INTRODUCTION: Aseptic loosening is one of the most serious problems to be solved after total hip arthroplasty (THA). The cause of loosening has been considered to be both mechanical stress and biologic reactions in the periprosthetic tissue against wear debris of ultrahigh molecular weight polyethylene, metal, and bone cement. Little is known about the role of extracellular matrix (ECM) proteins in aseptic loosening of THA.

Osteopontin (OPN), an ECM glycoprotein, is a potential inflammatory cytokine and modulates a variety of pathological conditions. The presence of thrombin-cleaved form of OPN is well correlated with various inflammatory disease activities [1]. We measured the synovial fluid levels of the N-terminal half of thrombin-cleaved osteopontin (OPN N-half) in rheumatoid arthritis (RA) directly, and demonstrated that OPN N-half levels were elevated in RA compared with osteoarthritis (OA) [2].

The purpose of this study was to determine the levels of OPN N-half and non-thrombin-cleaved osteopontin (OPN full-length) in pseudosynovial fluid from patients with aseptic loosening after THA and to compare the levels in synovial fluid from patients with OA undergoing primary THA.

METHODS: Pseudosynovial fluid samples were obtained by aspiration at the time of revision THA performed for aseptic loosening on 4 men and 20 women (n = 24). Their median age was 63 years with a median body mass index (BMI) of 23.7 kg/m². Eighteen THA were originally performed due to OA, 4 had idiopathic osteonecrosis of the femoral head, and 2 were required surgery due to trauma. The median time from primary THA to revision was 8.8 years. No evidence of infection was observed. As a control, synovial fluid samples were also obtained by aspiration at the time of primary THA (n = 15) in the patients with OA.

There were 1 man and 14 women with a median age of 60 years and a median BMI of 24.5 kg/m². The demographics between the 2 groups showed no differences. All patients gave informed consent and had serum C reactive protein concentrations within the normal range for healthy adults. The joint fluids were centrifuged at 15,000 x g for 15 minutes and the supernatants were stored at -80 °C until analyzed.

An enzyme-linked immunosorbert assay (ELISA) was applied to quantify the levels of OPN full-length and OPN N-half as previously described [2]. For the OPN N-half ELISA, two antibodies, O-17 and 34E3, were used. The OPN N-half ELISA system does not recognize full-length OPN and detects OPN after thrombin cleavage. To quantify the levels of OPN full-length, human osteopontin assay kit was used with two antibodies (O-17 and 10A16). The percentage of OPN N-half (% N-half) was expressed as a percentage (OPN N-half divided by all OPN [OPN N-half plus OPN full-length]).

Expression of thrombin-cleaved OPN in pseudosynovial membranes was determined by immunohistochemistry using the avidin-biotin complex method with anti-OPN N-half (34E3) mouse monoclonal antibody (n=16). The results of immunoreactivity for pseudosynovial membranes and OA synovial subintimal tissues (n=9) were identified using the point system of Salter [3], as follows: No staining = 0 points; focal weak staining = 1 point; focal strong staining = 2 points; and extensive strong staining = 3 points.

Statistical analyses were performed using the Mann-Whitney U-test, the Fisher’s exact test, and Spearman’s rank correlation test. Statistical significance was set at p < 0.05.

RESULTS: ELISA results showed the median levels of OPN full-length (Fig. 1A, p<0.001), OPN N-half (Fig. 1B, p>0.001), and % N-half (Fig. 1C, p=0.002) were significantly higher in loose artificial joint than in the synovial fluid from primary THA patients with OA. In the hips with loosening after THA, the OPN levels were not significantly affected by gender, age, BMI, and the interval from primary THA to revision.

Both samples from pseudosynovial membrane and OA synovial membrane showed positive labeling of OPN N-half. The mean ± standard deviation) scores of expression of OPN N-half was higher in pseudosynovial membranes in loose artificial joint (1.8 ± 0.8) compared with OA synovial tissues (1.2 ± 0.4, p=0.047). An intense immunoreactivity of pseudosynovial membrane in loose artificial joint was seen in multinucleate giant cells (Fig. 2). Under polarized microscopy, a strong immunoreactivity was found to be associated with polyethylene wear debris.