EFFECT OF SYNOVIAL FLUID pH ON PCR BASED DIAGNOSIS OF JOINT INFECTION

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INTRODUCTION

Polymerase chain reaction (PCR) based detection of microorganisms has been used to diagnose synovial infections. Recent studies have shown that many synovial fluid samples contain PCR inhibitors. Among the inhibitors reported, pH is an important one, which can directly inhibit DNA polymerase, a key enzyme in PCR amplification. On the other hand, pH value is a useful indicator of infection. Consequently, we studied the effect of joint infection on pH of synovial fluid and the effect of joint fluid pH on PCR amplification. The null hypotheses were (A) joint infection had no effect on pH of synovial fluid; and (B) pH of synovial fluid did not affect PCR amplification.

METHODS

155 joint fluid samples from 155 consecutive patients were used in this study. Modified protocol of Mariani et al. (1) was used. Briefly, 100 µl of joint fluid was mixed with 500 µl of lysis buffer (75 mM KCl, 3 mM EDTA, 0.75% Tween-20 and 150 mM Tris-HCl, pH 8.00). The mixture was then heated at 90°C for 30 minutes to release bacterial DNA. After a spin at 4°C, 16000g for 10 minutes, 20 µl of supernatant was added to 80 µl PCR mixture (10 mM Tris-HCl of pH 8.3, 50 mM KCl, 4 mM MgCl2, 200 µM dNTPs, 0.5 µM primers, 0.01% gelatin and 3 U Taq DNA polymerase). PCR primers were 5’ primer, CGGCAGGCCTAACACATGCAAGTCG and 3’ primer, GGTTGCGGCCGTACTCCCCAGG. The amplified target DNA was identified either in 1.2% agarose gel or in Southern blot using a specific probe.

Sterile saline spiked with bacteria Staphylococcus aureus or Escherichia coli were used as positive controls. Sterile saline spiked with genomic DNA isolated from human stem cells was used as a negative control. Sterile saline alone was used as contamination control. All the three controls were routinely used each experiment.

Effect of pH on PCR amplification was evaluated by varying pH value of PCR mixture. 10^3 bacteria of Escherichia coli in sterile saline was added to 5 microtubes containing PCR mixture. Then, the pH of the PCR mixture in the 5 microtubes was adjusted at 7.10, 7.40, 7.70, 8.00, 8.30 and 8.60, respectively. The effect of pH on PCR amplification was determined by comparing PCR yield at different pH with a control standard, which consisted of 10^3 Escherichia coli and had an optimal PCR yield at pH of 8.30. To evaluate possible pH change during sample preparation and to study effect of this change on the PCR based diagnosis, pH values of joint fluid, supernatant of its lysis product, pre-PCR mixture and post-PCR mixture were measured. Before added to the PCR mixture, the pH of all the three controls was adjusted to 8.00, the same value as that of lysis buffer. pH values were measured using a micro pH probe, which was capable of measuring volume as little as 10 µl.

RESULTS

The effect of pH on PCR amplification was shown in Figure 1. The figure shows an increase in PCR yield associated with an increase in pH, with an optimal PCR yield at pH 8.30.

Tab. 1 Comparison of pH values between positive and negative groups at different experimental steps. The values are means and standard errors of means. Unpaired t test was used to determine if any difference was significant.

Tab. 1 Comparison of pH values between positive and negative groups at different experimental steps.

<table>
<thead>
<tr>
<th>pH of PCR reaction mixture</th>
<th>Pre PCR mixture</th>
<th>Post PCR mixture</th>
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<tbody>
<tr>
<td>pH</td>
<td>8.240 ± 0.041</td>
<td>8.252 ± 0.024</td>
</tr>
<tr>
<td>PCR Yield (% of Optimal Yield at pH 8.30)</td>
<td>40 ± 6</td>
<td>40 ± 6</td>
</tr>
</tbody>
</table>

59 of 155 (38.7%) joint fluid samples were positive in PCR diagnosis. The pH value of both positive and negative samples was compared at each experimental step (Table 1). The difference in pH value between the positive and the negative groups was statistically significant. However, there was no difference in pH value between the positive and the negative groups at the other experimental steps.

DISCUSSION

Although the pH of infected joint fluid samples is significantly different from the pH of noninfected samples, this difference in pH does not affect PCR amplification. The difference in pH between positive and negative samples of joint fluid was buffered in the lysis and PCR reaction solutions (Tab.1). Therefore, PCR based diagnosis is not affected by pH difference in the range of synovial pH.

ACKNOWLEDGEMENT

We wish to thank Dr. Brian D. Mariani for his generous gift of PCR primers and valuable advice. This work was supported by the Department of Orthopaedic Surgery, Virginia Commonwealth University, and by Orthopaedic Research of Virginia.

REFERENCE

(1) Mariani BD et al., Clin Ortho Rel Res, 331:11-22, 1996

Bacterial infection often results in a low pH. However, many other conditions also give rise to low pH. Therefore, low pH is not a definite sign of infection. PCR based detection provides a decisive diagnosis due to its direct and specific nature.

Tab. 1 Comparison of pH values between positive and negative groups at different experimental steps. The values are means and standard errors of means. Unpaired t test was used to determine if any difference was significant.

<table>
<thead>
<tr>
<th>Joint Fluid pH</th>
<th>Positive (n=29)</th>
<th>Negative (n=126)</th>
<th>Difference</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.10 ± 0.040</td>
<td>7.50 ± 0.029</td>
<td>Yes, p = 0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.40 ± 0.040</td>
<td>7.95 ± 0.024</td>
<td>No, p = 0.081</td>
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<td></td>
</tr>
<tr>
<td>7.70 ± 0.040</td>
<td>8.25 ± 0.023</td>
<td>No, p = 0.201</td>
<td></td>
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</tbody>
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