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,
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 becomes 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.

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 to see the rising titre for the serodiagnosis of typhoid fever. Out of 26 cases, 15 (48.38%) were found to have rising titre 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.

<table>
<thead>
<tr>
<th>Study group</th>
<th>ICT positive cases</th>
<th>ICT negative cases</th>
<th>Total cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Typhoid patients</td>
<td>28 (90.3)</td>
<td>3 (9.7)</td>
<td>31 (100.0)</td>
</tr>
<tr>
<td>Febrile controls</td>
<td>3 (15.0)</td>
<td>17 (85.0)</td>
<td>20 (100.0)</td>
</tr>
<tr>
<td>Healthy controls</td>
<td>0</td>
<td>20 (100.0)</td>
<td>20 (100.0)</td>
</tr>
</tbody>
</table>

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.

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 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).
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.

<table>
<thead>
<tr>
<th>Study group</th>
<th>ICT M+</th>
<th>ICT G+</th>
<th>ICT M+G+</th>
<th>Total ICT positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Typhoid patients</td>
<td>8</td>
<td>3</td>
<td>17</td>
<td>28 (90.3%)</td>
</tr>
<tr>
<td>Febrile controls</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3 (15.0%)</td>
</tr>
<tr>
<td>Healthy controls</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Test validity</th>
<th>Result (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>90.3</td>
</tr>
<tr>
<td>Specificity</td>
<td>92.5</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>90.3</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>92.5</td>
</tr>
<tr>
<td>Accuracy</td>
<td>91.5</td>
</tr>
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</table>

to be detectable 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


