Frequency of HHV-6 and HHV-7 Infection Markers in Rheumatoid Arthritis and Osteoarthritis Patients and Changes in Cytokine Expression Levels

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HHV-6 and HHV-7

- *Roseolovirus* genus in Betaherpesvirinae subfamily
- Up to 90% of adults being seropositive
- Primary infection commonly occurs in early childhood
  - \( \downarrow \)
  - latency
  - \( \downarrow \)
  - reactivation under conditions of stress and immunocompromised state
  - \( \downarrow \)
  - contribution of further immunosupression

Rheumatoid arthritis

• The primary target for HHV-6 and HHV-7 replication is CD4+ T lymphocytes, pivotal cells in generation of humoral and cell-mediated adaptive immune response
• Both viruses have been shown to up-regulate TNF-alpha secretion by PBMC
• HHV-7 could also act as a trigger factor for HHV-6 activation

The aim

To assess the relevance of viral infection in RA we evaluated the frequency of HHV-6 and HHV-7 reactivation in RA and OA patients and changes in cytokine expression levels.
Matherials and methods

• **Patients and controls.**
  – 35 patients with RA (27 females and 8 males) with mean age 56.1 years (range 38-76),
  – 33 patients with OA (25 females and 8 males) with mean age 66.1 years (range 46-83)
  – 31 apparently healthy persons (mean age 53.6 years, range 38-72).

• The clinical features and laboratory parameters (hemoglobin, C reactive protein, rheumatoid factor and anti-cyclic citrullinated peptide) and disease activity score *DAS28* (only for RA patients) were examined for their potential relationship with HHV-6 and HHV-7 infections.
• The presence of HHV-6 and HHV-7 DNA in clinical samples was detected by nested PCR.

• Detection of viral DNA in cell-associated material – leucocytes and synovial tissues, has been used as a marker of latent/persistent infection while detection of viral DNA in cell-free plasma and synovial fluid as a marker of active infection (virus reactivation).

• Secretion level of IL-6, TNF-alpha, IL-1beta and IFN-gamma in peripheral blood was determined by quantitative ELISA (Immunotool, Germany).

• Statistical analysis. Data were analyzed by Fisher’s exact test. Values of p<0.05 were considered to be significant.
Prevalence of latent/persistent HHV-6 and HHV-7 infections among RA and OA patients

<table>
<thead>
<tr>
<th>Condition</th>
<th>HHV-6</th>
<th>HHV-7</th>
<th>HHV6/7</th>
</tr>
</thead>
<tbody>
<tr>
<td>without infection</td>
<td>5.7</td>
<td>8.6</td>
<td>22.6</td>
</tr>
<tr>
<td>with HHV-6 infection</td>
<td>6.1</td>
<td>3</td>
<td>9.7</td>
</tr>
<tr>
<td>with HHV-7 infection</td>
<td>28.6</td>
<td>41.9</td>
<td>57.6</td>
</tr>
<tr>
<td>with HHV-6/7 infection</td>
<td>25.8</td>
<td>57.1</td>
<td>57.1</td>
</tr>
</tbody>
</table>

RA: Rheumatoid Arthritis
OA: Osteoarthritis
HP: Healthy Population
Incidence of active HHV-6 and HHV-7 infection in RA and OA patients

Incidence of active HHV-6 and HHV-7 infection (%)

<table>
<thead>
<tr>
<th>Condition</th>
<th>RA</th>
<th>OA</th>
<th>HP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without activation</td>
<td>18.2</td>
<td>19.4</td>
<td>83.3</td>
</tr>
<tr>
<td>HHV-6</td>
<td>23.1</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>HHV-7</td>
<td>56.7</td>
<td>33.3</td>
<td>4.8</td>
</tr>
<tr>
<td>HHV-6/7</td>
<td>70</td>
<td>84.2</td>
<td>37.5</td>
</tr>
</tbody>
</table>
Incidence of HHV-6 and HHV-7 DNA in synovial fluid and sinovial tissues

HHV-6
- RA: 33.3%
- OA: 8.33%

HHV-7
- RA: 33.3%
- OA: 41.7%

HHV6/7
- RA: 16.7%
- OA: 41.7%

HHV-6 in synovial tissues
- RA: 42.9%
- OA: 50%
Medium results of laboratory parameters

**DAS28 in RA patients**

- **without infection**
  - RA: 4.72

- **latent persistent HHV-6 and/or HHV-7**
  - RA: 5.61

- **active HHV-6**
  - RA: 4.46

- **active HHV-7**
  - RA: 4.51

- **active HHV-6/HHV-7**
  - RA: 4.77

**C reactive protein**

- **without infection**
  - RA: 35.7
  - OA: 3.88

- **latent persistent HHV-6 and/or HHV-7**
  - RA: 20.3
  - OA: 2.4

- **active HHV-6**
  - RA: 26.9
  - OA: 4.45

- **active HHV-7**
  - RA: 11.88
  - OA: 4.8

- **active HHV-6/HHV-7**
  - RA: 6.56
  - OA: 2.89
Rheumatoid factor

![Rheumatoid factor bar chart](image)

Anti CCP

![Anti CCP bar chart](image)
without infection, latent persistent HHV-6 and/or HHV-7, active HHV-6, active HHV-7, active HHV-6/HHV-7
 Serum IFN-gamma level in RA patients

 Serum IL-1beta level in RA patients

 Serum IL-6 level in RA patients

 Serum TNF-alpha level in RA patients

Level of TNF-alpha (pg/ml)

Level of IL-1beta (pg/ml)

Level of IL-6 (pg/ml)

Level of IFN-gamma (pg/ml)

Serum IFN-gamma level in RA patients

Serum IL-1beta level in RA patients

Serum IL-6 level in RA patients

Serum TNF-alpha level in RA patients

Level of IL-6 (pg/ml)

Level of IFN-gamma (pg/ml)

Level of IL-1beta (pg/ml)

Level of TNF-alpha (pg/ml)

without infection

latent/persistent infection

active infection

without infection

latent/persistent infection

active infection

without infection

latent/persistent infection

active infection

without infection

latent/persistent infection

active infection

without infection

latent/persistent infection

active infection

without infection

latent/persistent infection

active infection
Conclusion

• Concurrent HHV-6 and HHV-7 reactivation may cause more aggressive progress of RA.
• Concurrent HHV-6 and HHV-7 reactivation in RA patients leads to significant increase of tumor necrosis factor alpha and interferon-gamma secretion.
• The role of HHV-6 and HHV-7 infection in the RA pathogenesis through immune dysregulation could not be excluded.