Enteroviral pharyngitis diagnosed by reverse transcriptase-polymerase chain reaction

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Abstract
The role of enteroviruses in childhood pharyngitis was investigated using enteroviral specific reverse transcriptase-polymerase chain reaction (RT-PCR). Viral/bacterial throat swabs were taken from 50 children with acute pharyngitis and 26 controls. A positive culture was identified in only 26% of children with pharyngitis (adenovirus 10%, group A streptococci 2%), and none of the controls. Enteroviral RT-PCR was positive in 8% of the pharyngitis group and none of the controls. Enteroviruses are an important cause of pharyngitis in childhood.

(Molecular Methods)
Briefly, 100 μl aliquots from the rhesus monkey kidney tissue culture specimens were thawed and pretreated with RNAsin and diisothreitol. Nucleic acid was extracted using phenol/chloroform, and the RNA precipitated using ethanol. All RT and PCR steps were performed blind. A 20 μl reverse transcriptase (RT) mix was made from 14 μl of sample, 2 μl of nucleoside triphosphates, 2 μl of RT buffer, 0.5 U of RNAsin, 0.2 μl of random hexamers, 50 μl murine Moloney leukaemia virus (MMLV) RT (BRL), and 1.5 μl of 50 mM MgCl₂. This was incubated at 42°C for 15 min, 99°C for 5 min, and 4°C for 5 min. First round PCR reaction was in 100 μl, using 0.2 units of SuperTaq (HT Biotechnology), with 0.5 μM of primer E1 and CX3 with a final magnesium concentration of 1.5 mM. Two minutes at 95°C were followed by 40 cycles of 1 min 95°C/1 min 60°C. Nested PCR used 2 μl of first round product in a 50 μl mix containing 0.15 U of SuperTag, 1.5 mM MgCl₂, 0.15 μM E2, and CX10 for 30 cycles. The PCR product (15 μl) was electrophoresed in 1.5% agarose gels. DNA was visualised with ethidium bromide and ultraviolet. First round products were 287 base pairs, and nested products were 154 base pairs. DNA was transferred to Zetaprobe by alkali transfer with 0.4 M NaOH. The oligo E3 (100 ng) was endlabeled with r32P CTP, and used to probe the filters. Prehybridisation was at 37°C for 1 h, and hybridisation for 2 h, in 5 ml 20×SSPE, 1 ml 1×Denhardt's solution, and 1 ml 10% SDS made up to 20 ml in distilled water. Membranes were washed in 2×SSC with 0.1% SDS up to 48°C then exposed overnight to x ray film. The detection limit of the enteroviral nested PCR was analysed using the positive control of coxsackie B3/030893 (10⁷ TCID₅₀/ml), and was shown to be approximately 0.5 TCID₅₀. As published primer pairs were used, not all enteroviral serotypes were tested. Several other positive tissue cultures infected with non-enteroviral DNA and RNA viruses were tested, but no positive results were obtained. Standard negative controls and precautions to avoid contamination were used.

Results
Over the one year period 50 children with pharyngitis were swabbed (26 M, 24 F; mean age 4·1 years, range 1 to 10 years). Control throat swabs were taken from 26 children (22 M, 4 F; mean age 4·7 years, range 1 to 10 years). All swabs from control children were negative for bacterial culture, viral cell culture, and...
Enteroviral pharyngitis diagnosed by RT-PCR

In this prospective study, a positive viral or bacterial diagnosis was obtained in only one third of children studied. Group A streptococcal infection was very rare (2%). Mycoplasma, chlamydia, Epstein-Barr virus, and anaerobic infections would not have been detected. The enteroviral nested PCR gave clear bands on agarose gel and Southern blots, detecting samples that were negative by cell culture. Although it is possible that these positives were due to contamination, all repeat PCR reactions gave identical results, despite their lack of diagnosis in cell culture. Only the RMK cell culture was analysed by PCR, as it is unusual for enteroviruses to grow just in HEP cell lines.

There have been few prospective studies of the relative importance of different viral infections in childhood pharyngitis. Adenovirus was the commonest pathogen identified in this study, confirming a previous report. Enterviral infections were the second commonest documented infection, confirming their importance as a cause of pharyngitis in childhood.


Frequency of organisms identified from pharyngitis group

<table>
<thead>
<tr>
<th>Organism isolated</th>
<th>Frequency (%)</th>
</tr>
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<tbody>
<tr>
<td>Streptococcus pyogenes</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Haemophilus influenzae</td>
<td>2 (4)</td>
</tr>
<tr>
<td>Influenza A</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Influenza B</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>5 (10)</td>
</tr>
<tr>
<td>Parainfluenza 3</td>
<td>2 (4)</td>
</tr>
</tbody>
</table>

Four samples (8%) were positive on enteroviral nested PCR. All four specimens were repeated on the RMK tube frozen specimen, and confirmed to be positive on a second nested PCR. All four samples that were positive on nested PCR were also positive on blotting. No sample was positive by blotting, and not by nested PCR.

Discussion

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