**Introduction:** Previous studies have indicated that bone resorption in RDC may occur through cytokine production from activated synoviocytes. Recent studies have detected osteoclastogenesis inhibitory factor (OCIF), which is identical to osteoprotegerin (OPG), a soluble member of the tumor-necrosis factor receptor family that inhibits osteoclastogenesis. In contrast, Osteoclast differentiation factor (ODF) has been found to be a member of the membrane-associated tumor necrosis factor ligand family and induce osteoclast-like cells formation from osteoclast progenitors. ODF is identical to RANKL. We therefore investigated the expression and localization of the OPG and RANKL mRNAs in the tissues obtained from patients with RDC using reverse transcription-polymerase chain reaction (RT-PCR) and in situ hybridization.

**Materials and Methods:** Tissues were harvested from 11 patients who had undergone total hip arthroplasty (THA) for RDC. Preoperative plain X-ray films showed acute bone resorption of the femoral head. Tissue for RNA analysis was frozen and stored at -80°C. Tissue for histological examination was fixed in 4% paraformaldehyde, embedded in paraffin, and subjected to in situ hybridization. Human OPG and RANKL complementary DNAs were used as a template and were subjected to 35 PCR cycles of 30 seconds of determination at 94°C, 1 minute of annealing at 60°C, and 30 seconds of extension at 72°C. This procedure was used to prepare a digoxigenin-labeled PCR probe, which was labeled with biotin. After hybridization for 15 hours at 37°C, the probe was reacted with streptavidin and developed with DAB. Then cell nuclei were stained with methyl green.

**Results:**
1. RT-PCR: Expression of the RANKL and OPG was determined in the tissue by using RT-PCR method. RT-PCR analysis revealed expression of RANKL in 450 base pairs and OPG in 370 base pairs in all specimens. We simultaneously confirmed the β-actin band in the 275 bp area as a positive control.
2. In situ hybridization: In the in situ hybridization study, the localization of RANKL and OPG was examined. Higher-magnification images of the results obtained by in situ hybridization are shown in Figure 1.

RANKL mRNA was expressed in the cytoplasm of fibroblast-like synoviocytes, and a few multinucleated giant cells, but no expression was detected in T-lymphocytes. In contrast, OPG mRNA was expressed in the cytoplasm of fibroblast-like synoviocytes.

**Discussion:** We detected RANKL expression on fibroblast-like synoviocytes and multinucleated giant cells using in situ hybridization. Thus, not only the fibroblast-like synoviocytes but also the multinucleated giant cells may be important stimulators in the bone resorption observed in patients with RDC. Although RANKL has already been implicated in rheumatoid arthritis, it has not previously been considered a factor in RDC.

Mizuno et al. reported that OPG knockout mice exhibit severe osteoporosis due to enhanced osteoclastogenesis, demonstrating that OPG is a key factor acting as a negative regulator against osteoclastogenesis. Recently, Kong et al. reported that inhibition of RANKL through OPG can completely prevent bone and cartilage loss in an animal model with arthritis. Thus, the present findings suggest that interventions aimed at controlling the balance of OPG and RANKL may prove to be useful for preventing bone resorption in RDC. The control of expression of RANKL and/or OPG in the tissues will provide directions for the development of new treatment strategies for inhibition of bone resorption in RDC.

In conclusion, the present findings indicate that the RANKL and OPG genes may be deeply involved in the persistent chronic inflammation and bone resorption in RDC.