable and rapid diagnostic test is needed for clinicians especially in areas where laboratory services are limited. **Objective:** To evaluate sensitivity and specificity of typhidot (IgM), a serological test to identify IgM antibodies against salmonella typhi. **Study Design:** This was a prospective study. **Setting:** Northern Institute of Medical Sciences (NIMS) and Ayub Teaching Hospital Abbottabad. **Period:** 1st November 2009 to 31st August 2010. **Methods:** A total of 100 patients with clinically suspected typhoid fever were studied and divided into three main groups as A, B and C, with definite typhoid fever, typhoid suspects plus non-typhoidal illnesses and healthy controls respectively. Blood culture and typhidot (IgM) tests were conducted for all subjects included in the study. The validity of typhidot (IgM) test has been evaluated by determining the sensitivity, specificity, positive and negative predictive values. **Results:** In our study, majority (75%) were males and (25%) females with M to F ratio of 3:1. The mean age of study group was 26.31±11.8 (SD) years. Among 100 clinically diagnosed typhoid fever patients, 19 had positive blood culture for S.typhi and 71 were typhidot (IgM) positive. Out of 19 culture positive patients, 18 (94.73%) were true typhidot (IgM) positive, which was also falsely positive in 05 (20.83%) among 24 non- typhoidal febrile controls. None of the healthy controls was positive for typhoid (IgM) test. The sensitivity, specificity, negative and positive predictive values of typhidot (IgM) test using blood culture as gold standard were 94.73%, 90%, 97.72% and 78.26% respectively for patients having typhoid fever. **Conclusions:** Typhidot (IgM) test is a simple, reliable, rapid and valid diagnostic tool for typhoid fever especially in areas where laboratory services are limited.

Key words: Typhoid fever, typhidot (IgM) test, sensitivity, specificity, salmonella typhi, blood culture.

.INTRODUCTION

Typhoid fever is widely recognized as a major public health problem in developing countries. It is a severe systemic infection caused by Salmonella typhi. The disease is endemic in the Indian sub-continent, South-East Asia, Africa, the Middle-East, South and Central America, where provision of pure water supplies and sewage control are inadequate[1]. Incidence of typhoid fever has been estimated as approximately 22 million cases with at least 200,000 deaths occurring annually[2]. The disease may occur in all ages, with the highest incidence found particularly in children[3]. According to WHO (World Health Organization), the overall incidence of typhoid fever is 412 cases per 100,000 population per year in Pakistan[4]. Typhoid fever is known to be associated with significant morbidity and mortality due to emerging multidrug-resistant strains of salmonellae and also delay in diagnosis and so start of appropriate therapy[5].

This study was important in context of Abbottabad where typhoid fever had caused large number of patients to visit tertiary care hospitals because majority of them drank raw-water from tube-wells and deep-borred wells and also didn't wash hands properly after using toilets with soap. In addition, most of them are illiterate, living in unhygienic surroundings and have poor socio-economic status hence further predisposing for disease prevalence. Therefore, we conducted this study in need of a reliable laboratory test for early and rapid diagnosis of typhoid fever.

Isolation of Salmonella typhi (S.typhi) via blood culture is still used as gold standard test for typhoid fever, but

culture yield has been obtained in only 70-75% cases. However, bacterial culture facilities are often unavailable, expensive and time-consuming especially in rural and hilly areas of our country. In addition, easy and open access to antibiotics without medical prescription in community makes it very difficult to isolate organisms from blood culture and alternative methods of diagnosis such as bone marrow culture may be needed, but are invasive and difficult to conduct routinely. Therefore, a simple and rapid serological diagnostic test for typhoid fever would be great benefit in circumstances where more sophisticated laboratory support has not been practiced.

Such a simple and reliable test has now become commercially available as typhidot test (dot-EIA). The dot-enzyme immunoassay (dot-EIA) is a relatively new serological test based upon the presence of specific-IgM antibodies to a specific 50-kD outer membrane protein (OMP) antigen on salmonella typhi strains[6]. It incorporates nitrocellulose strips impregnated with OMP antigen and hence separately identify IgM antibodies. Typhidot (IgM) test become positive as early as in first week of fever and results can be interpreted visually and available within one hour[6,7]. However, its validity in early serologic diagnosis of typhoid fever has not been studied in this region. Therefore, aim of our study was application of typhoid test in order to detect S.typhi specific-IgM antibodies in the serum and results were compared with blood culture to observe its sensitivity and specificity at tertiary care settings in Abbottabad.

PATIENTS AND METHODS

This prospective descriptive study has been conducted at Northern Institute of Medical Sciences (NIMS) and Ayub Teaching Hospital Abbottabad from 1st November 2009 to 31st August 2010. The local Ethical Committees of the institutes approved the study protocol and all patients gave written and informed consent.

Study Population

Patients included in this study were between 18-45 years old. All study subjects having fever after being admitted in hospital, their venous blood sample taken for blood culture and typhidot (IgM) test. Baseline complete blood count (CBC) and urinalysis were taken to help for probable diagnosis. If infection other than typhoid was suspected, other necessary laboratory investigations have also been done.

For the purpose of analysis, patients were divided into three groups; Group A- consisted of adults with a definitive diagnosis of typhoid fever as confirmed by positive blood culture. Group B- called as febrile controls and consisted of two sub-groups; first sub-group comprised of patient's whose clinical course was compatible with typhoid fever but have negative blood cultures (Clinical typhoid or typhoid suspect). Patients in this group were selected on six of the following criteria; a history of fever for five days or more prior to admission plus documentation of fever ($\geqslant 38^{\circ}C$) in the hospital (both are essential for diagnosis), headache, constipation, diarrhea, rose spots, splenomegaly, hepatomegaly, abdominal pain, nausea, signs of toxemia, leukopenia and /or leukocytosis, response to treatment (i.e. lysis of fever after four or more days of antibiotic therapy recommended for typhoid fever); second sub-group include patients with fever due to non-typhoidal illnesses (i.e. pneumonia, malaria, urinary tract infection etc). Group C- consisted of healthy controls.

Isolation of S.typhi:- Blood samples were incubated in liquid broth. They were sub-cultured on blood agar and Maconkey agar. If turbidity detected, then salmonella organisms were identified. Serotyping was then used to identify specific salmonella strains.

Typhidot (IgM) test: - One micro-milliliter containing 0.3µg of 50 kD protein of S.typhi has been double dotted onto nitrocellulose test strips. After probing with 1:100 dilution of serum, the strips were washed with distilled water and antigen-antibody complexes visualized one hour after addition of horse-reddish peroxide-conjugated anti-serum to human IgM. A substrate, 4-chloro-1-naphthol, has been added as coloring agent. The test results were interpreted as; positive test- when double dots on test strips were as dark as or darker than their corresponding dots on positive control strip; negative test- absence of visible dots on test strips; equivocal-discrepant appearance of duplicate dots on test strips.

The equivocal test results have to be repeated when duplicate dots were fainter than control.

Data Analysis

The validity of typhidot (IgM) has been measured by using blood culture as gold standard. Sensitivity, specificity, positive and negative predictive values were computed with positive blood culture for S.typhi. Statistical data was analyzed by SPSS version 10.0. Sensitivity and specificity were calculated by following formulae;

Sensitivity = 100 x a/a+c
Specificity = 100 x d/b+d
Positive Predictive value =a x 100/a + b
Negative Predictive value = d x 100/c +d.

a = True positive
b = False positive
c = False negative
d = True negative.

RESULTS

A total of 100 patients with clinically suspected typhoid fever were studied during nine months period. All patients who fulfilled the inclusion criteria have immediately undergone for blood culture and typhidot (IgM) tests respectively. The mean age of study group was 26.31±11.8 years and majority (75%) were males than females (25%) having M to F ratio 3:1. More patients (85%) were admitted before tenth day of illness during this study.

Among 100 clinically diagnosed typhoid cases, 19(19%) have positive blood culture for S.typhi and 71(71%) were typhidot (IgM) positive.

Table-II. Diagnostic accuracy of typhi dot (IgM) test.

Parameters	Accuracy
Sensitivity	94.73%
Specificity	90%
Negative predictive value	97.72%
Positive predictive value	78.26%

Out of 19 culture positive patients, 18(94.73%) were true typhidot (IgM) positive, which was also falsely positive in 05(20.83%) among 24 non- typhoidal febrile controls. Of these 05 false positive patients, 02 had infection with salmonella paratyphi A which was proved by S.paratyphi A blood culture, while rest of 03 had other infections (i.e. malaria 01, urinary tract infection 01, pneumonia 01). None of the healthy controls was positive for typhidot (IgM) test. This has been shown in table I. The sensitivity, specificity, negative and positive predictive values of typhidot (IgM) test using blood culture as gold standard were 94.73%, 90%, 97.72% and 78.26% respectively for patients having typhoid fever. This has been shown in table II.

DISCUSSION

Typhoid fever still remains a major endemic public health problem in Pakistan 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 rural and hilly areas of our country, where disease is more prevalent. In addition, culture method is expensive,

Table-I. Results Showing validity of typhidot (IgM) in different study groups (n=100).

Study groups	No. of patients	Typhidot (IgM) positive	Typhidot (IgM) negative
Group A (Definite typhoid fever)	19	18 (94.73%) (True positive)	1 (5.26%) (False negative)
Group B (Typhoid suspect plus non-typhoidal illness)	24	05 (20.83%) (False positive)	19 (79.16%) (True negative)
Group C (Healthy controls)	24	-	24 (100%) (True negative)

time-consuming and usually negative due to prior usage of antibiotics. Therefore, in present scenario, typhidot (IgM) test is an ideal alternative providing rapid and early diagnosis of typhoid fever.

Our study demonstrated that, among 100 clinically diagnosed typhoid fever cases, 19(19%) were blood culture positive for salmonella typhi. This is quite closer to results of studies by Saha MR[8] and Hossain MS[9], whom found isolation rates of 21.1% and 16.67% respectively. In contrast to these studies, others have reported low isolation rates of only 8.40% and 6.92%[10,11]. During these studies[10,11], low sensitivity of blood culture in diagnosing typhoid fever was mainly due to indiscriminate use of antibiotics and difficulties in obtaining adequate volume of blood for culture from children[12,13].

Males were predominant and mean age of study group closely matches to results by Khoharo KH[17]. Also in our study, 18(94.73%) out of 19 culture-positive cases were positive for typhidot (IgM) test. Similar findings were also reported by Bhutta ZA[12] from Pakistan showing 43(93.47%) typhidot positives out of 46 culture-positive typhoid cases, Sherwal BL[14] from India showing 35(92.10%) out of 38 culture-positive typhoid cases and Begum Z[15] from Bangladesh showing 13(92.85%) out of 14 culture-positive cases, were positive for typhidot (IgM) test. Only 01(5.26%) out of 19 culture-positive cases, of our study has false negative typhidot (IgM) and he was 35 years old male taking corticosteroids for chronic bronchial asthma. He attends the hospital on 3rd day with high grade fever and vomiting. The probable reason for false negative typhidot (IgM) test in this patient was immunosuppression from longstanding corticosteroid therapy. During this study, 05(20.83%) of our patients have falsely positive typhidot (IgM) test. This is quite closer to results of study by Bhutta ZA[12], who found false positive typhidot (IgM) test in 6(23.07%) out of 26 non-typhoidal febrile controls. Out of 05 cases in our study, 02 had infection with S.paratyphi A and rest of 03 had non-typhoidal fever. The false positive typhidot in S.paratyphi A infection may be due to cross-reactivity between outer membrane protein (OMP) antigen of S.typhi and S.paratyphi A[16]. In addition, false positive results of typhidot (IgM) in rest of 03 patients having non-typhoidal fever probably either by persistently elevated antibodies titer from previous infection or recent sub-clinical infection[16]. This explains why typhoid fever is highly endemic in this region.

In our study, typhidot (IgM) test has high sensitivity and specificity values comprising of 94.73%, 90% respectively. Sensitivity and specificity results of our study are comparable with the studies conducted by Begum Z[15], Sherwal BL[14], Jesudason MV[11] and Khoharo KH[17], whom showed sensitivities and specificities of 92.85% and 90%, 92% and 87.5%, 92.3% and 98.8%, 93% and 98.8% respectively hence showing usefulness of typhidot (IgM) test in early diagnosis of typhoid fever. In contrast, a local study by Shaikh KR[18] has shown a very low sensitivity of 72.4% for typhidot test. This may be due to high rate of false positive results of typhidot (IgM) test among non-typhoidal fever patients or genomic diversity between S.typhi isolates in the region.

CONCLUSIONS

Our study concludes, although blood culture is the gold standard for diagnosis of typhoid fever but typhidot (IgM) test can be a simple, reliable and valid alternative diagnostic tool especially in areas where laboratory services are limited. It has high sensitivity, specificity and negative predictive values.

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Correspondence Address:
Dr. Atif Sitwat Hayat
MBBS, M.D (Medicine)
Consultant Physician
Assistant Professor of Medicine,
Northern Institute of Medical
Sciences (NIMS) Abbottabad,
NWFP, Pakistan.
ashayat@hotmail.com

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"A man who doesn't trust himself can never truly trust anyone else."

(Cardinal de Retz)