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Original Article

IgM flow assay for rapid detection of Typhoid Fever

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Abstract

A case-control study was carried out in the Department of Microbiology & Immunology at BSMMU, Dhaka from July 2007 to June 2008 to validate IgM flow assay commercial diagnostic kits to assess the usefulness for the rapid diagnosis of typhoid fever. A total of 437 febrile patients clinically suspected of having typhoid fever were studied. Sixty cases were taken as controls, in which 30 were febrile controls (non-typhoidal febrile illness) and 30 were healthy controls. Among these 437 patients, *Salmonella typhi* was isolated from 58 (13.27%) cases .The isolation rate of *S. typhi* from blood was higher 21 (22.34%) in pediatric age group than that of the adult 37(10.78%); which is statistically significant (P<0.003).The detection of specific LPS antibody (IgM flow assay) were evaluated in 58 culture proven cases,42 high Widal titre patients and 60 controls. The sensitivity of LPS antibody and Widal test was 91% and 54% respectively and specificity was 100% and 91.66% respectively. The serological assays based on the detection of IgM antibodies against serotype Typhi LPS had a significantly higher sensitivity and specificity than Widal test when used with a single acute phase serum sample(P<0.007). So, these test involving detection of anti-LPS antibodies could be of use for the diagnosis of typhoid fever in patients who have clinical typhoid fever but are negative in culture or in regions where bacterial culturing facilities are not readily available.

Key words: LPS, Widal test, Culture, Typhoid fever

Introduction

Typhoid fever is an enteric fever of humans caused by infection with Salmonella enterica serovar Typhi (ST). It is an acute generalized infection of the reticuloendothelial system, intestinal lymphoid tissues and gallbladder[1]. Diagnosis of typhoid fever can be difficult because its non-specific symptoms and signs can be easily confused with those of other acute and sub acute infectious and non infectious febrile disease, such as dengue fever, malaria, viral fever and leptospirosis, which are often prevalent in the same areas. The diagnosis of enteric fever is ultimately based on isolation and identification of bacterium in cultures but though the clinical setting, serological tests and hematological abnormalities may suggest the diagnosis. Blood cultures are the gold standard diagnostic method for diagnosis of enteric fever. The sensitivity of blood culture is highest in the first week of the illness and reduces with advancing illness. Stool and urine cultures are not recommended for diagnosis because of poor sensitivity[2]. The Widal test has been used very extensively in the serodiagnosis of typhoid fever in developing countries particularly, remains the only practical test available. Numerous studies, however, have produced data which have cast serious doubts on the value of the Widal test in the diagnosis of typhoid fever. The test has only moderate sensitivity and specificity. Several factors have contributed to this uncertainty. These include poorly standardized antigens, the sharing of antigenic determinants with other Salmonella and the effects of treatment with antibiotics and previous immunization with TAB vaccine [3].

Recently, a typhoid fever IgM flow assay, using LPS antigen has been introduced as a rapid, qualitative, unique two-site sandwich immunoassay for the detection of IgM antibody. The test is based on the binding of S.typhi specific IgM antibodies to S.typhi Lipopolysaccharide (LPS) antigen and the staining of the bound antibodies by an antihuman IgM antibody conjugated to colloidal dye particles and utilizes the principle of immunochromatography and carried out using a

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nitrocellulose membrane. This test will be useful in places where culture facilities are not available as it can be performed without formal training and in the absence of specialized equipments. It has high specificity (89%) and has a high positive predictive value (95%) [4].

So the present study was aimed to compare the usefulness of different serological diagnostic tools for rapid and early detection of typhoid fever.

Material and methods

A case-control analytical study carried out in the department of microbiology, at Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, from July 2007 to June 2008. The patients were selected from outpatients department of Bangabandhu Sheikh Mujib Medical University Hospital; Dhaka. Patients were selected according to the clinical data and detail information. Healthy controls consisted of individuals who did not have any history of fever within last one year [5]. The followings were excluded from the control study: immunized with typhoid or paratyphoid vaccines, Patients suffering from immune complex mediated disease like rheumatic fever, systemic lupus erythematosis and glomerulonephritis, Patients under therapy on immune suppressive drugs. Single blood sample was taken for primary blood culture by conventional method and serological test from each patient within first week to fourth week of illness. Widal agglutination test was done in all patients and controls using single samples by rapid slide agglutination method, containing O and H antigens of *Salmonella typhi* and O and H antigens of *S .paratyphi* A and B. On the basis of culture results and Widal test results the study patients were divided into two groups .In Group-I: Bacteriologically proven typhoid (n= 58). Serum samples of patients in whom the diagnosis was confirmed by the isolation of *Salmonella typhi* from blood. Group-II: includes culture negative typhoid patients (n=42). Serum of patients in whom the diagnosis of typhoid was based on clinical suspicion and high (TO/TH ≥ 320) in sera by Widal agglutination test but without confirmation by isolation of *Salmonella typhi* from blood. Two other study groups were: Group III: febrile controls (n=30). Serum from patients with non-typhoidal febrile illness. Group IV: Healthy control (n=30). ICT method was done as per manufacture's instruction using serum sample from all groups of patient and controls.

Rusults

A total of 437 febrile patients clinically suspected of having typhoid fever were studied. Sixty cases were taken as controls, in which 30 were febrile controls (non-typhoidal febrile illness) and 30 were healthy controls. Among these 437 patients, *Salmonella typhi* was isolated from 58 (13.27%) cases .The isolation rate of S.typhi from blood was higher 21 (22.34%) in pediatric age group than that of the adult 37(10.78%) which is statistically significant (P<0.003). LPS (IgM) antibody was positive in 54 (93.10%) out of 58 culture positive typhoid cases and 37 (88.09%) out of 42 culture negative typhoid cases. No positive result came from febrile and healthy control (Table I)

Table I: Result of LPS (IgM) antibody among study groups (n=160)

Study groups	No. of Individual	IgM positive	Percentage
I	58	54	93.1%
II	42	37	88.09%
III	30	0	0
IV	30	0	0

Among the 58 culture positive patients 12 (20.68%) patients were both positive to LPS antigen and Widal test. Remaining 46 patients were Widal test negative but 42 of them were positive for antibody response to LPS.(Table-II)

Out of 42 culture negative patients 16 had only TO and 13 patients had only TH and remaining 13 had both TO and TH (≥320) titer high. Out of 16 TO titer high patients 14 were positive for LPS antibody. Out of 13 TH titer high patients 10 were LPS antibody positive. All 13 patients were positive for LPS antibody who had high titer for TO and TH (Table-II).

Table II: Comparison of Widal test and LPS (IgM) antibody among typhoid patients (n= 100)

Typhoid patients	Widal Antigen	No. of Patient	Positive LPS antibody
Group I	TO≥320	12(20.6)	12(20.6)
N= 58	TH≥320	03(5.17)	03(5.17)
	TO&TH<320	46(79.3)	42(72.4)
Group II	TO≥320	16(38.09)	14(33.3)
N=42	TH≥320	13(30.9)	10(23.8)
	TO &TH ≥320	13(30.9)	13(30.9)

TableIII. showed the comparative results of antibody response to LPS, culture and Widal test among 100 typhoid patients according to duration of fever. 31 patients who had fever within 1 week , all were culture test positive but none of them were widal test positive. Out of these 31 patients 28 (90.32%) were LPS antibody positive. Among 43 patients who were presented with 2nd weeks of fever 24 (55.8%) were culture positive, 29 (67.44%) were widal positive, 42 (97.6%) were LPS antibody positive.

Out of 14 patients who were presented in the 3rd week of fever 11(78.5%) were LPS antibody positive, 13(92.8%) were Widal test positive, and only 3 (21.4%) were culture positive patients.

In the 4th week none out of 12 patients were culture positive but all of the patients were positive for Widal test. Out of those 12 patients 10 were LPS antibody positive.

Table III: Comparison of culture and serological tests in different weeks of fever (n=100)

Duration of fever(week)	No. Of Individuals	Culture positive patients (%)	LPS antigen	Widal test (%)
1st	31	31(100)	28(90.32)	00(0)
2nd	43	24(55.8)	42(97.67)	29(67.6)
3rd	14	03(21.4)	11(78.57)	13(92.8)
4th	12	00(0)	10(83.3)	12(100)
Total	100	58	91	54

The sensitivity of LPS antibody was found to be much higher than Widal test (91% VS 54%) in the early serodiagnosis of typhoid fever. Specificity of LPS antibody and Widal test were 100%, and 91.66% respectively.(Table-IV)

Table IV. Sensitivity and Specificity of different diagnostic tests.

Test Validity	LPS (IgM)	Widal (TO/TH ≥1:320)
Sensitivity	91%	54%
Specificity	100%	91.66%
Positive predictive value	100%	91.52%
Negative predictive value	86.95%	54.45%

Discussion

In the recent study, the Typhoid fever IgM flow assay (LPS antibody) was done on 100 confirmed typhoid patients (Group I &II). They included both blood culture positive cases and blood culture negative but "significant titer" high Widal cases. IgM flow as say was compared with the results of culture positive, Widal≥320 titer cases and control sera.

In 58 culture positive patients; 54 (93.1%) were positive for IgM flow assay (Table-I).Our results are remarkably consistent with the finding of Gasem *et al*. (2002)[6]; who found that 32 (86.5%) out of 37 culture positive typhoid patients were IgM flow assay positive. In contrast, a study from Indonesia reported that positivity of the IgM flow assay ranges from 41.25% to 89.5% depending on the duration of illness; 41.2% sensitivity was found in the 4th day of illness which increased 89.5% in the 7th day [7]. Similarly, Hatta *et al*. (2002)[4] also found that IgM assay was positive in 43.5%, 92.9%, and 100% for the sample collected in 4-6 days,6-9 days and >9 days after the onset of fever respectively. In our study, positive result in the IgM flow (LPS antibody) assay was obtained in 28 (98.32%) out of 31 patients admitted during the first week of illness. 24 (100%) out of 24 patients during the second week and 2(66.6%) out of 3 patients those admitted during the third week of illness (Table-III). Closely similar findings were also reported by Gasem et al. (2002)[6] who found that IgM assay was 84.6% positive in the first week, 75.4% in the second week and 69.3% in the third week among the culture proven cases. These results were not significanty different. Only four patients who tested negative in IgM flow assay in this group was attend in the 2nd and 3rd day of fever having insignificant titer (TO/TH<1:80) in the widal test (Table-III). The negative IgM flow assay in these four cases was probably due to the failure of IgM assay to detect the antibodies or perhaps the antibodies did not yet reach the detectable level in these patients.

In our study it was found that IgM flow assay becomes positive from the 4th day of fever in maximum number of cases i.e. 90.32% and on the 2nd week onwards it becomes 97.67% positive. Whereas Widal test becomes positive from the 2nd week of fever (Table-III). This findings correlated with the traditional view that Widal agglutination titre begins to appear after about first week of illness [8].

Conclusion:

IgM flow assay against LPS antigen, a new serologic test is highly sensitive for the early serodiagnosis of typhoid fever when the Widal test is often negative. It also offers the advantage of specificity and reliability over the Widal test and testing of a single serum specimen is often sufficient. Its high negative predictive value in an endemic area is also an advantage and the IgM assay is easy to use, rapid test and does not require any special technology. Thus, the application of the IgM flow assay clearly offers the opportunity to start the appropriate treatment at the same day the patients come to hospital. So, in typhoid fever IgM flow assay might replace the Widal test as a routine technique for early and accurate diagnosis of typhoid fever in areas where the disease is endemic.

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