Laboratory Diagnosis of Rubella Infection in Pregnant Women: A Study at Siriraj Hospital

Pilaipan Puthavathana Ph.D.
Chantapong Wasi M.D.
Uraiwan Kositanont M.S.
Presert Thongcharoen M.D., Dr. Med.

Abstract

Solid-phase immunosorbent hemadsorption (SPIHAd) for specific IgM determination has been introduced for rubella serodiagnosis in combination with hemagglutination inhibition test (HAl) during the outbreak prevailed from September 1983 to August 1984 with its peak in March. By HAl test, 92.7% of patients with apparent rubella infection whose first blood was obtained within 3 days after rash showed seroconversion as compared to their paired sera bled 7 days later. The remaining cases already developed high HAl titer (>80) and diagnosis was done by further investigation for specific IgM. Rubella IgM determination by SPIHAd was valid for sera drawn within 4-5 weeks after rash or 8 weeks after contact with the index cases. During the epidemic, both tests have diagnosed clinical rubella in 70.0% of the patients with maculopapular eruption, and subclinical infection in 5.1% of the contact cases.

การตรวจวินิจฉัยโรคหัดเยรูมทั้งทรงปฏิบัติการในหญิงตั้งครรภ์: การศึกษาที่โรงพยาบาลศิริราช

ฟิโลฟันธ์ ภูสถิตานนท์, อัตถางศ์ ลัดชัย, อุไรวรรณ โภคภัณท์, ประเสริฐ ทองเจริญ
ภาควิชadalวิทยา, คณะแพทยศาสตร์ศิริราชพยาบาล, มหาวิทยาลัยธรรมศาสตร์

Rubella IgM will be detected in sera collected within 4-5 days after the rash onset. A titre of 80 or greater was considered as a positive result. The convalescent HAl titre was determined at least 2 weeks after the appearance of rash.
INTRODUCTION

Rubella is an acute, self-limited disease with maculopapular rash, which frequently accompanied by mild upper respiratory symptoms and suboccipital lymphadenopathy. The infection is usually mild and even elicit inapparently. Infection in utero might occur if mother had primary infection with or without the clinical symptom. Generally, termination of pregnancy is recommended for pregnant women who have rubella infection within the first 16 weeks of gestational age.1-3

Clinical diagnosis given without laboratory confirmation is inaccurate, since maculopapular eruption can be caused by various etiology. The method of choice preferably performed in most laboratories is the hemagglutination-inhibition test (HAI). HAI demonstration demonstrates seroconversion in paired sera collected at 1-2 weeks apart. HAI antibody reaches its peak so rapid; when first blood samples are obtained late after infection, the HAI test can not demonstrate the seroconversion. Therefore, HAI alone can not differentiate the current or recent from previous infections.4,5

IgM is the first immunoglobulin class detected early after infection. IgM level declines gradually to undetectable level in 4-6 weeks or later depending on individual and the sensitivity of the testing method. Presence of specific IgM in single serum specimen clearly indicates current or recent infection. IgM does not cross placenta, so the presence of specific IgM in cord blood is a criteria for laboratory diagnosis of congenital rubella infection.6,7

The present communication reports our experience in using SPIHAd for specific IgM detection in adjunct to the conventional HAI method to diagnose rubella infection in pregnant women.

MATERIALS AND METHODS

Subjects

Subjects participated in this study were 1) 619 pregnant women who attended the ante-natal clinic of Siriraj Hospital during 1979-1981; and 2) 1211 pregnant women who visited Siriraj Hospital and other institutes in and outside Bangkok and informed illness suspected of rubella or history of rubella contact during 1983-1984.

Laboratory investigation

From 1979-1981, pregnant women who attended the ante-natal clinic of Siriraj Hospital were checked for their rubella immune status by HAI test. SPIHAd for rubella IgM determination was not available at that time.

During the epidemic period 1983-1984, serodiagnosis for rubella infection was given by the demonstration of a four-fold rising of HAI antibody titer in paired sera, or the presence of rubella specific IgM detected by SPIHAd in sera which did not show seroconversion but had the high HAI antibody titer (> 80).

Blood sample collection for rubella serodiagnosis

In clinical rubella, the first blood specimen was drawn at the earliest day usually 1-3 days after rash, and second blood sample at a week later. Paired sera were tested simultaneously by HAI.

In contact case, the first blood specimen was drawn at the first visit and assayed promptly for the HAI antibody titer. If the HAI titer was equal to or higher than 10, the second blood would be collected at 7 days later. The one whose first serum sample showed HAI titer less than 10 would be bled at 1 month after contact with the index case. Time was allowed for incubation period and increasing in the antibody level.

Rubella hemagglutination-inhibition test

The rubella HAI test was performed using the method being described elsewhere.6,7 Briefly, serum nonspecific inhibitor was treated with heparin and manganous chloride, the nonspecific agglutinator was removed by adsorption with 50% pigeon red blood cells, and then, the heat labile component was eliminated by inactivation at 56°C for 30 min. HEPES saline albumin gelatin buffer pH 6.2, rubella hemagglutinating antigen (Flow Laboratories Inc., USA), and 0.25% pigeon red cells were used in the test system.

Solid phase immunosorbent hemadsorption test

SPIHAd was modified after Krech in 19798 and Goldwater in 1980.9 Principle of the technique was based on “IgM capture” by which IgM of various specificities in the tested sera would be trapped by anti-μ antibody coating on solid phase. However, only rubella specific IgM reacted to the rubella HA antigen which in turn “adsorbed” pigeon erythrocytes adding in later step. Thus, the name “solid phase immunosorbent hemadsorption” was derived. In the well of negative sera, “hemagglutination” between rubella antigen and erythrocytes was formed on the coated plate.

Hemagglutination and hemadsorption reactions were not distinguishable by their appearance until the plate was subjected to spinning. After centrifugation, film of hemadsorption was still sustained by binding force between the antigen and the specific IgM captured. In the well without specific IgM, complex between the antigen and red blood cells could not resist to centrifugal force, and then settled to bottom of the well in pattern of red button.

Technique Polystyrene microtiter plate of U-
shape (Immunoplate II, Nunc Inc., Denmark) was used as solid phase. Each well of the plate was coated with 50 µl of rabbit anti-human IgM specific for µ-chains (Dakopatts, Denmark) diluted at 1:200 in carbonate buffer 0.05 M pH 9.6. The plate was incubated at 37°C for 1-2 hours and kept overnight at 4°C. Then, the plate was washed 3 times with 0.1% Tween 20 in phosphate buffer saline (PBS-Tween) and the treated serum (as in the HAl test) was diluted in HEPES saline albumin (HSA) buffer pH 6.2 starting from the dilution of 1:10 to 1:1280. After incubating at 37°C for 2 hours the plate was washed, and 25 µl of rubella HA antigen at the concentration of 2 hemagglutination units was added to all wells except the first row of sera at the dilution of 1:10 which would be served as serum control was added with HSA. The volume in all wells was brought-up to 50 µl by adding with 25 µl of the pretested rubella negative human serum at dilution of 1:200 in HSA. After overnight incubation, 50 µl of 0.25% pigeon red blood cells was added to all wells, further incubated at 4°C for 2-3 hours and then spun at 700xg for 1 minute at 4°C.

IgM confirmatory test The rubella IgM positive serum was further confirmed by the anti-IgM blocking test. Briefly, the positive treated serum was diluted in duplicate in vertical rows of the anti-µ-coated plate starting from the dilution of 1:5 to 1:640 in the volume of 25 µl, then 25 µl of anti-human IgM at the dilution of 1:100 was added in every well of the tested row and HSA was added in the control row, and further incubated at 37°C for 2 hours. The following steps were as above. The row added with HSA should retain high titer of rubella specific IgM, while the one with anti-human IgM would show blocking effect on activity of the specific IgM by reduction of the IgM antibody titer.

RESULT
Rubella HAI antibody titers at different days after rash

Kinetics of HAI antibody titers in paired sera of 165 rubella infected cases as diagnosed by HAI or SPIHAd were analysed (Fig. 1). If the first blood sample was collected early after onset of illness, seroconversion would be shown in the second blood obtained 7 days later. Sera collected within 3 days after rash usually yielded low level of HAI titers; and when collected 7 days or later after rash, all sera had HAI titer at the level of 80 or more. However, the high HAI antibody titers at 80 and 160 were obtained in 20 patients who were bled 1-3 days after rash; 8 of them showed seroconversion in their paired sera, but the remaining cases who were accounted for 7.3% of the total cases studied (12 of 165) did not.

HAI antibody titers in rubella and non-rubella women

All convalescent sera from 195 rubella cases as proved by HAI had the antibody titers equal to or higher than 80, i.e., 6 cases (3.1%) had the titers at 80 and the remaining cases had the titers at ≥160. Thus the HAI titer at 80 was considered to be the cut-off value for further specific IgM investigation when HAI itself could not diagnose rubella infection.

Nevertheless, the high HAI titers were also found in some immune population. From 1979 to 1981, 217 of 619 (35.1%) healthy, pregnant women had the HAI titers equal to or higher than 80, the titers were in such level in 448 of 1016 (44.1%) non-rubella pregnant women studied during 1983 to 1984.

It was shown in Table 1 that the HAI titers of 80 and 160 were frequently found in the immune women, but the titers of 320 or above were unusual.

Application of specific IgM determination by SPIHAd

Validity for IgM detection by SPIHAd had been performed in 142 serum samples from 71 rubella cases who showed seroconversion by HAI. Among acute sera, rubella IgM was not found in all 54 specimens with HAI titer less than 10, and it was present in 13 of 17 sera with the HAI titers between 10-160.

All convalescent sera had the HAI titers equal to or higher than 80, and also all of them had the rubella specific IgM with titers ranging from 80 to 1280, or even as high as 20480 in some specimens (Fig. 2). Presence of rubella specific IgM of any titer even in single serum was adequate for the diagnosis of current
Table 1 Rubella HAI antibody titers in clinical rubella and non-rubella cases

<table>
<thead>
<tr>
<th>Group studied</th>
<th>No. tested</th>
<th>&lt;10</th>
<th>10</th>
<th>20</th>
<th>40</th>
<th>80</th>
<th>160</th>
<th>320</th>
<th>&gt;640</th>
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<tbody>
<tr>
<td>Rubella cases</td>
<td>195</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>59</td>
<td>79</td>
<td>51</td>
</tr>
<tr>
<td>(1983-1984: convalescent titer)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-rubella cases</td>
<td>619</td>
<td>144</td>
<td>32</td>
<td>100</td>
<td>126</td>
<td>127</td>
<td>69</td>
<td>21</td>
<td>0</td>
</tr>
<tr>
<td>(1979-1981)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-rubella cases</td>
<td>1,016</td>
<td>279</td>
<td>23</td>
<td>73</td>
<td>188</td>
<td>262</td>
<td>164</td>
<td>22</td>
<td>0</td>
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<tr>
<td>(1983-1984)</td>
<td></td>
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</table>

Table 2 Rubella serodiagnosis by HAI and SPIHAd

<table>
<thead>
<tr>
<th>No. diagnosed by</th>
<th>No. studied</th>
<th>No. infected</th>
<th>HAI</th>
<th>HAI &amp; SPIHAd</th>
</tr>
</thead>
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<tr>
<td>Clinical suspected cases</td>
<td>584</td>
<td>409 (70.0%)</td>
<td>319 (54.6%)</td>
<td>90 (15.4%)</td>
</tr>
<tr>
<td>Contact cases</td>
<td>1,071</td>
<td>55 (5.1%)</td>
<td>19 (1.8%)</td>
<td>36 (3.4%)</td>
</tr>
</tbody>
</table>

Fig. 2 Correlation between HAI titer and rubella IgM titer by SPIHAd

or recent rubella infection. The laboratory then, reported the result as "negative" or "positive" rubella IgM.

Increased sensitivity of rubella serodiagnosis by using SPIHAd in adjunction with HAI

Specific IgM detection by SPIHAd has been introduced to Virology Laboratory, Siriraj Hospital in 1983. SPIHAd in combination with HAI when compared to HAI alone had markedly increased sensitivity of rubella diagnosis as has been shown in Table 2.

Diagnosis of rubella infection was given to 409 of 584 (70.0%) cases suspected of the disease; 319 cases (54.6%) were diagnosed by HAI alone and 90 cases (15.4%) by both techniques. Among 1071 contact cases, 55 (5.1%) women developed subclinical infection which 19 of them (1.8%) were diagnosed by HAI alone and 36 (3.4%) by HAI and SPIHAd.

DISCUSSION

Laboratory investigation in co-operation with the clinical findings for the accurate diagnosis of rubella infection is needed for proper management of the patients and also for control of the diseases. There are many cases that HAI alone can not support the clinical diagnosis, and variety of methods for specific IgM determination have been developed in the past decade as diagnostic aids for a current and a recent infection. The methods employing whole serum and using labelled second antibody as detector as in the cases of indirect immunofluorescence, enzyme immunoassay and radioimmunoassay will encounter problems of, 1) competition of specific IgG with specific IgM for the same epitopes which leads to false-negative result; 2) fixation to specific IgG in the antigen-antibody complex by rheumatoid factor which is also able to bind to the second antibody leads to false-positive result. These problems have been solved by using pure IgM preparation in the test. IgM can be separated from whole serum by 1) fractionation e.g., by sucrose gradient centrifugation,
column chromatography; 2) absorption of IgG e.g., by using staphylococcus protein A; and 3) capturing with anti-human IgM coating on solid phase as has been done in the present study. IgM capture test has excluded false-positive and false-negative results and since red blood cells are used as detector, the test is designated "solid-phase immunosorbent hemadsorption".

SPIHAd has advantage for its high sensitivity and economical cost. Its disadvantage is the not-clear-cut hemadsorption pattern in sera with low level of specific IgM which can be corrected by duplication of the test.

In the present study, SPIHAd was performed when rubella infection could not be diagnosed by HAI, and only sera with high HAI titers would be tested. Establishment for cut-off value of high HAI titers at \( \geq 80 \) has been based on the value of the rubella HAI titers in convalescent blood (Fig. 1). In another word, the ones whose HAI antibody titers in paired sera did not exceed 40 were not suggestive for current or recent rubella infection.

The high HAI titers were also present in some immune pregnant women. During 1979-1981, it was found that 35% (217 of 619) of non-rubella pregnant women had the high HAI titers and it was 44% (448 of 1016) in the study during the epidemic years 1983-1984. Immune persons even with high antibody titers had no specific IgM, while the currently and recently infected cases did.

IgM has the average half-life about 5 to 6 days. Duration of specific IgM production after rubella infection is an important parameter for the diagnosis of current and recent infections. Follow-up study for rubella IgM persistence is being investigated. At the present time, from small scale of our prospective study, we have found that rubella specific IgM would persist up to 4-5 weeks after rash or about 8 weeks after contact with the rubella index cases. Interpretation for the negative result obtained from sera collected beyond this period of time was considered inconclusive, on the contrary the positive result was still meaningful for the diagnosis. Nevertheless, persistence of the specific IgM does not only depend on nature of the test but also on biological variation in each infected individuals as we have found that few clinical cases had prolonged synthesis of the specific IgM up to 3-4 months after rash.

During the rubella outbreak prevailed from September 1983 to August 1984, 70.0% of the clinical suspected cases were laboratory confirmed. Among 464 laboratory proved cases, 126 (27.2%) needed definite diagnosis by SPIHAd (Table 2). This technique was very useful especially in the investigation for subclinical infection which occurred in 55 (5.1%) of 1071 contact cases studied.

At the present time, SPIHAd is one of the best methods used to detect rubella IgM. This technique is widely used in many laboratories. Its application in the diagnosis of congenital rubella will be reported later.

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REFERENCES