

## THE EVALUATION OF EPIPHYSEAL PLATE HISTOLOGICAL CHANGES IN OSTEOPETROTIC OP/OP MICE.

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### ABSTRACT

This study was designed for evaluation of epiphyseal plate histological changes of femur bones in osteopetrotic op/op mice. In this study 5 osteopetrotic op/op mice which were purchased from the commercial source were used. The animals were killed by overdose of chloroform and their femur bones were extracted. The bones were fixed in 10% formaldehyde and decalcified by HCl (0.6N), and routine histological processing were performed. The sections were stained by H&E methods and studied by conventional light microscopy. The results showed that, proliferative zone (PZ) and especially hypertrophic zone (HZ) were much thickened. In the ossification zone, trabecular bones were irregular and atypical osteoblast cells were observed. The osteoclast cells were not attached to trabecular bones. The bone marrow cavity was restricted and bone marrow cells were poor and scattered. Findings of the present investigation are similar to those reported about epiphyseal plate in osteosclerotic (OC) mice in which epiphyseal plate especially hypertrophic zone was thickened and chondrocytes were not substituted for osteoblasts in calcified cartilage area. Also, osteoclast cells had been inactive or absent in OC mice. For prevention of other complication due to the epiphyseal plate changes in new borne, suitable and punctually treatment protocols such as prescription of Macrophage Colony Stimulating-Factor (MCS-F) could be useful.

**Key Words:** Chondroblast cells, Epiphyseal Plates, Osteopetrotic OP/OP Mice

### INTRODUCTION

Osteopetrosis is a rare hereditary bone metabolic disease that was distinguished for the first time in 1904 by Albers-Schoberg (1) and for this reason was called Alber-Schoberg disease. Also, because of the high density of bones, some physicians called it Marble bones or marble bone disease (2, 3). This disease results from the lack of the activity or absence of osteoclast cells, severe decrease of monocytes and peritoneal macrophage due to the decrease in Macrophage- Colony Stimulating Factor (M-CSF) in this mutation (4, 5). Osteopetrosis appear in different types and different ages so have different treatment (6). The clinical appearance of osteopetrosis because of their accompanied immunitic and hematologic complication is different and while some cases die after birth because immunologic and hematogenic disorder, others continue the life for a long time (7, 8). The severe type of osteopetrosis in human is distinguished 2

months after birth by optical and hematological signs. Also, 2 types of osteopetrosis, lethal and nonlethal, are distinguished in adult people (9). In mice, 4 types of osteopetrotic mutation, happen at different chromosomes, and have been distinguished as follows: grey lethal (gl), microphthalmic (mi), osteosclerotic (oc) and osteopetrotic (op). The grey lethal (gl) and microphthalmic (mi) might results to death 5 weeks after the birth (10). Four other types mutations are discovered in rats as follows: Incisor absent (ia), osteopetrotic (op), toothless (tl) and microphthalmic blanc (mib) (11, 12, 13). In these mutations growth plate and its specific zones, proliferation zone, hypertrophic zone, calcified cartilage and substitution of chondrocytes by osteoblast cells are rare. The growth plate in this disease is affected, normal endochondral ossification is restricted, bone marrow cavity is limited and bone marrow cells are in weak condition and scattered (4, 14, 15). Marks and his coworker arranged a project for

histologic study of epiphyseal cartilage and ultrastructure of osteoclast cells in OC mice and reported that in OC mice growth plate particularly hypertrophic zone were vacant and substitution of chondrocytes by osteoblast cells were delayed (16). The chondroblast arrangement in hypertrophic zone was irregular, cells organism and external shape in light microscopy changed atypically. Also, hypertrophic zone vascularization was in a poor condition in OC mice (16). Also, Ricket like condition in human osteopetrosis has been reported (17, 18, 19, 20) and treatment of disorders has not been successful by vitamin D and other ricket protocols treatment in human osteopetrosis (21). For evaluation of superior epiphyseal plate histologic changes of femur bone, this study was designed on osteopetrotic op/op mice.

#### **MATERIALS AND METHODS**

In the present investigation, 40 days osteopetrotic op/op mice were purchased from Jackson laboratory (Bar Harbor Me USA). The animals were dieted by special soft nutrition at the animal house for one week and standard health conditions were arranged. For histological studies animals were killed by chloroform overdose and the femur bones were extracted. The harvested specimens were repeatedly fixed by 10% formaldehyde solution for one week and decalcified by HCl (0/6 N) for 2 weeks. The upper epiphyseal plate region was dissected. After routine histological processing the selected part of femur bone were embedded in hard paraffin longitudinally and were sectioned at 6 micron thickness (17). The sections at intervals of 60 micron were stained with Hematoxilin and Eosin (H&E). By the above-mentioned condition sections were prepared from normal animals and stained by H&E. Also, semi thin sections were prepared and stained by Toluiden blue for 2 groups. The slides were studied microscopically and photos were obtained.

#### **RESULTS**

In osteopetrotic op/op group by Hematoxilin and Eosin staining sever thickness of proliferative zone (PZ) and especially Hypertrophic zone (HZ) in longitudinal view were observed in contrast to control group (Fig 1a, 1b). The normal columns of chondroblasts, in the hypertrophic zone (HZ), were changed and irregular in the op/op groups (Fig 2a, 2b). The chondrocytes by Hematoxilin and Eosin staining were appeared vacant at calcified cartilage (CC) region in osteopetrotic op/op mice (Fig 3a, 3b). Osteoblast cells were

abnormal in external morphology, and typical epitheloid osteoblast cells were not observed and regulation of trabecular bones in op/op mice were destroyed (Fig 3a, 3b). Bone marrow cavities in experimental group were very restricted and bone marrow cells were poor and scattered. Also, no osteoclast cells in op/op group could be detected (Fig 3a, 3b). By toluiden-blue staining, the chondrocytes in terminal portion of hypertrophic zone were large and were accompanied by reach granules (Fig 4a, 4b).

#### **DISCUSSION**

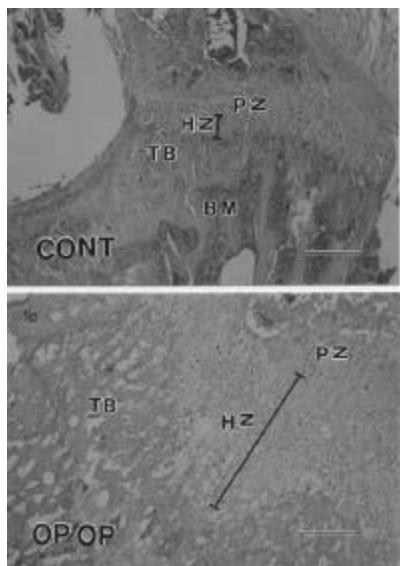
Osteoporosis is a rare congenital disorder which is observed in one of 200000 children. It prevents formation of bone marrow cells and results in abnormal long bone development, blindness (22), stounded growth, abnormal dental development and fragile bones (23). Radiologically, this disease is characterized by generalized increases in skeletal density in which bones look solid on X-ray with defect in metaphiseal modeling (24, 25). Sever forms of osteopetrosis have been described with overlapping of clinical and radiographic features (25). Irregular conditions of the bone at the metaphyses may produce the appearance of parallel plates of dense bone at the end of long bones (16). In this investigation, sever thickness of proliferative zone (PZ) and especially hyperthrophic zone (HZ) of growth plate were observed. These findings resemble to the rickets condition that has been explained by other investigations (20, 26) and in other type of osteoporosis (27). The chondrocytes by H&E staining appeared vacant and was accompanied with granules by Toloiden blue staining. Marks and his coworkers by arrangement of the results of histological study of epiphyseal plate in oc mice reported similar results (28). In this investigation typical epitheloidal osteoblasts cells were not observed and regulation of trabecular bones were destroyed. Poppof has reported this characters in rabbits (29). In the present investigation bone marrow cavity was restricted and marrow cavities cells deficiency were similar to those of previous reports (30, 31). Osteoclast cells attached to bone trabeculae at present study could not be distinguished. Recently, Amling and his coworker reported that, in the absence of bone resorption because of osteoclastic function, bone formation continues and leads to progressive accentuation of the osteopetrotic phenotype in c-src-deficient mice (32). According to Nomura and his coworker idea, osteoclast normal function was dependent to the presence of a target gene for

Microphthalmia mutation (Mi) that is essential for the proliferation/differentiation of osteoclasts (33). It is believed that decrease in the amount of macrophage colony stimulating factor in these mutations, cause absence or inactivity of osteoclast cells (34, 35, 36, 37, 38).

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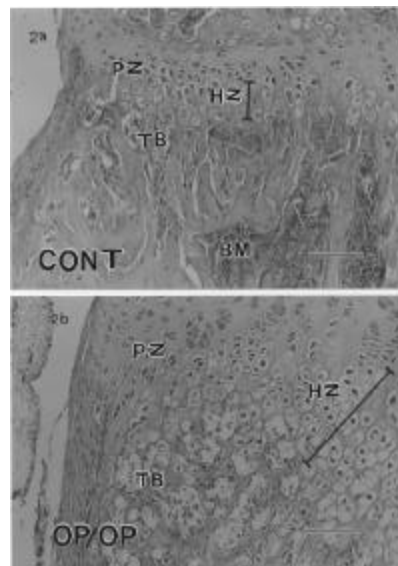
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**Figure 1-** Light microscopic view from longitudinal section of proximal epiphyseal plate (Femure) in osteopetrotic op/op (a) and normal animals (b).

The proliferative zone (PZ) and especially Hypertrophic Zone (HZ) in osteopetrotic op/op mice is too thickend in contrast to the normal animals. Also, Trabecular bone (TB) is irregu-lated in op/op mice.

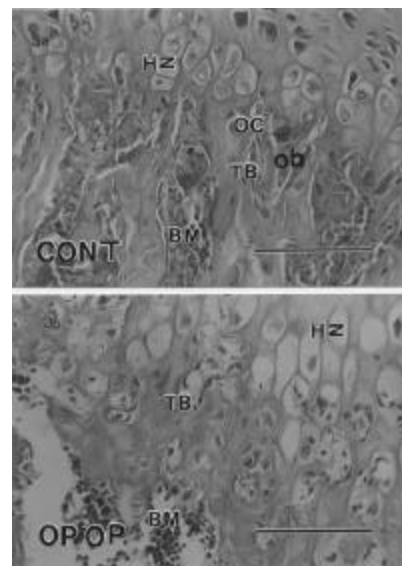
Staining: Hematoxilin and Eosin  
Original magnification: X100



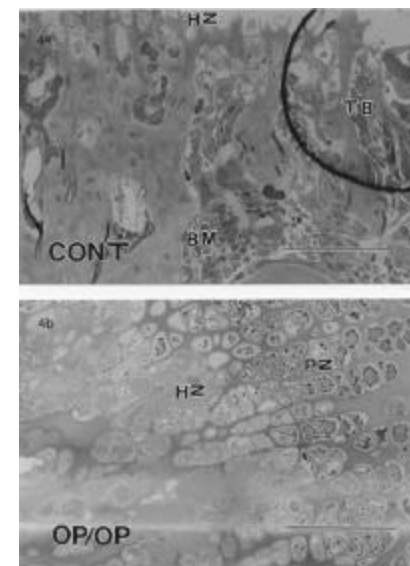
**Figure 2-** Light microscopic view from longitudinal section of proximal epiphyseal plate (Femur) in osteopetrotic op/op (a) and normal animals (b).

The trabecular bones (TB) in osteopetrotic op/op mice are irregulated in contrast to normal animals. Normal typical epitheloidal osteoblast cells were not shown in op/op group. Bone marrow cavities (BM) are restricted and bone marrow cells are not enough in op/op group.

Staining: Hematoxilin and Eosin.  
Original magnification: X 200



**Figure 3-** High magnified light microscopic view from longitudinal section of proximal epiphyseal plate (Femur) in osteopetrotic op/op (a) and normal animals (b). The chondrocytes in calcified cartilage (cc) region were observed vacant in op/op group by Hematoxilin and Eosin staining. The trabecular bone (TB) is irregulated and normal epitheloidal osteoblast cells are not appeared in op/op group. Osteoclast cells are not shown in op/op group. Staining: Hematoxilin and Eosin. Original magnification: X400



**Figure 4-** Light microscopic view from longitudinal section of proximal epiphyseal plate (Femur) in osteopetrotic op/op (a) and normal animals (b).

The chondrocytes in terminal portion of hypertrophic zone (HZ) in the region of calcified cartilage (cc) is accompanied by some granules in op/op group. But, in normal animals these granules how ever did not appear.

Staining: Toluidin Blue  
Original magnification: X400