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Comprehensive investigation into the endothelium in Legg-Calvé-Perthes with clinical, molecular, and histopathological examinations reveals arteriolar wall thickening and proliferative synovitis: A preliminary report

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Background and study aims: **The exact cause of spontaneously healing idiopathic osteonecrosis in the Legg-Calvé-Perthes disease (LCPD) is still unknown. Although it is postulated that thrombotic processes that occur as a result of genetic, epigenetic and environmental factors are at the forefront, the root cause cannot be fully elucidated. The aim of this study is to investigate underlying endothelial inflammation as a trigger of these pathophysiological mechanisms leading to LCPD.**

Materials: **The levels of ICAM-1 and E-selectin in the local blood vessels around the femoral neck and peripheral veins of seven surgically treated LCPD patients and similar age controls were compared. These were reviewed together with the clinical and histopathological findings of the LCPD patients.**

Results: **There were no statistically significant differences between each group's ICAM-1 and E-selectin levels in the femoral neck region or the peripheral vessels ($p>0.05$). Histopathological examination revealed arterioles with thickened walls and luminal obliteration. Chronic synovitis with plasma cell and lymphocyte infiltration of different severity was detected in more than half of the patients. Proliferative synovitis with papillary proliferations was seen in two patients. The patients who had these histological findings also had endothelial marker levels above the cohort mean.**

Conclusions: **In summary, this study provided a comprehensive examination of LCPD patients. We observed arteriolar involvement and proliferative synovitis in multiple patients in the group.**

Clinical Relevance: **This study provides important information about the pathophysiological basis of LCPD by combining clinical, molecular, and histopathological data. It is a comprehensive study in an uncommon population of surgically treated LCPD patients and similar age controls. To our knowledge, the findings of proliferative synovitis and arteriolar wall thickening have not been previously reported in the LCPD literature.**

Keywords: Legg-Calvé-Perthes, endothelium, endotheliitis, proliferative synovitis, ICAM-1; E-selectin.

INTRODUCTION

Factors that can lead to a prothrombotic state have been shown to play a role in the pathogenesis of Legg-Calvé-Perthes disease (LCPD), such as hereditary thrombophilia[1], increased thrombomodulin levels and global fibrinolytic capacity[2], and decreased protein C and antithrombin activity[1]. In recent years, an increase in endothelium-related E-selectin levels[3] and nitric oxide synthase gene polymorphisms[4] have also been described, suggesting potential endothelial dysfunction and inflammation as a potential cause.

Inflammation and swelling of the inner surface of vascular structures indicate endotheliitis. Endotheliitis has been observed and studied in the literature, particularly in conditions such as transplant rejection. Among the markers that rise in endothelial inflammation are intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), E-selectin, P-selectin, von Willebrand factor (vWF), plasminogen activator inhibitor-1 (PAI-1), and homocysteine[5,6].

ICAM-1 is a cell surface glycoprotein and adhesion receptor belonging to the immunoglobulin superfamily,

which regulates the accumulation of leukocytes from the circulatory system to inflammatory sites. This molecule, which is always expressed in leukocytes and endothelial cells, increases in concentration following cytokine stimulation. Additionally, it is involved in transmembranous signaling of the cell through its interaction with actin in the cell cytoskeleton[7]. The soluble form of this molecule, sICAM-1, is used as an inflammatory biomarker. sICAM-1 has been found to be elevated in the sera of animal models and in humans with certain diseases, including atherosclerosis, sepsis, and cancer.

E-selectin is a selectin cell adhesion molecule that is only found in endothelial cells and is not stored in cells. The production of E-selectin requires stimulation through P-selectin expression[8]. Due to their significant role in inflammation, it is believed that elevated levels of E-selectin and P-selectin can have an impact not only on inflammatory and thrombotic diseases but also on metastases[9]. In LCPD, elevated levels of E-selectin and P-selectin have been observed compared to controls, suggesting their potential involvement in the pathogenesis of the disease[3].

Morphological findings related to endotheliitis are generally not specific nor pathognomonic; however, they have been documented and frequently reported in the literature, particularly during transplant rejection. In the hyperacute phase of inflammation, endothelial damage and fibrin-platelet thrombus formation can occur due to the accumulation of immunoglobulins and complement in the endothelial wall, along with neutrophil infiltrations around arterioles and capillaries. In the acute phase, swollen endothelial cells in blood vessels and lymphocyte infiltration between the endothelium and vessel wall can be observed. Intimal thickening accompanying inflammation and mononuclear cell infiltrations, including natural killer (NK) cells and plasma cells can be seen in chronic endotheliitis[10].

The endotheliitis-thrombosis relationship in femoral head osteonecrosis in LCPD has not been fully elucidated. In this study, we developed a comprehensive technique to analyze a previously overlooked aspect of LCPD to answer our research questions: (1) Is there a role of endothelial inflammation in the pathogenesis of LCPD? (2) Is there a difference between the local and systemic endotheliitis markers between LCPD patients and other children? (3) Are there any morphologic clues that are indicative of endotheliitis in the histopathological findings of LCPD? This study aims to investigate the presence of endotheliitis that may predispose to thrombosis in the pathogenesis of LCPD by comparing the levels of endotheliitis markers, ICAM-1 and E-selectin, in the local blood around the femoral neck and peripheral vessels against controls in a similar age group, and to investigate the findings in detail with clinical data and histopathological examinations. We hypothesized finding increased markers of endothelial inflammation in LCPD with corresponding histopathological findings.

MATERIALS AND METHODS

Institutional review board approval was obtained from the authors' institution (Date: 06/29/2021, Number: 21/828). Written informed consent was taken from the parents or guardians of the study participants. The children were also informed about the procedures in accordance with their ages and understanding capabilities.

LCPD patients who were scheduled for operative procedures were included in the study. These included tenotomies with hip capsulotomies, surgical dislocations, and proximal femoral or pelvic osteotomies. Children who underwent similar surgical procedures within the same age group but had other orthopedic disorders were selected as the control group. Patients with other non-orthopedic systemic diseases were excluded from the study.

Bone and/or soft tissue samples were collected from patients who underwent surgery in the LCPD group. Since normal histological findings of these tissues are already known and widely reported in the literature, specimens were not collected from controls. The bone samples were shaved off from the drills and/or other surgical instruments that were used during the osteotomy procedures. In the patients that underwent capsulotomies, a thin 0.5x3 mm strip of the capsule was removed before repair or capsulorraphy. After routine preparation for pathology and hematoxylin and eosin staining, the samples were examined by a senior pathologist (K.K.) for endothelial and vascular pathologies under light microscopy. In both the LCPD group and the control group, the levels of two endothelial inflammation markers, E-selectin and ICAM-1, were investigated in peripheral venous blood (peripheral blood) and the surgical site (local blood). 0.5 cc samples were collected from both areas intraoperatively for this analysis. To avoid iatrogenic insult to the femoral head vasculature, only incidentally bleeding areas from the periarticular anastomosis of the hip joint were collected as local blood samples. The wounds were dried with laparotomy sponges to avoid dilution

before sample collection, and fresh blood oozing from vessels was suctioned into syringes. Local and peripheral blood samples were placed in tubes containing citrate and Ethylenediaminetetraacetic acid (EDTA), respectively. After centrifugation at 1000 x g for 15 minutes, the plasma was separated and stored in a -80°C refrigerator. The holding times varied based on patient's surgical dates and final sample analysis. The maximum holding time was 18 months, and the minimum was one month.

A micro-ELISA (enzyme-linked immunosorbent assay) analysis was used to determine if there was a difference in endothelial biomarker levels between the local and peripheral blood samples. After thawing the samples, the levels of ICAM-1 (Human ICAM-1/CD54 Allele-specific Quantikine ELISA Kit, R&D Systems, Minneapolis, MN, USA) and E-selectin (Human sE-selectin/CD62E Quantikine ELISA Kit, R&D Systems, Minneapolis, MN, USA) were calculated using the ELISA method. Both tests adopt the quantitative sandwich enzyme immunoassay technique in which the lower layers of the micropellets were coated with monoclonal antibodies specific to the antigen of interest. When the samples were added to the micropellets, they bound the immobilized antibodies. In the ICAM-1 test, at this stage, a conjugate solution was added, sandwiching target ICAM-1 molecules between immobilized antibodies and enzyme-bound antibodies (in the E-selectin test, the conjugate was added after the initial incubation process and washing). After 90 minutes of incubation, tubes were washed with a buffer solution to remove unbound substances. The substrate solution was added, and the tubes were incubated for 30 minutes. Then, the stop solution was added to change the color from blue to yellow. After the color turned yellow, the tubes were placed in the microplate reader. A point-to-point graph was created for each analysis by the optical density measurements of standard solutions at different concentrations. The concentrations (ng/ml) corresponding to the optical densities of the samples were calculated based on this reference curve.

Tissue samples collected for the histopathological examination were placed in different storage containers labeled with the anatomical regions from where they were obtained during surgery (interarticular cartilage-epiphysis, epiphysis-metaphysis, synovium). After fixation with formaldehyde and decalcification, 4-micron-thick sections of the samples were stained routinely with Hematoxylin and Eosin and examined under a light microscope. During the examination, possible correlations between the patients' E-selectin and ICAM-1 levels and histopathological findings were investigated on a case-by-case basis. The laboratory staff and the pathologist were blinded to the cases and relevant clinical information at analysis and workup.

SPSS® Statistics 23 (IBM Corp., Armonk, NY) was used for the statistical analysis of the study. Descriptive statistics were used to calculate the mean and range values of the variables. All p-values were two-tailed, and values <0.05 were considered statistically significant. A Kolmogorov-Smirnov test revealed normal distribution of the data. A power analysis was omitted because of the scarcity of specimens due to the rare surgical indication and the pandemic restrictions, rendering the achievement of large numbers in the study period impractical. Therefore, we decided to analyze the specimens that were collected during the study period that was feasible regardless of the numbers to provide descriptive information as well as a framework method for future multicenter collaborations. The presence of statistically significant differences between the mean values of E-selectin and ICAM-1 in the LCPD group and the control group was investigated using independent samples t-tests.

RESULTS

Demographic and clinical data

Seven patients met the inclusion criteria and were included in the LCPD group. The control group consisted of 7 patients in a similar age range with different diagnoses who underwent similar surgical procedures. Both groups comprised exclusively of male patients (100%). The condition affected the left hip in 3 children (42.9%) and the right hip in 4 children (57.1%) in the LCP group. The average age was 9.42 (range, 8-11) years in the LCP group, and 9.14 (range, 5-14) years in the control group. The control group consisted of 6 patients with Gross Motor Function Classification System (GMFCS) I-III cerebral palsy patients and one patient with developmental dysplasia of the hip (DDH). None of the patients in the control group had underlying inflammatory disorders or were on anti-inflammatory medications preoperatively.

Every patient in the LCPD group presented with unilateral hip involvement (100%). 3 patients had the disease in the left hip (42.8%), and 4 patients had the disease in the right hip (57.1%). The average symptom duration before surgery was 16.14 (range, 12-36) months. The mean postoperative follow-up was 10 (range, 2-18) months. Five patients were classified as lateral pillar group C (71.4%). One

patient was identified as lateral pillar group B (14.2%), and one patient was group B/C (14.2%). According to the Waldenström classification, the most common stage was Stage 2 (71.4%), followed by Stage 1 (14.2%) and Stage 3 (14.2%). Three patients underwent medial hip capsulotomy (42.8%). Iliopsoas and adductor tenotomies were performed on four patients (57.1%). All patients underwent proximal femoral varus osteotomies (100%) for containment. One patient additionally had a hump resection of the femoral head with safe surgical dislocation (14.2%). Four patients in the control group underwent varus osteotomies of the proximal femur (57.1%), and three patients underwent iliopsoas and adductor tenotomies as part of single-event multilevel surgeries (42.8%).

The LCPD group was evaluated for potential indicators of poor prognosis. Gage sign was positive in 3 patients (42.8%). Metaphyseal cysts were observed in two patients (28.5%), and two other patients had horizontal physes (28.5%). Lateral hip subluxation was observed in all patients (100%). The mean medial joint space in the LCP group was measured as 11.86 mm (range, 9.3-15.6). The mean Reimer index was 32 (range, 16-48) (Table I).

Endothelial markers

E-selectin values measured in peripheral blood samples were found to be higher than the values measured in the local blood samples for all patients in the LCPD group. The average concentration of peripheral E-selectin in the LCPD group was 28.0 ng/ml (range, 7.9 – 48.9). Local E-selectin levels had an average value of 11.423 ng/ml (2.4 – 27.7). ICAM-1 concentrations of the peripheral blood samples in the LCPD group were higher compared to the local blood samples in all individuals. The average concentration of peripheral ICAM-1 in the LCPD group was 211.3 ng/ml (range, 151.6-254.2). The mean local ICAM-1 level was 144.088 ng/ml (range, 95.3-195.5) (Table II).

In the control group, similar to the LCPD group, E-selectin concentration in the peripheral blood samples was found to be higher than the values measured in local blood samples for all patients. The average concentration of peripheral E-selectin in the control group was 23.1 ng/ml (range, 12.7-49.2). Local E-selectin levels had a mean of 11.4 ng/ml (range, 2.5-19.7). Notably, one individual in the control group had a lower ICAM-1 concentration in his peripheral blood sample than that in his local blood sample (14.2%) (Table III). In the remaining patients, peripheral ICAM-1 concentrations were found to be higher compared to local samples (85.7%). The average concentration of peripheral ICAM-1 was calculated as 210.5 ng/ml (range, 123.2-276.3). The average local ICAM-1 levels were calculated as 149.4 ng/ml (range, 94.0-205.0). Although the mean peripheral E-selectin levels were higher in the LCPD group with a mean difference of +4.85 ($p=0.53$, 95% CI [−13.30, 23.01]) and Cohen's d

Table I. — Age, Gage sign presence, metaphyseal cyst, lateral subluxation, medial joint space (mm), and Reimer index of the patients in the LCPD group.

Patient	Age	Gage Sign	M0hyseal cyst	Horizontal Physis	Lateral Subluxation	Medial Joint Space	Reimer İndex (%)
#1	8	-	-	-	+	13.2	16
#2	10	+	+	-	+	10.7	22
#3	10	+	+	-	+	11.1	29
#4	10	-	-	+	+	9.3	48
#5	11	-	-	+	+	13.5	38
#6	9	-	-	-	+	9.6	41
#7	8	+	-	-	+	15.6	34

Table II. — E-Selectin ve ICAM-1 concentrations in the peripheral and local blood samples from the LCPD group (ng/ml).

Patient	Peripheral E-Selectin	Local E-Selectin	Peripheral ICAM-1	Local ICAM-1
#1	42.482	27.738	254.2	173.94
#2	14.878	8.644	216.6	143
#3	7.933	2.405	176.25	95.331
#4	33.8	10.684	186.9	132.52
#5	16.265	5.111	151.69	107.17
#6	48.975	4.734	244.64	161.14
#7	31.697	20.645	248.866	195.517

Table III. — Age, E-Selectin ve ICAM-1 concentrations in the peripheral and local blood samples from the control group (ng/ml).

Patient	Age	Peripheral E Selectin	Local E-Selectin	Peripheral ICAM-1	Local ICAM-1
#1	5	24.309	15.625	221.778	155.341
#2	14	12.793	2.513	123.254	94.093
#3	10	18.829	12.622	250.274	138.203
#4	7	22.007	9.748	276.302	205.058
#5	9	21.225	19.009	165.776	189.754
#6	7	13.661	6.041	222.838	113.469
#7	12	49.236	19.702	213.640	149.899

effect size of 0.35, the difference did not reach statistical significance. Similarly, no statistically significant differences were observed in local E-selectin which had a mean difference of −0.76 (p=0.86, 95% CI [−11.29, 9.77], d = -0.09). Peripheral ICAM-1 had a mean difference of +0.75 (p=0.98, 95% CI [−59.63, 61.14], d = 0.02), and local ICAM-1 concentrations had a mean difference of −5.31 (p=0.80, 95% CI [−54.45, 43.83], d = -0.14), neither of which was statistically significant.

Histopathological evaluation

One patient had medial hyperplasia (#1) (Fig. 1) characterized by an onion-skin appearance causing luminal obliteration in the vessel wall, and another patient had arteriolar wall thickening (#3) as well (28.5%) (Fig. 2). Synovitis was not observed in either of these patients, despite the presence of this finding that is indicative of chronic vascular damage. No endotheliitis was observed in any patient. Osteonecrosis was detected in a total of 3 patients (42.8%), including two patients with epiphyseal involvement (#4, #5) and two patients with metaphyseal involvement (#4, #6). Additionally, medullary necrosis was found in the metaphyseal bone marrow of one patient (#5) (14.3%). No other bone

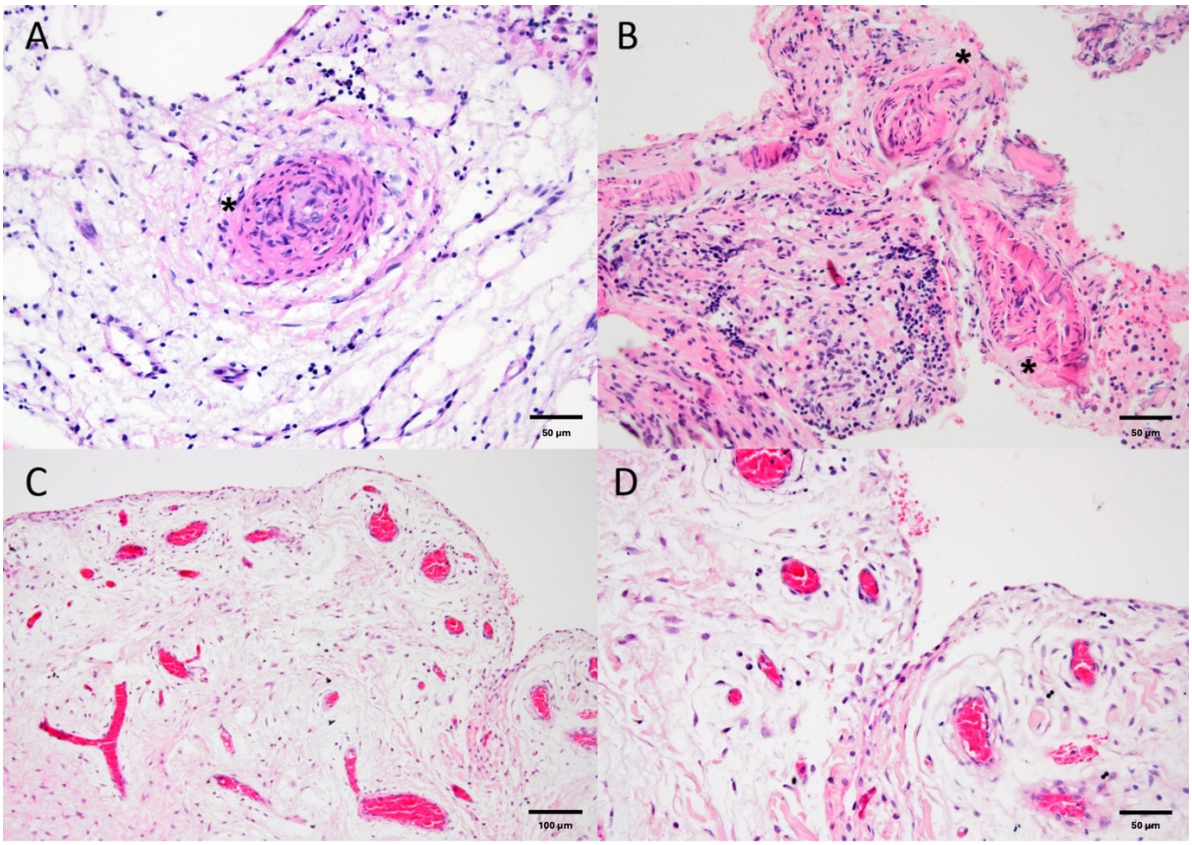

Fig. 1 — (LCPD Patient #1): Onion-skin arterioles (*) with medial hyperplasia and luminal obliteration are seen in the bony architecture (A, B). Although there is minimal edema and congestion in the synovium, the findings are not indicative of synovitis (C, D).
A: 200xHE B: 200xHE C: 100xHE D: 200xHE (HE: Hematoxylin and Eosin).

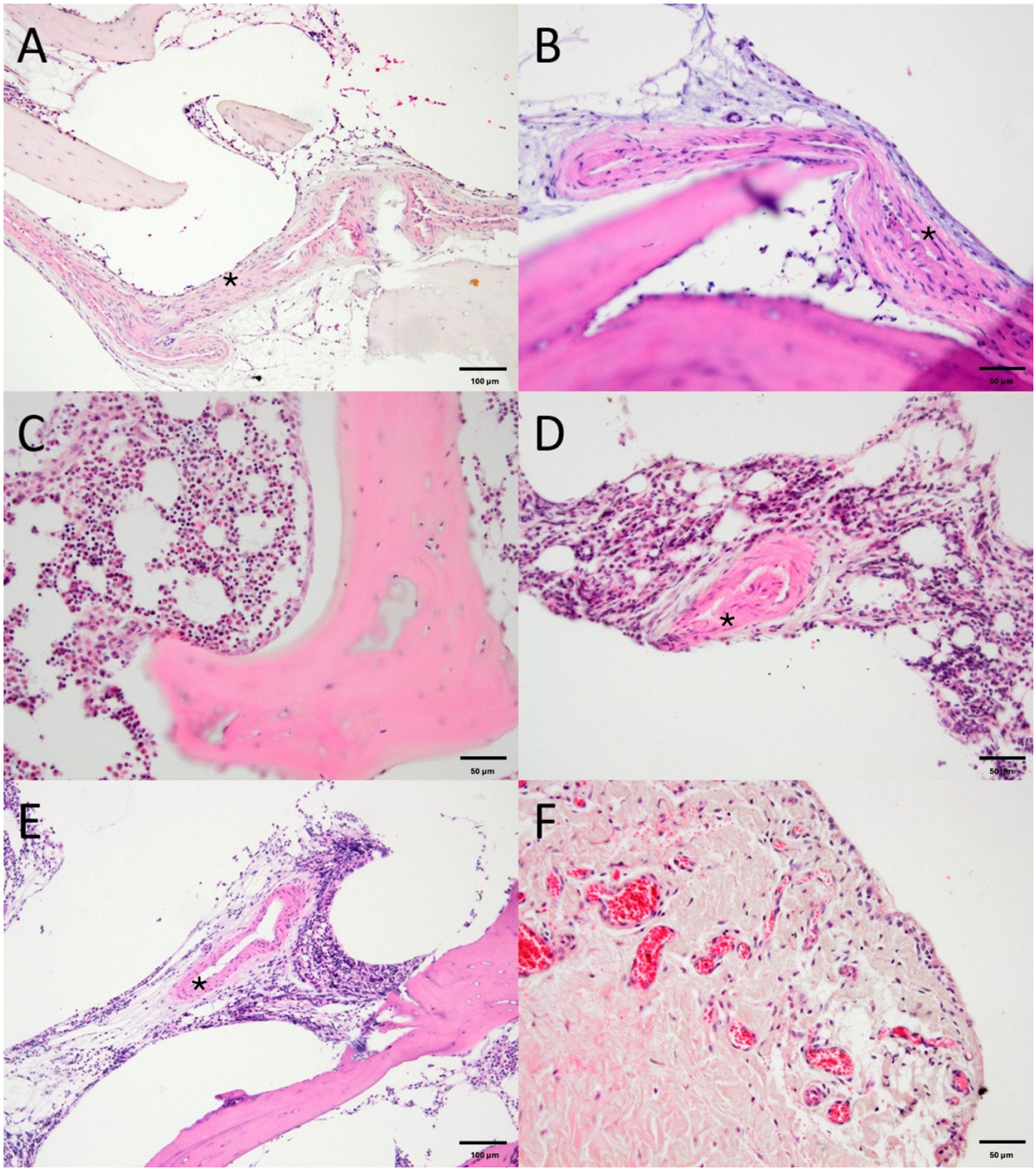

Fig. 2 — (LCPD Patient #3): Arteriolar wall thickening (*) is seen with normocellular bone marrow (A-E). There is synovial edema and congestion without synovitis (F).
A: 100xHE B: 200xHE C: 200xHE D: 200xHE E: 100xHE F: 200xHE (HE: Hematoxylin and Eosin).

pathology was observed in the other patients, and the findings were consistent with normocellular bone marrow. One patient had histopathological evidence suggestive of degenerative arthritis in the femoral head cartilage (#7) (14.3%) (Table IV).

Histology consistent with chronic synovitis rich in plasma cells and lymphocytes was observed in a total of 4 patients (57.1%). In the patient with the most severe synovitis findings (#7), the formation of lymphoid follicles was prominent (14.3%). Chronic proliferative synovitis with papillary synovial proliferation was observed in two patients (#6, #7) (28.5%) (Fig. 3) (Fig.4). Active chronic synovitis with neutrophil infiltration on top of chronic synovitis was observed in one case (#4) (14.3%).

DISCUSSION

In the present study, E-selectin and ICAM-1, which are markers of endothelial dysfunction and

Table IV. — The histopathological examination of the patients revealed arteriolar wall thickening, synovitis, and osteonecrosis in varying degrees of severity.

Patient	Histopathological findings
#1	Onion-skin arterioles with thickened walls in the epiphysis and metaphysis
#2	Chronic synovitis
#3	Arterioles with thickened walls in the epiphysis and metaphysis
#4	Epiphyseal osteonecrosis and chronic active synovitis
#5	Epiphyseal osteonecrosis
#6	Metaphyseal osteonecrosis and chronic proliferative synovitis
#7	Degenerative cartilage and chronic proliferative synovitis

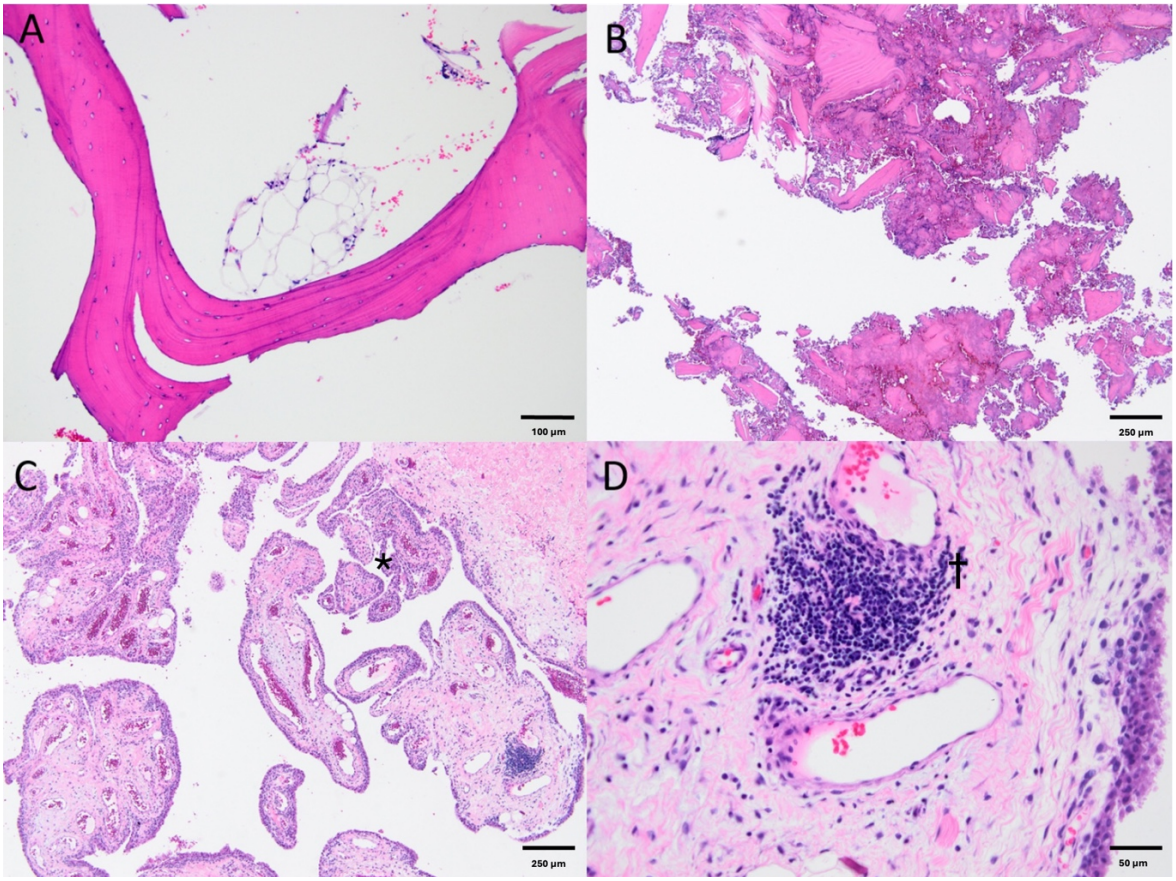

Fig. 3 — (LCPD Patient #6): Although there are some healthy areas without abnormalities (A), osteonecrosis is notable in the metaphyseal region (B). There is chronic proliferative synovitis rich in plasma cells and lymphocytes (†) with accompanying papillary synovial proliferations (*) (C, D).
A: 100xHE B: 40xHE C: 40xHE D: 200xHE (HE: Hematoxylin and Eosin).

inflammation, were investigated in LCPD patients and controls of similar age. Samples were collected from the peripheral blood and the blood vessels supplying the femoral head, and these two markers were compared between the groups to determine if there were any differences. Although some important clues regarding the role of endothelial inflammation have been observed among the findings of this study, which illustrates the pathophysiological basis of LCPD, statistical significance could not be demonstrated. Nevertheless, the combined analysis of radiographic, histopathological, and molecular data acquired from pediatric LCPD patients provides valuable contributions to the literature as an original study.

Neither local nor peripheral blood levels of E-selectin and ICAM-1 showed statistically significant differences between the LCPD and control groups. The peripheral marker levels were generally higher, which could be explained by the involvement of local biomolecules interacting and binding these agents, as well as the inclusion of tissue fluid. Although all samples were obtained from dry surgical beds, cross-contamination is still a possibility, especially at a

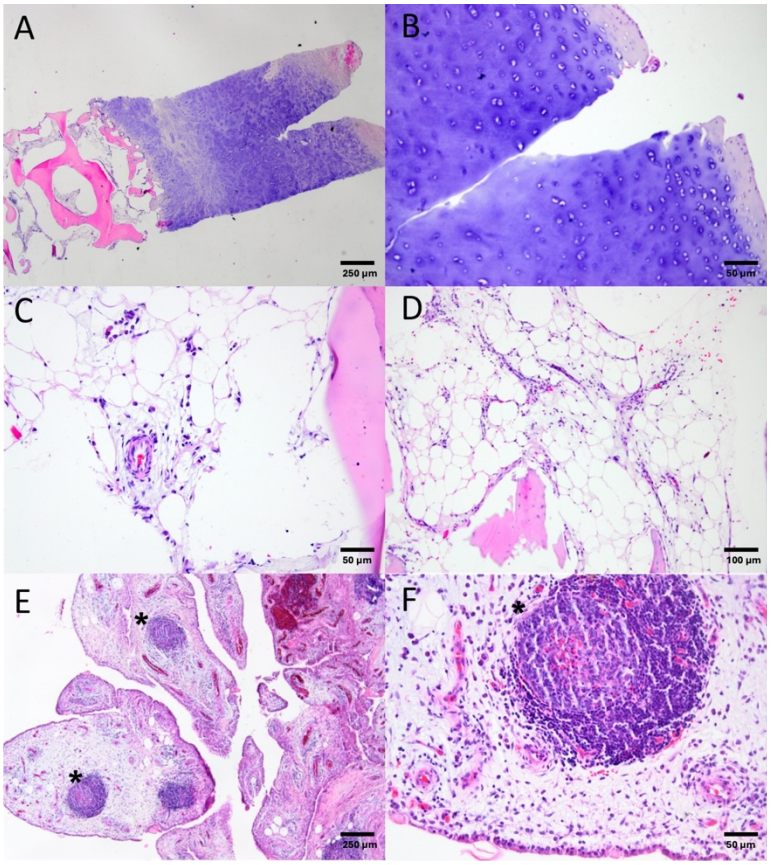

*Fig. 4 — (LCPD Patient #7): Findings suggestive of degenerative arthritis appeared in the femoral head cartilage. (A, B). There are no histopathological abnormalities in the epiphysis (C) or the metaphysis (D). Chronic proliferative synovitis rich in plasma cells and lymphocytes with accompanying papillary synovial proliferations, and the formation of lymphoid follicles (E, F) can be observed. *: Lymphoid follicles*
A: 40xHE B: 200xHE C: 200xHE D: 100xHE E: 40xHE F: 200xHE (HE: Hematoxylin and Eosin).

molecular level. In spite of this, our method remains valid to answer the study questions, as we compared LCPD and controls in separate analyses of peripheral and local marker levels. The levels of E-selectin in the peripheral blood of the LCPD group were 28.0 ng/ml, while in the control group, they were 23.1 ng/ml (p=0.525). In a study comparing E-selectin levels in peripheral blood between LCPD and control groups, the marker was found to be 54.9±18.8 pg/mL (0.054 ng/mL) in the LCPD group and 45.5±15.3 pg/mL (0.045 ng/mL) in the control group (p=0.02)[3]. Although our study showed a higher average E-selectin level in peripheral blood in LCPD, the lack of a significant difference might be due to an insufficient number of samples. On the other hand, our histopathological observations demonstrated unexpected findings. Onion-skin arterioles in one patient (#1) and thick-walled arterioles in another patient (#3) are consistent with chronic involvement of these vessels. Long-standing insults to these structures may predispose them to wall thickening, and the resulting ischemia of the femoral heads may lead to the clinical findings of LCPD, which may translate to shape changes and lead to sequela and morbidity in adulthood[10,11]. Similar cases of occlusive arteriolopathy have been proposed to cause a seronegative antiphospholipid syndrome-like condition in adults[13]. A study conducted on spontaneously hypertensive stroke-prone rats revealed narrowing and ballooning in arteriole lumens in scanning electron microscopy images of the arterioles supplying the femoral head[14]. Furthermore, transmission electron microscopy revealed decreased smooth muscle cells, increased collagen fibers, and hypertrophy in the vascular wall, resembling arteriolosclerosis. With our current knowledge, it is hard to pinpoint a specific mechanism for these pathologies. The arteriolar changes may potentially occur secondary to endothelial inflammation,

however, it is likely that some other cause may be behind these findings. To our knowledge, there are no publications in the literature that have reported these in LCPD patients. It should be noted that the peripheral and local E-selectin levels of patient #1, the most severe case, were above average compared to the LCPD group.

Due to the significant role of endothelium in the development of inflammation, its importance in the pathogenesis of synovitis has also been postulated[15]. The exact mechanism of chronic synovitis in LCPD has not been fully elucidated[16,17]. According to a recent study, 12 out of 242 children diagnosed with transient synovitis had chronic hip pain, and 3 of them were diagnosed with LCPD despite a normal appearing radiograph[18]. This emphasizes the need for targeted follow-up in transient synovitis patients who continue to have hip pain. A possible relationship between transient synovitis and LCPD has been debated with controversy, and authors have been proposing that transient synovitis could lay the etiological groundwork for LCPD. In contrast, prospective series and animal studies found no increased risk of femoral head ischemia after transient synovitis[19,20]. Regardless, the effect of prolonged high intra-articular pressure in cases of chronic synovitis should not be ignored, as it could cause a tamponade effect on the femoral head[21]. Histopathological evidence in favor of concurrent chronic synovitis was present in 57% of the patients in this series. Two of them showed chronic proliferative synovitis with papillary synovial proliferation (#6, #7), and in one case, the formation of lymphoid follicles was also observed (#7). The marker levels of the two patients with observed chronic proliferative synovitis were generally higher than the group averages, except for local E-selectin levels in #6. Severe synovial inflammation coinciding with high levels of endothelial markers might suggest a relationship between endothelium and synovitis as well. Furthermore, endotheliitis has been proposed to manifest as lymphocyte infiltration and "spotty" necrosis in chronic liver diseases[22]. The presence of active chronic synovitis and osteonecrosis rich in neutrophils and lymphocytes in a patient (#4) could be the result of such a mechanism.

Proliferative synovitis is the formation of papillary structures and stromal proliferation following inflammatory processes[23]. Proliferative synovitis may exhibit a similar appearance to pigmented villonodular synovitis (diffuse-type tenosynovial giant cell tumor), which is a neoplastic process, but they are entirely distinct entities. An example of proliferative synovitis can be seen in the pannus observed in patients with rheumatoid arthritis. Extreme proliferative synovitis leading to synovial hypertrophy has been associated with high synovial vessel density in ultrasound examinations of rheumatoid arthritis[24]. In a study involving rats, inhibiting synovial angiogenesis in the early stages of proliferative synovitis was found to reduce the progression and chronicity of the synovitis[25]. Similar mechanisms are believed to slow disease progression in rheumatoid arthritis. Therefore, investigational drugs like paclitaxel, anti-integrin alpha(V)beta3 antibodies, and LM-609 are being tested in proliferative synovitis[26,27]. There may be a potential role of these agents in the management of LCPD in the future. Moreover, one study highlighted a link between vascular remodeling and synovial proliferation in mouse models for hemophilic arthropathy[28]. They concluded that the chronic inflammation led to an ongoing reparative process in the vascular supply. A similar mechanism could play a role in the pathogenesis of LCPD. Future research should focus on the basis of synovitis in the pathogenesis of LCPD, and the influence of synovitis and ischemia on the management of severe disease[29]. Our current knowledge of LCPD is yet inadequate to elucidate the patients who progress to have deformity in the femoral head, which prompts us to perform complex surgeries to salvage the hip[30].

This study has some limitations. Firstly, the small number of patients due to the collection of tissue from a relatively uncommon population reduced the statistical power. The evaluation of patients at different stages and the collection of samples from different surgeries could also impact the results. Only one pathologist reviewed the slides; as a result, we were not able to analyze interobserver reliability. However, there are strengths in our study. The detailed examination of individuals, including clinical, molecular, and histopathological data, provided valuable insight. The absence of another report in the literature with a similar methodology contributes to the originality and significance of the findings of this study. Since histopathology is rarely utilized in the routine diagnostic approach in LCPD, this method has been overlooked in research. We believe the storage of viable samples that are salvaged from surgical instruments should be adopted more commonly, and larger study populations should be created with multicenter collaborations.

In summary, this study provided a comprehensive examination of LCPD patients. Although endothelial markers were not different, histology suggests

microvascular remodeling and proliferative synovitis warrant larger confirmation. Given these findings, this study should serve as a preliminary study that should be repeated with larger patient groups and multicenter studies. Further studies are needed to investigate the role of these entities in the pathophysiology of LCPD.

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