Diagnostic Usefulness of Vi-Indirect Fluorescent Antibody Test (Vi-IFAT) for Typhoid Fever

— A Prospective Study —

June Myeong Kim¹, Eung Kim¹, Yunsop Chong² and Chein Soo Hong¹

Although the confirmative diagnosis of typhoid fever is by culture of the causative organism, usually from blood, a serological test is still necessary to provide a more rapid method of diagnosis. The indirect fluorescent antibody test, using a Salmonella typhi Vi antigen and a FITC-conjugated rabbit anti-human polyvalent immunoglobulin, was evaluated for the diagnosis of typhoid fever. Serum specimens were collected from patients with febrile diseases on admission.

Of the 32 patients with titers of 1:64 or more, 22 were confirmed to have typhoid fever by blood culture and 7 had fever of undetermined origin that was considered to be typhoid fever clinically. Three patients were diagnosed to have salmonellosis other than typhoid fever.

Of the 121 patients with titers of 1:32 or less, 105 patients had non-typhoidal febrile disease, 15 patients had fever of undetermined origin, and one patient was confirmed to have typhoid fever by blood culture.

When a Vi antibody titer of 1:64 or more was taken as serological evidence for the diagnosis of typhoid fever, the sensitivity and specificity were 95.7% and 97.2%, respectively.

The incidence of positive test results following fever onset was 70.0% within 1 week of fever onset, 88.9% from 1 to 2 weeks, and 100% after 2 weeks.

In conclusion, the Vi-indirect fluorescent antibody test (Vi-IFAT) can be employed as a useful serologic test in the diagnosis of typhoid fever.

Key Words: Typhoid fever, fluorescent antibody test, Vi antibody estimation

Typhoid fever remains endemic in many developing countries and is still an important disease in the differential diagnosis of febrile infectious diseases.

The most widely accepted method for conclusive diagnosis is the demonstration of Salmonella typhi by blood culture. But it requires at least 2 to 3 days for completion and may give a false negative result when there are too few organisms in the blood due to indiscriminate use of antibiotics (Robertson et al. 1968; Keusch 1986; Kim et al. 1986).

Also, a serologic method by the Widal test has been widely used for the diagnosis of typhoid fever, but it has shown limited value because of high false-negative and false-positive rates (Schroeder 1968; Wicks et al. 1971; Brodie 1977).

Other serological techniques have been studied, including enzyme-linked immunosorbent assay (ELISA) (Carlsson et al. 1972; Sippel et al. 1978; Nardiello and Pizzella 1984; Hwang et al. 1987), radioimmunoassay (Tsang et al. 1981; Chau et al. 1981), coagglutination assay (COAG) (Mikhail et al. 1983; Shetty et al. 1985) and counter-immunoelectrophoresis (Gupta and Rao 1981; Sundaraj et al. 1983). However, the clinical application of these techniques has been limited because of difficulties with the methods.

Therefore, a new, simple and accurate diagnostic method was recently needed for the diagnosis of typhoid fever.

In this respect, Doshi and Taylor (1984) attempted to apply an indirect fluorescent antibody test using a Salmonella typhi Vi antigen in the diagnosis of typhoid fever and showed excellent results in sensitivity and specificity.
Thus, we introduced the Vi-indirect fluorescent antibody test (Vi-IFAT) in the diagnosis of typhoid fever and reported good results obtained with it in a retrospective study in 1987 (Kim et al. 1987). In this study, the Vi-IFAT was performed as a prospective study to confirm the results of the retrospective study.

MATERIALS AND METHODS

Serum specimens

Sera were abstained from 153 patients who were admitted due to febrile diseases to Yonsei Medical Center, Yonsei University College of Medicine from May 1987 to February 1988.

Serum specimens were collected immediately on admission. Additional specimens were collected twice a week for two weeks following admission and then once a week.

Vi antigens

A strain of Vi-positive Salmonella typhi (YS 0682) was used for the preparation of Vi bacterial cell antigen. The phage type of Vi antigen used in this study was M1.

Cells of the strain were grown on blood agar by incubation at 37°C for 18 hours. The bacterial cells were harvested by centrifugation at 400×g for 20 min and washed two times in phosphate-buffered saline solution (PBS) with 0.3% formalin.

The suspension was adjusted to a concentration of 100 to 200 cells per high-power dry field in 0.05% homogenised normal yolk sac suspension which facilitates adherence of the antigen to the microscope slides.

Finally, a whole Salmonella typhi Vi suspension was obtained.

Indirect fluorescent antibody test (IFAT)

Smears of whole Salmonella typhi Vi suspension were made within a circle 5mm in diameter on clean slides. The smears were fixed with acetone and either used immediately or kept at −60°C until used.

Serum specimens were diluted two fold in PBS from 1:16 to 1:512. Volumes of 0.02ml of serum dilutions were added to circles containing bacterial cell antigen and incubated in a wet chamber at 37°C for 30 min. The slides were rinsed and washed in PBS for 10 min.

Polyvalent anti-human immunoglobulin antiserum conjugated to fluorescein isothiocyanate (FITC; Axell

<table>
<thead>
<tr>
<th>Interpretation of Vi-indirect fluorescent antibody test (Vi-IFAT)</th>
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<tbody>
<tr>
<td>+++ : Strong fluorescence with brilliant margin</td>
</tr>
<tr>
<td>++  : Weaker marginal fluorescence</td>
</tr>
<tr>
<td>+   : Bright fluorescence with demarcated margin</td>
</tr>
<tr>
<td>±    : Fairly weak fluorescence with diffuse margin or barely distinguishable fluorescence</td>
</tr>
<tr>
<td>−    : Negative fluorescence</td>
</tr>
</tbody>
</table>

Reagents) was diluted in Tween 80-PBS to 1 in 160. Volumes of 0.02 ml of the diluted fluorescent antiserum were added to the circles on the slides, which were again incubated in a wet chamber at 37°C for 30 min. The slides were then washed in PBS for 10 to 15 min and dried gently.

Finally, they were examined under a fluorescent microscope.

Interpretation

Fluorescence intensity was scored ++++, ++, +, ±, and −−. The end point was the last dilution giving + fluorescence (Table 1).

A titer of 1:64 or more was taken as a positive result for the diagnosis of typhoid fever.

RESULTS

Patients with positive results of Vi-IFAT

Of 32 patients with titers of 1:64 or more, 22 patients had culture proved typhoid fever and 7 patients had fever of undetermined origin that was considered to be typhoid fever clinically.

However, 3 patients with salmonellosis other than typhoid fever presented titers of 1:64 or more (Table 2).

Patients with negative results of Vi-IFAT

Of 121 patients with titers of 1:32 or less, 105 patients had non-typhoidal febrile diseases and 15 patients had fever of undetermined origin.

However, one patient with culture proved typhoid fever presented a titer of 1:32 or less (Table 3).

Sensitivity and specificity of Vi-IFAT

Of the 23 patients with culture proved typhoid fever, 22 patients showed a positive reaction of a titer of 1:64 or more and one patient presented a negative
Diagnostic Usefulness of Vi-FAT for Typhoid Fever

Table 2. Vi-FAT titers in 32 patients with positive reaction to Vi-FAT

<table>
<thead>
<tr>
<th>Diseases</th>
<th>No. of cases</th>
<th>Vi-FAT reciprocal titer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>&lt;16</td>
</tr>
<tr>
<td>Typhoid fever</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Salmonellosis Group-A*</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Group-B</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Group-D</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>F.U.O.**</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>32</td>
<td></td>
</tr>
</tbody>
</table>

* Salmonella paratyphi-A infection
** Fever of undetermined origin

Table 3. Vi-FAT titers in 121 patients with negative reaction to Vi-FAT

<table>
<thead>
<tr>
<th>Diseases</th>
<th>No. of cases</th>
<th>Vi-FAT reciprocal titer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>&lt;16</td>
</tr>
<tr>
<td>Salmonellosis Group-A*</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Group-B</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Group-C</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Group-E</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>G.I tract infections</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Urinary tract infections</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Respiratory tract infections</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Viral infections</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Rickettsial infections</td>
<td>5</td>
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</tr>
<tr>
<td>Collagen diseases</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Others</td>
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</tr>
<tr>
<td>Typhoid fever</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>F.U.O.**</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>121</td>
<td></td>
</tr>
</tbody>
</table>

* Salmonella paratyphi-A infections
** Fever of undetermined origin

reaction of a titer of 1:16. Thus, the sensitivity was 95.7%.

Of the 108 patients with non-typhoidal febrile disease, 105 patients showed a negative reaction of a titer of 1:32 or less and 3 patients presented a positive reaction of a titer of 1:64 or more. Thus, the specificity was 97.2%.

Incidence of positive test results following fever onset of typhoid fever

In 22 culture proved typhoid fever patients that showed positive reaction, the incidence of positive test results following fever onset was 70.0% within 1 week of fever onset, 88.9% from 1 to 2 weeks, and 100% after 2 weeks (Fig. 1).

DISCUSSION

The role of the Vi antigen, the capsular polysaccharide of Salmonella typhi, in the pathogenesis of and immunity to typhoid fever remains the subject of controversy. Vi-positive Salmonella typhi resist
phagocytosis and the action of serum complement, both of these actions are initiated by antibodies to Vi antigen (Felix et al. 1934; Kauffmann 1935).

Among more than one thousand serotypes of Salmonella species, Salmonella typhi, Salmonella paratyphi C, and Salmonella dublin have Vi antigen. Among Enterobacteriaceae other than Salmonella species, Citrobacter species has Vi antigen. But these species are rarely pathogenic to humans. Thus, an assay of serum antibodies to purified Vi antigen is a reliable method for the diagnosis of typhoid fever in respect of avoidance of cross reactions with other Enterobacteriaceae.

The detection of Salmonella typhi Vi antibodies by an agglutination test was first recommended by Felix et al. (1935). However, limitations of the value of the test have since been noted by various researchers (Anderson an Richards 1948; Brodie 1977).

Other serological techniques for the detection of Vi antibodies have been studied, including counter-immunoelectrophoresis (Tsang and Chau 1981), ELISA (Beasley et al. 1981), and haemagglutination test (Chau and Chan 1976; Nolan et al. 1981).

The indirect fluorescent antibody test has been studied by Chau and Chan (1976) and by Chikara and Urquhart (1979), who concluded that this technique was superior to the agglutination test in both sensitivity and specificity. Also, the IFAT is able to detect antibodies of all major classes, whereas the agglutination test detects antibodies of the IgM class preferentially (Kumar and Malaviya 1974). The IFAT has the advantage of using reagents which are readily available and it is technically simple.

In 1984, Doshi and Taylor introduced an indirect fluorescent antibody test using a whole Salmonella typhi Vi suspension as the antigen in the diagnosis of typhoid fever. Results using sera from 140 patients with culture proved typhoid fever showed the test to have good sensitivity (96.4%) when a titer of 1:64 or more was taken as positive response. Also titers of 1:32 or less were seen in 282 of 284 blood donors and in 108 of 110 patients with infections due to agents other than Salmonella typhi. Two patients that presented false-positive responses had legionellosis and tuberculosis, respectively. Thus, the specificity was 99.0%.

In our retrospective analysis (Kim et al. 1987), when a titer of 1:64 or more was taken as serological evidence for the diagnosis of typhoid fever, 59 of 61 sera from patients with culture proved typhoid fever were positive. Also, 118 of 119 sera from patients with non-typhoidal febrile diseases were negative. One patient that showed a false-positive result had acute pylonephritis due to Escherichia coli. So, the sensitivity and specificity were 96.7% and 99.2%, respectively. These results were similar to those obtained by Doshi and Taylor (1984).

In this prospective study, 32 patients showed a positive reaction of a titer of 1:64 or more on admission. Of these, 22 patients had culture proved typhoid fever and 7 patients had fever of undetermined origin that was considered to be typhoid fever clinically. But, 3 patients with group A, B, and D salmonellosis showed a false-positive result.

A negative reaction of a titer of 1:32 or less was shown by 121 patients on admission. Of these, 105 patients had non-typhoidal febrile disease and 15 patients had fever of undetermined origin, but 1 patient with proved typhoid fever showed a false-negative result. Thus, the sensitivity and specificity were 95.7% and 97.2%, respectively, and these results were similar to those of Doshi and Taylor (1984) and Kim et al. (1987). The positivity of the Widal test was 53.8% in this study.

One patient with proved typhoid fever showed a false-negative result that suggested an infection by Vi-negative Salmonella typhi strain (Robbins and Robbins 1984). Three patients with false-positive tests all had salmonelloses (group A, B, and D) other than typhoid fever, but showed negative reactions in our retrospective study (Kim et al. 1987). The strain of Salmonella group A, which induced Vi-antibody production, was a Salmonella paratyphi-A. The two strains of...
Salmonella group B and D couldn't be defined by exact serotypes. Thus, we need to have further studies related to Salmonellosis other than typhoid fever. However, patients with tuberculosis or rheumatoid factor associated disease who sometimes showed a false-positive result in other serologic tests (Doshi and Taylor 1984; Choe et al. 1985; Kim et al. 1986) still showed negative results as in our retrospective study (Kim et al. 1987).

Doshi and Taylor (1984) showed that four sera taken within 3 days of fever onset presented titers of 1:128 or more, suggesting that the IFAT becomes positive early in the course of the disease.

In this study, two sera were taken at 3 days after fever onset. One of these sera had a titer of 64, and the other had a titer of 32. These results support the findings of our retrospective study (Kim et al. 1987), which showed that the antibody titers to Vi antigen increased within 1 week of fever onset, reached a maximum at 2 to 3 weeks, and then gradually decreased to normal or near normal levels.

In the analysis of initial sera on admission, four of 23 patients with proved typhoid fever showed titers of 1:32 or less, suggestive of a negative reaction (Table 4). One patient had a fever duration of 3 days, two of 5 days, and one of 10 days. However, three patients with a fever duration of 5 days or less showed titers of 1:64 or more in the follow-up study taken after 3 or 4 days. For example, a patient with a fever duration of 5 days presented a titer of 1:16 on admission, but showed a titer of 1:128 in the follow-up study taken after 3 days (Table 5). But one patient with a fever duration of 10 days showed continuous negative results in follow-up studies.

In 22 culture proved typhoid fever patients that showed positive reaction, the incidence of positive test results following fever onset was 70.0% within 1 week of fever onset, 88.9% from 1 to 2 weeks, and 100% after 2 weeks. These results were similar to those obtained in our retrospective study that showed 71.4% within 1 week of fever onset, 90.0% from 1 to 2 weeks, and 100% after 2 weeks (Kim et al. 1987). Park and Kwon (1985) showed Widal test positivity that was 10% within 1 week of fever onset, 28.6% from 1 to 2 weeks, and 66.7% from 2 to 3 weeks in the tube agglutination test, and was 25% within 1 week and 70 to 75% from 1 to 3 weeks in the microagglutination test. Thus, with regard to the diagnosis of patients with fever duration of less than one week that seem to have insufficient antibody production, the Vi-IFAT is considered to be superior to Widal test.

We requested a phage typing of the Vi antigen used in this study through the Korean National Institute of Health to the Central Public Health Laboratory in London. The result showed that the Vi antigen is M1 type, which is widely distributed in Korea (Lee et al. 1983).

Based on these results, we conclude that the Vi-indirect fluorescent antibody test (Vi-IFAT) is a highly sensitive and specific serologic test even in the early stage of the illness and therefore this method could serve as a useful serologic test in the diagnosis of typhoid fever. However, the results reported here on the specificity of Vi-IFAT must be extended in further studies on sera from chronic typhoid carriers and in healthy subjects with typhoid vaccination.
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