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Full Length Research Paper

Detection of anti-salmonella antibodies by Immunochromatographic assay at Rajshahi Medical College, Bangladesh

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The public health burden of typhoid fever can be substantially reduced by early diagnosis and appropriate antibiotic therapy. This study was conducted in an attempt to assess the reliability of immunochromatographic test (ICT) for the early diagnosis of typhoid fever. Immunochromatographic test to detect IgM, IgG or combined IgM/IgG in serum in 1st week of fever was done for the diagnosis of typhoid fever. Blood samples were taken for culture and Widal test of 100 clinically suspected cases of typhoid fever in 1st week of illness of patients at Outpatient Department of Rajshahi Medical College Hospital (RMCH), Bangladesh. Forty (40) controls of comparable age and sex were also taken including 20 febrile (non-typhoidal) and 20 healthy persons. Blood culture positive cases (16) and Widal test positive cases (15) were considered as typhoid fever patients and the total number was 31. ICT was performed for 31 typhoid fever patients and 40 controls. In this study, out of 31 cases, ICT was found positive in 28 (90.32%) typhoid fever patient, while 03 (15.00%) of the controls also showed ICT positivity indicating its specificity of 92.50%. It is evident from this study that ICT as a reliable diagnostic tool for early diagnosis of typhoid fever was found highly sensitive, rapid and easy to perform. It can be a versatile test for the screening of clinically suspected case of typhoid fever. therefore, ICT have been found to be encouraging in this study.

Key words: Anti-salmonella antibodies, immunochromatographic assay, diagnostic tool, early diagnosis of typhoid fever.

INTRODUCTION

Enteric fever is still a significant public health burden in many developing countries and the incidence has been estimated at 540 cases per 100,000 of the population per year (Krishna et al., 2011). Typhoid fever is associated with significant morbidity and mortality worldwide, especially in tropical countries.

Incidence of typhoid fever has been estimated at approximately 22 million cases with at least 200,000 deaths occurring annually (Crump et al., 2004). The disease is endemic in many developing countries particularly in the Indian subcontinent including Bangladesh (Saha et al., 1996). In Bangladesh, the overall incidence of typhoid fever is 390 cases per 100,000 populations per year (Brooks et al., 2005). Diagnosis of typhoid fever still remains a puzzle. Although culture of blood remains to be the gold standard in the diagnosis of typhoid fever,

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but facility for culture is not widely available and also time consuming. Moreover, easy and open access to antibiotics without medical prescription in community makes it difficult to isolate organisms from blood culture and alternative methods of diagnosis, like bone marrow culture may be needed, but are invasive and difficult to conduct routinely (Hayat et al., 2011). The unsatisfactory yielding rate in culture for *Salmonella* has always urged laboratory scientists to hunt for rapid and inexpensive laboratory tests for early and accurate diagnosis of patients with typhoid fever, prompting the exploration of variety of rapid antibody detection methods.

As a means of immunodiagnostic procedure, Widal test which is widely used, readily available and inexpensive has been used for many years, but with lowered specificity problems due to background levels of IgG to *Salmonella typhi* in the regions of endemicity (Kawano et al., 2007). Classically, a fourfold or greater rise demonstrated in paired sera is considered diagnostic but it is not feasible and the diagnosis become retrospective one. Thus, there is always a need for the development of a simple, rapid, reliable and sensitive diagnostic method for the early diagnosis of typhoid fever especially in the endemic areas (Hossain, 2001).

The rapid and early immunodiagnosis of typhoid fever can be done by the detection of anti-salmonella antibodies by immunochromatographic test (ICT) which does not require any specialized laboratory or highly skilled personnel and can be done in field areas also. Its usefulness has been shown to detect the anti-salmonella antibodies as early as 4 days of fever onset (Collee et al., 1996). False positivity of immunochromatographic tests in control population detecting anti-salmonella antibodies is very low (Collee et al., 1996).

Antihuman gamma globulin against IgG and IgM fixed in the strip can detect both IgM and IgG of anti-salmonella antibodies in the serum. Thus, detection of anti-salmonella antibodies (IgM and IgG) by immunochromatographic test may be an appropriate adjunct for the clinical diagnosis of typhoid fever. The purpose of the present study was to assess the reliability of immunochromatographic test (ICT) for the early diagnosis of typhoid fever.

METHODOLOGY

This prospective study was carried out from July, 2006 to June, 2007 in Microbiology Department of Rajshahi Medical College, Bangladesh. The research protocol was approved by the Institutional Review Board (IRB) of Rajshahi Medical College for issues of ethical clearance.

Study population

Patients

One hundred (100) clinically suspected cases of typhoid fever patients attending the OPD of Rajshahi Medical College Hospital 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, etc. Cases having fever with at least one of the above clinical features within 1st week of illness were considered as typhoid suspects.

Controls

Twenty (20) non febrile healthy persons of comparable age and sex without having fever and past history of symptom suggestive of typhoid fever in the last six month were considered as healthy controls. Another twenty febrile controls included pulmonary tuberculosis, respiratory treat infection and urinary tract infection in which the diagnosis was confirmed earlier by relevant investigations (Hossain, 2001).

Laboratory procedure

One blood sample was taken from each suspected case at the first week of illness for culture and serological test. A second blood sample was collected from randomly selected 26 culture negative cases 7-10 days after collection of the first sample to see the rising titer. Blood sample was taken from all controls to perform ICT according to the manufacturer's instruction (IDL Biotech AB, Sweden). Blood sample was taken from all controls to perform ICT on single occasion. Blood culture was done by conventional methods using the Trypticase soya broth with sodium polyanethol sulfonate. Isolated bacteria were identified according to the recommended standard protocol. Immunochromatographic tests were done for typhoid fever patients and controls. Blood culture positive cases and cases with rising titer of widal tests were considered as typhoid fever cases. Controls include both non-typhoidal febrile controls and non febrile healthy controls.

RESULTS

A total of 100 clinically suspected cases of typhoid fever and 40 controls were studied. Among the controls, 20 were febrile controls and 20 were healthy controls. Out of 100 suspected cases of typhoid fever, blood culture positive for *S. typhi* were 16 (16%) and remaining 84 (84%) were negative (Figure 1). Widal tests were performed for all 100 typhoid suspected cases during first week of illness. Further, 26 cultures negative cases were randomly selected for paired sera in Widal test to see the rising titre for the serodiagnosis of typhoid fever. Out of 26 cases, 15 (48.38%) were found to have rising titer and considered as Widal positive patients. ICT were performed to detect IgM or IgG or both in serum in the first week of illness in the case of culture positive patients (16) and Widal positive patients (15). To evaluate the sensitivity and specificity of this device, ICT was also done in the case of healthy controls and febrile controls. ICT were found positive in 28 (90.32%) out of 31 typhoid fever patients (16 culture positive patients and 15 Widal positive patients). The tests were also positive in 3 (15%) febrile controls. None of the healthy control was positive by ICT (Table 1). Among the 16 culture positive patients, ICT were positive in 15 cases while 9 (57%) were positive

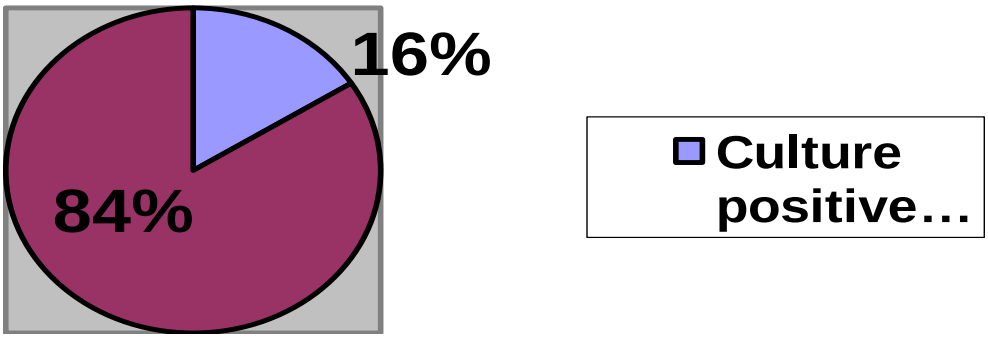

Figure 1. Rate of isolation of *S. typhi* in blood culture. Among 100 suspected cases of typhoid fever, 16 (16%) were found positive for *S. typhi* and 84 (84%) were found negative in blood culture.

Table 1. Detection of anti-salmonella antibodies by immunochromatographic assay (ICT) in typhoid patients at Rajshahi Medical College, Bangladesh.

Study group	ICT positive cases	ICT negative cases	Total cases
Typhoid patients	28 (90.3)	3 (9.7)	31 (100.0)
Febrile controls	3 (15.0)	17 (85.0)	20 (100.0)
Healthy controls	00	20 (100.0)	20 (100.0)

Figure in parenthesis indicates percentage.

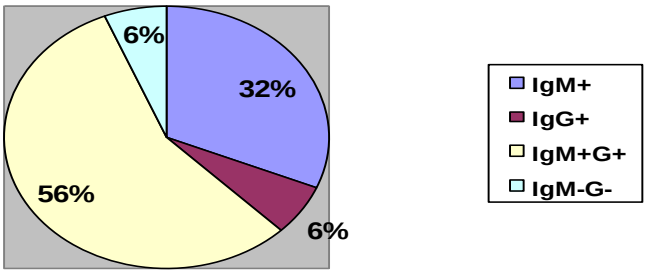

Figure 2. Results of ICT in culture positive patient. Among 16 culture positive patients, ICT were positive in 15 cases where 9 (57%) were positive for both IgM and IgG, 5 (31%) were positive for IgM, and 1 (6%) was positive for IgG only whereas 1 (6%) was negative for IgM and IgG.

for both IgM and IgG, 5 (31%) were positive for IgM and 1 (6%) was positive for IgG only, whereas 1 (6%) was negative for IgM and IgG (Figure 2). Out of 15 Widal positive patients, ICT were positive in 13 cases while 8 (54%) were positive for both IgM and IgG, 3 (20%) were positive for IgM, 2 (13%) were positive for IgG only, whereas 2 (13%) were negative for IgM and IgG (Figure 3). Among the 31 typhoid patients, 17 (54.83%) were positive for both IgM and IgG, 8 (25.80%) were positive for IgM and 3 (9.69%) were positive for IgG. Out of the 20 febrile controls, 2 were positive for both IgM and IgG and 1 showed positivity for IgG only (Table 2).

DISCUSSION

In the present study, out of the 100 clinically diagnosed typhoid fever, 16 were blood culture positive for *S. typhi*. Similar findings were also reported from another study in Bangladesh where the isolation rate is 14% (Begum et al., 2009). This lower values in positivity of blood culture could be due to the rampant use of antibiotics by private practitioners. This value is too low to satisfy the criterion of a diagnostic test, irrespective of the reasons for its low yield (Beig et al., 2010). In this study, ICT was done on 31 typhoid fever patients (16 blood culture positive patients and 15 Widal positive patients) and was found positive in 28 patients in the first week of illness. A positive IgM or IgG either alone or in combination, was regarded as a positive ICT in this study. Detected IgM antibody is suggestive of recent infection and IgG indicates a current or previous infection (Parry et al., 2011). The reason for considering IgG as positive for ICT is that when typhoid infection occurs the immune response mechanism produces both IgM and IgG as well. That is why positivity of IgG is considered as positive ICT. Fifteen (93.75%) out of 16 culture positive patients were found positive for ICT in our study. The only patient who tested negative in ICT in this group was a 3 year old child diagnosed in the 2nd day of fever which also had insignificant titer (TO/TH=20/20) in Widal test. The negative ICT in this case may be due to the inadequate antibodies production

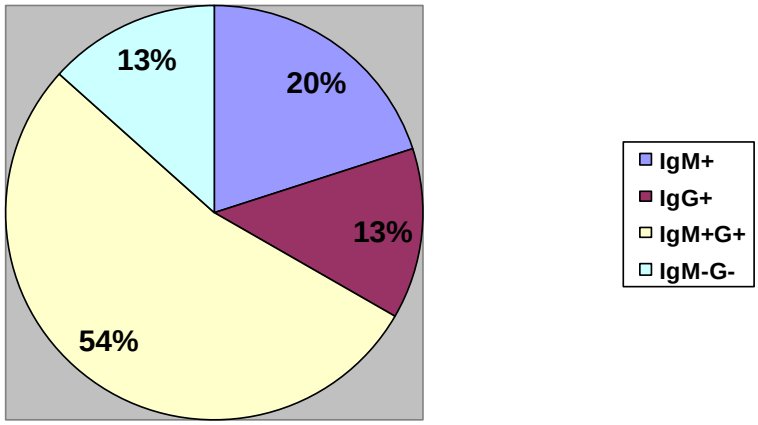

Figure 3. Results of ICT in Widal positive patient. Figure shows that out of 15 Widal positive patients, ICT were positive in 13 cases whereas 8 (54%) were positive for both IgM and IgG, 3 (20%) were positive for IgM, 2 (13%) were positive for IgG only whereas 2 (13%) was negative for IgM and IgG.

Table 2. Positivity of IgM, IgG or combined IgM/IgG in ICT among typhoid patients and controls.

Study group	ICT			Total ICT positive
	M+	G+	M+G+	
Typhoid patients	8	3	17	28 (90.3%)
Febrile controls	0	1	2	3 (15.0%)
Healthy controls	0	0	0	0

Table 3. Validity of ICT for detection of typhoid fever.

Test validity	Result (%)
Sensitivity	90.3
Specificity	92.5
Positive predictive value	90.3
Negative predictive value	92.5
Accuracy	91.5

to be delectable by ICT (Table 3).

On the other hand, 13 (88.88%) out of 15 Widal positive patients were found positive for ICT. Only 2 Widal positive patients were found negative for ICT. The negative ICT in these cases were perhaps due to the antibodies which did not yet reach detectable levels in the first week of fever. Among the 20 non-typhoidal febrile patients, ICT was found positive for 3 (15%) cases.

In this study, the sensitivity, specificity, positive and negative predictive value of the test was found as 90.32, 92.50, 90.32 and 92.50%, respectively. The results are remarkably consistent with the findings of another investigator in Bangladesh who noted sensitivity, specificity, positive and negative predictive value as 91.42, 90.00, 88.88 and 92.30%, respectively (Begum et al., 2009).

It is generally stated that, conventional blood culture is time consuming procedure with a very low yielding rate though it is considered still as the gold standard for the detection of typhoid fever. During blood culture it is important to consider adequacy of blood and it should be at least 1:5 dilution with the tryptica soy broth with blood. It is not a routine practice in common laboratory while Widal titer in a single blood sample is only presumptive. Whereas almost certain diagnosis of typhoid fever can be done by doing ICT in a single blood sample in the first week of illness.Thereby, ICT is a more sensitive and specific test which is easy to perform and more reliable as compared to the Widal test and is it useful in early therapy and similar report was also mentioned in another study (Khoharo, 2011).

Although, bacterial isolation remains to be conclusive for the diagnosis of typhoid fever but failure to perform culture does not rule out the existence of infection. Laboratory diagnosis of typhoid fever always put a dilemma since conventional blood culture is a cumbersome, time consuming procedure with unsatisfactory yielding rate. Though Widal test is performed widely it has some limitation due to poor standardization and difficulty in interpretation on a single sample (Kawano et al., 2007). Considering the practical situation of laboratory diagnosis, detection of anti-Salmonella antibodies by ICT has been found to be quite reliable, easy to perform and may be a good adjunct to clinical suspect in early days of

fever.

Conclusion

The study concludes that ICT is a reliable diagnostic method for early and rapid detection of typhoid fever and was found to be highly sensitive, rapid and easy to perform. It can be a versatile test for the screening of clinically suspected case of typhoid fever. So, ICT have been found to be encouraging in this study.

REFERENCES

Begum Z, Hossain A, Shamsuzzaman AKM, Monjurul AM, Musa AKM, Mahmud C, Sumona AA, Ahmed S, Jahan NA, Khaleque A (2009). Evaluation of Typhidot (IgM) for Early Diagnosis of Typhoid Fever. Bangladesh J. Med. Microbiol. 03(01):10-13.

Begum Z, Hossain MA, Musa AK, Shamsuzzaman AK, Mahmud MC, Ahsan MM, Sumona AA, Ahmed S, Jahan NA, Alam M, Begum A (2009). Comparison between DOT EIA IgM and Widal Test as early diagnosis of typhoid fever. Mymensingh Med. J. 18(1):13-17.

Beig FK, Ahmad F, Ekram M, Shukla I (2010). Typhidot M and Diazo test *vis-à-vis* blood culture and Widal test in the early diagnosis of typhoid fever inchildren in a resource poor setting'. Braz. J. Infect. Dis. 14(6):589-593.

Brooks WA, Hossain A, Goswami D, Sharmeen AT, Nahar K, Alam K, Ahmed N, Naheed A, GB Nair, Luby S, Breiman RF (2005). Bacteremic Typhoid Fever in Children in an Urban Slum, Bangladesh. Emerg. Infect. Dis. 11(2):326-329.

Collee JG, Fraser AG, Marmion BP, Simons A (1996). Old DC. Salmonella infection. In: Mackie and McCartney PracticalMedical Microbiology, 14th ed. New York: Churchil Livingstone pp. 385-402.

Crump JA, Luby SP, Mintz ED (2004). The global burden of typhoid fever. Bull World Health Org. 82:1-24.

Hayat AS, Shah SIA, Shaikh N (2011). Evaluation of Typhidot(IgM) in early and rapid diagnosis of typhoid fever. Professional Med J. 18(2):259-264.

Hossain MS (2001). Comparative study of Widal test and DOT enzyme immunoassay for early serodiagnosis of typhoid fever(Thesis).Dhaka, Bangladesh: Bangabandhu Sheikh Mujib Medical University.

Kawano RL, Leano SA, Agdamag DMA (2007). Comparison of serological test kits for diagnosis of typhoid fever in the Philippines. J. Clin. Microbiol. 45(1):246-247.

Kawano RL, Leano SA, Agdamag DMA (2007). Comparison of serological test kits for diagnosis of typhoid fever in the Philippines. J. Clin. Microbiol. 45:246-247.

Khoharo HK (2011). A comparative study of the typhidot (Dot-EIA) and Widal tests in blood culture positive cases of typhoid fever. Trop. Doct. 41(3):136-138.

Krishna S, Desai S, Anjana VK, Paranthaaman RG (2011). Typhidot (IgM) as a reliable and rapid diagnostic test for typhoid fever. Ann. Trop. Med. Public Health. 4(1):42-44.

Parry CM, Wijidoru L, Arjyal A, Baker S (2011). Utility of Diagnostic Tests for Enteric fever in Endemic. Expert Rev. Anti. Infect. Ther. 9(6):711-725.

Saha SK, Ruhulamin M, Hanif M, Islam M, Khan WA (1996). Interpretation of the Widal test in the diagnosis of typhoid fever in Bangladeshi children. Ann.Trop. Paediatr. 16:75-78.