

# Histological, histochemical and ultrastructural study of slipped capital femoral epiphysis

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## Abstract

**Purpose** The purpose of our study was to investigate the histological, histochemical and ultrastructural aspects of the proximal femoral growth plate in slipped capital femoral epiphysis (SCFE).

**Methods** Eight core biopsies of the proximal femoral growth plate were performed during *in situ* epiphysiodesis in patients with SCFE that was at the pre-slipping stage in two cases and at the mild slipping stage (Southwick angle < 30°) in six cases. After fixation, the specimens were processed for either histological or histochemical or ultrastructural studies.

**Results** The proximal femoral growth plate was thicker than normal in the SCFE cases, and the 3:1 ratio between the thickness of the resting zone and the other zones of the plate was reversed. Chondrocytes of the proliferating, maturation, hypertrophic and degenerating zones were arranged in large clusters rather than in columns, which were separated by loose fibrillary septae that appeared moderately alcian blue positive and metachromatic. The collagen fibrils of the longitudinal septae were uniformly thin, measuring about 200 Å, whereas in the normal plate collagen fibrils were in the range of 300 to 1200 Å in thickness. Chondrocytes were elongated and smaller than normal, with a dark cytoplasm. In the degenerating zone, mineralisation of the longitudinal and transversal septae was scanty and enchondral ossification was impaired, with a few small osteoblasts forming thin bone trabeculae on the cartilage septae of the degenerating zone.

**Conclusion** In SCFE, the proximal femoral growth plate undergoes several histological, histochemical and ultrastructural changes that precede slipping of the epiphysis since they are already present at a pre-slipping stage of the disease. The loss of solidity of the extracellular matrix and the disarrangement of the normal architecture of the physis very likely cause the consequent slipping of the proximal femoral epiphysis. SCFE aetiology remains unknown.

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**Keywords:** slipped capital femoral epiphysis; SCFE histopathology; SCFE histochemistry; SCFE ultrastructure; SCFE pathogenesis

## Introduction

Several histological studies have shown that the normal morphology of the proximal femoral growth plate is greatly altered in slipped capital femoral epiphysis (SCFE).<sup>1-8</sup> One basic alteration is the increased thickness of the proximal femoral growth plate that in some areas may reach fivefold the thickness of the normal plate. The second basic alteration is the reversed ratio between the thickness of the resting zone and the proliferating, maturation, hypertrophic and degenerating zones, that is 3:1 in the normal plate. The third basic alteration is the loss of the columnar arrangement of the chondrocytes of the proliferating, maturation, hypertrophic and degenerating zones. In SCFE, the chondrocytes are arranged in large clusters separated from each other by longitudinal septae whose extracellular matrix has a loose fibrillar appearance and in which several clefts are scattered, filled with necrotic debris and extravasated blood cells.

Since the abnormalities described could be interpreted as secondary to the slip rather than primitive, we obtained some of the specimens from pre-slipping cases in which no displacement of the proximal femoral epiphysis had occurred and the main radiographic change was widening of the growth plate. In this study, we describe the histological, histochemical and ultrastructural alterations observed in the proximal femoral growth plate of patients with SCFE in either the pre-slipping or mild-slipping stage.

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## Materials and methods

Eight core biopsies of the proximal femoral growth plate were performed during *in situ* epiphysiodesis in patients with SCFE (Fig. 1). Six patients were boys and two were girls; the age range was 11 to 13 years. The patients had had pain and limping from one to three months at the time of the operation. In two patients, SCFE was at the radiographic stage of pre-slipping whereas in six, the Southwick angle was  $< 30^\circ$  (mild slipping) (Fig. 1). Two normal specimens were obtained from a 12-year-old girl and a 13-year-old boy who had resection of the proximal femur for malignant bone tumours. The specimens were cut into small samples including the metaphyseal bone, the whole growth plate and the epiphyseal bone.

For both the histological and histochemical studies, the specimens were fixed in 10% buffered paraformaldehyde, decalcified in 2% formic acid, dehydrated in graded alcohols, cleared in xylene and embedded in paraffin. Seven-micron thick sections were stained with: 1) haematoxylin and eosin (H&E) for routine histological examination; 2) periodic acid-Schiff (PAS) reaction for highly hydroxylated collagen and glycoprotein, after  $\alpha$ -amylase digestion to eliminate glycogen from the tissue; 3) alcian blue and toluidine blue for proteoglycans; and 4) Von Kossa for calcium-phosphate crystals. For the ultrastructural studies, the specimens were cut into small samples, fixed in 2% gluteraldehyde in cacodylate buffer at pH 6.5 for two hours, post-fixed in 0.5% osmic acid for one

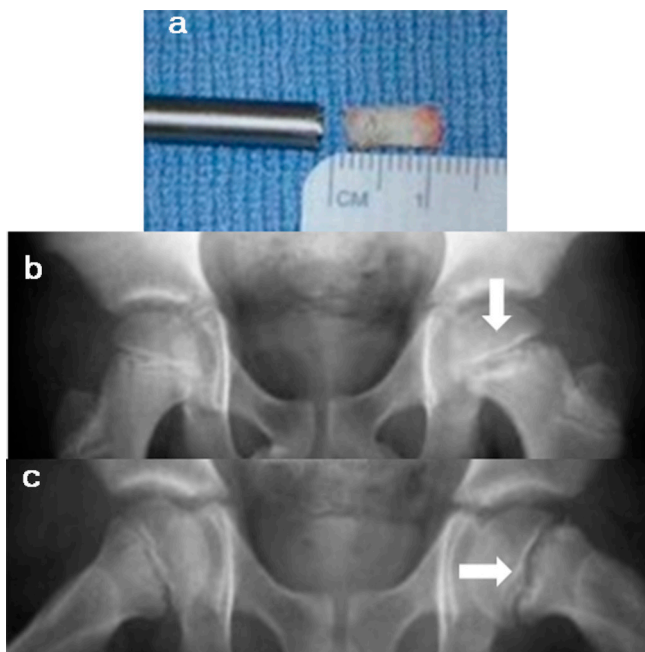
hour, dehydrated in a graded series of alcohol, clarified in toluene and embedded in Epon. Semi-thin sections were stained with toluidine blue, while ultra-thin sections were stained with uranyl acetate and lead hydroxide and observed and photographed with a Siemens electron microscope.

## Results

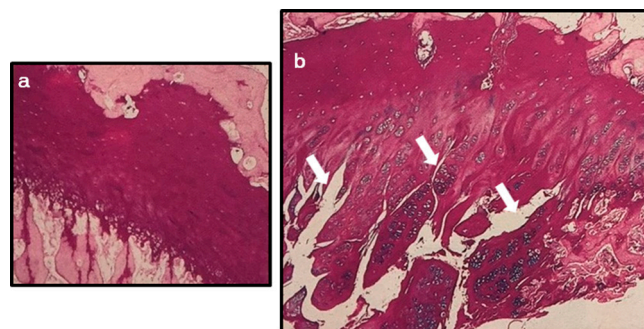
In no cases did the biopsy influence either the clinical course or the outcome of the disease.

### Histology

The histological appearance of the proximal femoral growth plate varied from case to case and from area to area of the same case. In the pre-slipping cases, there were areas that presented a normal appearance with the resting zone representing the thickest part of the growth plate, accounting for about 60% to 70% of its height (Fig. 2). In the normal appearing resting zone, chondrocytes were arranged in small nests containing two or three cells. Just below, proliferating, hypertrophic and degenerating chondrocytes were arranged in short columns undergoing normal and chondral ossification. The overall thickness of those areas was in the range of 2.5 mm to 6 mm as in the normal proximal femoral growth plate (Fig. 2). Abnormal areas adjoined the normal ones, showing distortion of the growth plate architecture. In the abnormal areas, the resting zone was thin, accounting for about 20% to 40% of the height of the growth plate. Chondrocytes were often arranged in nests containing numerous cells. In the proliferating and hypertrophic zones that were very thick (accounting for 60% to 80% of the height of the plate), chondrocytes were arranged in large clusters. Enchondral ossification was scanty underneath the degenerating zone. The overall thickness of the plate was irregular, in the range of 2.5 mm to 12 mm.



**Fig. 1** (a) Core biopsies of the proximal femoral growth plate were performed during *in situ* epiphysiodesis in SCFE patients either in (b) a pre-slipping stage or with (c) a mild slipping (Southwick angle  $< 30^\circ$ ).



**Fig. 2** (a) Normal plate and (b) SCFE plate. The SCFE plate is more than twice as thick as normal, with the resting zone thinner than normal; chondrocytes of the proliferating and hypertrophic zones are arranged in large clusters separated from each other by loose septae containing longitudinal clefts (arrows). The lacunae of chondrocytes are alcian blue positive while the extracellular matrix is mainly PAS-positive. (Stain: alcian blue – PAS – H&E;  $\times 110$ ).

In the cases of mild slipping, the whole proximal femoral growth plate presented the same distorted architecture as the abnormal areas of the pre-slipping cases (Fig. 2). The large clusters of chondrocytes of the proliferating and the hypertrophic zones were separated from each other by large areas of extracellular matrix, whereas in the other zones they were separated by loose fibrillary septae. Large longitudinal clefts containing extravasated blood cells and amorphous debris were often present within the loose-appearing septae separating the clusters of chondrocytes (Fig. 2). In three cases, fractures running along the growth plate at the border between the hypertrophic and the degenerative mineralising zone were present. Mineralisation of the hypertrophic and degenerating zones was scanty and irregular, and thin bone trabeculae were formed on the poorly mineralised cartilage.

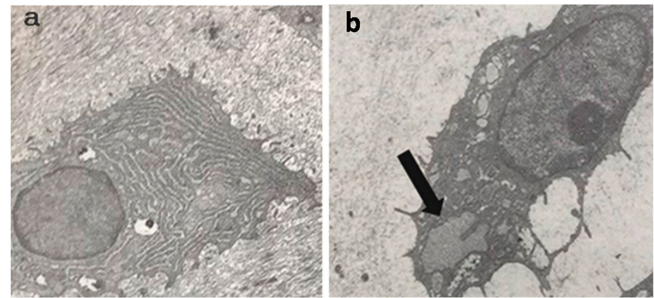
#### Histochemistry

The extracellular matrix of the resting zone was uniformly and strongly positive to the PAS reaction in both the normal and abnormal areas of the growth plate. The alcian blue staining as well as metachromasia by toluidine blue staining was present in the perilacunar rim of the chondrocytes (Fig. 2).

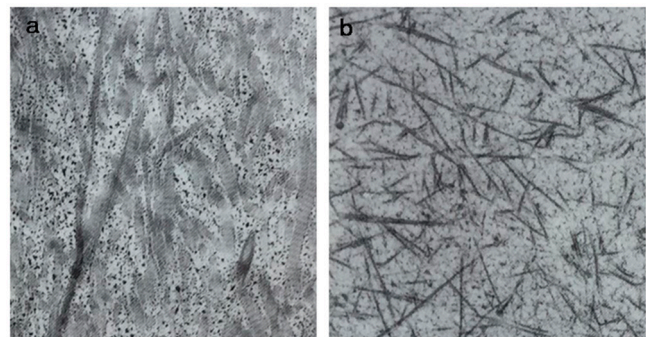
In the clusters of proliferating and hypertrophic chondrocytes, the extracellular matrix had a thick appearance and it was strongly PAS-positive, whereas the perilacunar matrix was strongly alcian blue positive and metachromatic as well. The longitudinal septae separating the clusters of chondrocytes with a loose fibrillar appearance were alcian blue positive and metachromatic, while the amorphous material contained in the longitudinal clefts was PAS-positive.

#### Ultrastructure

The chondrocytes of the resting zone of the SCFE plates were often elliptical in shape and smaller than normal. They contained enlarged rough-surfaced endoplasmic reticulum cisternae and an enormously developed Golgi apparatus filled with an amorphous filamentous material (Fig. 3). Cytoplasmic projections were more numerous and longer than normal, and the lacunae were filled with a thick radiodense floccular material. Collagen fibrils, thinner than normal, measuring about 200 Å, were intermingled with normal 300 to 1200 Å thick collagen fibrils. In the proliferating and hypertrophic zones, several chondrocytes were undergoing apoptotic changes. Very few mitochondria were present in the hypertrophic cells. The extracellular matrix of the longitudinal septae that was alcian blue positive at the light microscopic level was always more loosely arranged than in the normal plate. The randomly oriented collagen fibrils were thinner than normal, measuring about 200 Å, and were uniform in

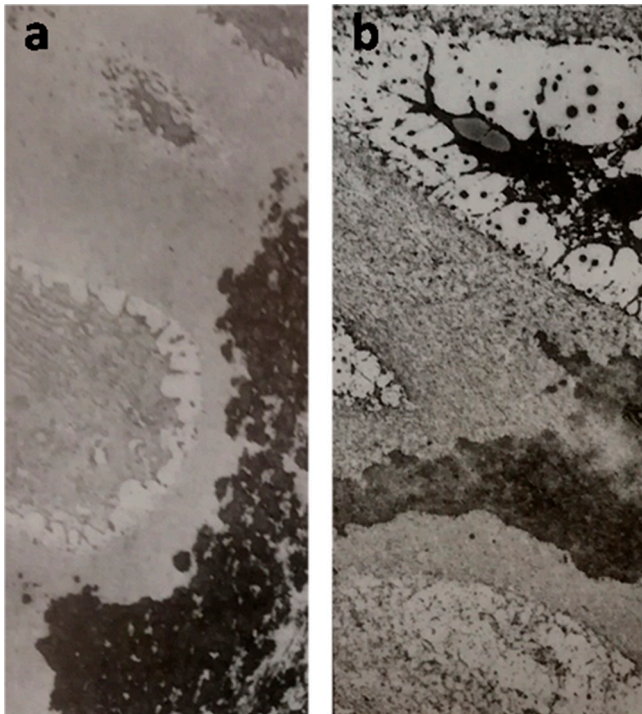


**Fig. 3** (a) Normal plate and (b) SCFE plate resting zone. The chondrocytes of the SCFE plate are smaller, more elongated and darker than normal. The endoplasmic reticulum is arranged in long and circonvoluted cisternae in normal chondrocytes and the Golgi apparatus is normally represented. The chondrocytes of the SCFE plate have the endoplasmic reticulum arranged in short and enlarged cisternae containing a floccular material (arrow), while the Golgi apparatus is markedly developed. (Stain: uranyl acetate – lead hydroxide;  $\times 4500$ ).



**Fig. 4** (a) Normal plate and (b) SCFE plate longitudinal septae. In the normal plate, collagen fibrils, with diameters in the range of 300 to 1200 Å, were longitudinally oriented along the intercolumnarseptae with intermingled largeradiodense granules of proteoglycans. In the SCFE plate, thin collagen fibrils with a diameter of about 200 Å were randomly oriented with intermingled amorphous floccular material. (Stain: uranyl acetate – lead hydroxide;  $\times 8000$ ).

diameter, while the spaces between the collagen fibrils were filled with the same floccular material present in the lacunae of the chondrocytes (Fig. 4). In the normal plate, 300 to 1200 Å thick collagen fibrils were longitudinally oriented with intermingled radiodense granules of proteoglycans (Fig. 4). In the degenerating zone, dying chondrocytes with dense cytoplasm had shrunk, forming numerous dark round bodies. There was little or no mineralisation of the longitudinal and transversal septae. Crystals of hydroxyapatite were aggregated in patches among the thin collagen fibrils whereas large crystals of hydroxyapatite were filling the intercellular septae of the normal plate (Fig. 5). A few small osteoblasts were synthesising scanty osteoid matrix on the poorly mineralised cartilage septae, with the formation of thin irregular bone trabeculae.



**Fig. 5** (a) Normal plate and (b) SCFE plate degenerating zone. In the SCFE plate, both hypertrophic and degenerating chondrocytes have a very dark cytoplasm, and numerous radiodense matrix vesicles are present around the cells; a few patches of mineral crystals are scattered in the matrix of the intercellular septae whereas hydroxyapatite crystals are diffusely and uniformly disposed within the intercellular septae of the normal plate. (Stain: uranyl acetate – lead hydroxide; a, b:  $\times 1800$ ).

## Discussion

The histopathology of SCFE was first described by Sutro in 1935.<sup>1</sup> He described the 'epiphyseal plate irregular in contour as well as in thickness'. He interpreted one area of the normal plate interposed between two wider clustered abnormal areas plugged into the metaphyseal bone (Fig. 1C of his paper) as 'the plate had buckled or bent on itself so that the proliferating zones were facing each other'. His conclusion was that 'trauma caused many of the microscopic observations'.

One year later, Kleinberg and Buchman<sup>2</sup> described the SCFE epiphyseal plate as 'very irregular. The columnar arrangement of the proliferating cartilage cells is all but lost. The resting cartilage... presents splits, some of which are invaded by blood vessels... while others are filled by granulation tissue. The cartilage plate... presents more increased areas of myxoid degeneration'.

In 1941, Howorth<sup>3</sup> divided SCFE into four stages. He named the first stage 'pre-slipping' in which there is no radiographic evidence of slipping but only 'widening and irregularity of the epiphyseal line with decalcification of the proximal end of the neck'. Eight years later, he

published a histopathological study,<sup>4</sup> but he interpreted the pathological changes of the proximal femoral growth plate as a 'process of degeneration and repair... at junction of plate with femoral neck', and the abnormal chondrocyte clusters as 'a mass of young cartilage cells growing into a defect in the adjacent bone'.

In 1951, Lacroix and Verbrugge<sup>5</sup> described the histopathological aspects of two SCFE cases. The plate had a fibrocartilaginous appearance and it was partly ossified. The impression is that those cases represent a very late stage of the disease.

Five years later, Ponseti and McClintock<sup>6</sup> reported the histopathology of three SCFE cases in which core biopsies were taken at the time of pinning operations. The epiphyseal plate was much wider than normal. Chondrocytes were grouped in clusters separated from each other by fibrillated septae in which extensive clefts containing amorphous debris were present. 'Adjacent to the metaphysis there were areas in which enchondral ossification appeared to proceed normally whereas in other areas, it had ceased completely'.

Further histopathological reports published later,<sup>7,8</sup> including the present study, did not add anything new to what had already been described. Only Adamczyk et al<sup>9</sup> in 2005, by using the TUNEL reaction, showed that chondrocytes of the SCFE plate are more vulnerable than those of the normal plate undergoing apoptosis, from 31% to 50% in SCFE versus 6% to 19% in the normal plate.

So far, very few histochemical and ultrastructural studies of the SCFE plate have been published.<sup>7,8,10-12</sup> The histochemical identification of proteoglycans by alcian blue and toluidine blue showed the presence of alcianophilic and metachromatic material in the loosely arranged fibrillated septae that separate chondrocyte clusters, suggesting an increased accumulation of proteoglycans in those areas. In the same areas, ultrastructural studies showed the presence of very thin collagen fibrils with interposed radiodense floccular material very likely representing abnormal proteoglycans. Since various types of collagen are normally synthesised by growth plate chondrocytes, namely type II, IX, X and XI,<sup>13</sup> we might postulate that thin collagen fibrils may be formed by collagen types different from type II. In this connection, Scharschmidt et al<sup>14</sup> have shown that in the SCFE plate type II collagen mRNA is under-expressed, by only 13.7% of normal collagen. In the same study, the gene responsible for aggrecan synthesis was under-expressed by 26% of normal collagen. As a consequence, the abnormal metachromatic and alcian blue floccular material might represent lower molecular weight proteoglycans like decorin, annexin II, V and VI, also synthesised by growth plate chondrocytes.<sup>13</sup>

Chondrocytes were abnormal in all the zones of the SCFE plate. They were smaller and more elongated than normal, with a dark cytoplasm. They contained an

enormously developed Golgi apparatus and very enlarged cisternae of the rough-surfaced endoplasmic reticulum containing an amorphous filamentous material similar to that found in the lacunae surrounding the chondrocytes. Dark, elongated and small chondrocytes were also found in the hypertrophic and degenerating zones. Cellular abnormality in SCFE might be responsible for the abnormal matrix synthesis as well as for the increased apoptotic changes.<sup>9</sup>

There was little or no mineralisation of the longitudinal and transversal septae in the degenerating zone: this finding might be the consequence of under-expression of alkaline phosphatase by the abnormal chondrocytes. As a result, bone formation was impaired as shown by the small osteoblasts synthesising scanty osteoid matrix on the poorly mineralised cartilage septae.

The histological and ultrastructural studies carried out by Guzzanti et al<sup>15</sup> and by Falciglia et al<sup>16</sup> deserve a particular mention. Basing the rationale of their investigations on the radiographic remodelling of the proximal femoral growth plate after *in situ* fixation, they showed a marked improvement of the SCFE plate histological picture following surgical stabilisation. In two cases, a nearly normal physal architecture was observed after fixation. We believe that this is a very sound demonstration of how the mechanical factor may strongly affect the metabolism of the SCFE chondrocytes.

In conclusion, the evidence of marked histological, histochemical and ultrastructural alterations of the proximal femoral growth plate in SCFE at the Howorth pre-slipping stage<sup>3</sup> puts an end to any criticism of the primitive alterations of the SCFE plate. Without slips, we exclude by definition any alterations secondary to the mechanical effect of epiphyseal slipping. The alterations observed are complex, including distortion of the cellular morphology and metabolism,<sup>14</sup> with important repercussions on the extracellular matrix composition. Thinning and loosening of collagen fibrils as well as the decrease of large proteoglycan aggregates (aggrecans), together with the marked under-regulation of the synthetic pathway for both type II collagen and aggrecan, might very likely account for the reduced mechanical strength of the proximal femoral growth plate under the shear force caused by body weight. In fact, it must be remembered that the proximal femoral physis is the only growth plate obliquely oriented in relation to the lower limb mechanical axis and consequently subjected to shear forces.

We cannot rule out the possibility that the basic alteration which causes SCFE might also be present in other physes of the lower limb but the absence of strong mechanical stimuli might block its progression. The observation that the histological alterations might be completely or partly recovered by providing mechanical stability to the physis<sup>15,16</sup> may give further proof supporting the theory

that the mechanical factor is a very important component of the aetiopathogenesis of the disease.

Further histochemical and metabolic studies on the proximal femoral growth plate will be needed to clarify SCFE aetiopathogenesis. The absence of any complications in our patients following trocar biopsy, as also reported by other authors who have performed morphological studies on core biopsies,<sup>6,8</sup> must be emphasised in order to reassure future investigations.

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## COMPLIANCE WITH ETHICAL STANDARDS

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### ETHICAL STATEMENT

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Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent: Informed consent was obtained from all individual participants included in the study.

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