Ossification of the choroid: three clinical cases and literature review of the pathogenesis of intraocular ossification

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Abstract
Objective: A presentation of the clinical and pathogenic aspects of choroidal ossification. Cases Presentation: We report three clinical cases of choroidal ossification: choroidal osteoma, ossified choroidal hemangioma and total ossification of the choroid. The three patients underwent complete eye examination. The optical microscopy of a sample of ossified choroidal tissue revealed a spongy, osseous structure consisting of circular osseous lamellae, osteocytes, canaliculi and adipose tissue with microfoci of calcification. Discussion: Choroidal ossification is characterized by reduced frequency of occurrence, accessible clinical diagnosis, and unspecified pathogenesis. Several of the factors identified in the pathogenesis of intraocular ossification may play a role in the ossification of the choroid: chronic inflammatory cells, bone morphogenetic proteins, growth factors and mesenchymal stem cells. In addition to these factors, pericytes have a special role in the pathogenesis of choroidal ossification. Under the influence of bone morphogenetic proteins and growth factors, mesenchymal stem cells differentiate into osteoblasts. They secrete bone matrix (ostoid), whose regeneration and remodeling lead to the formation of bone tissue. The spongy bone structure of choroidal tissue points to a model of endoconjunctive/desmal ossification. Conclusions: The knowledge of the clinical aspect of ossification of the choroids is required for the differential diagnosis with the posterior pole affections, and also for the prevention and treatment of secondary complications.

Keywords: ossification of the choroid, choroidal osteoma, ossified choroidal hemangioma.

Introduction
Ectopic ossification is the formation of extraskeletal bone tissue at the level of soft, richly vascularized tissues. Intraocular ossification, a type of ectopic ossification, congenital or acquired, presents itself as structured bone tissue, controlled by osteoblasts and osteoclasts. It includes several clinical forms: epibulbar osteoma, epibulbar osseous choristomas with scleral involvement, and several types of ossification of the choroid, among which choroidal osteoma, ossified choroidal hemangioma, and total ossification of the choroid.

Choroidal osteoma is a benign osseous tumor, with a very low frequency of occurrence, a number of only 142 cases being reported in specialized literature. In a reference centre, only 61 cases have been identified over a period of 26 years [1]. The disease is bilateral in 34% of the cases and it affects predominantly women, with a 6:1 female to male ratio. Patients are asymptomatic in 80% of the cases, with good visual acuity, even though the female to male ratio. Patients are asymptomatic in 80% of the cases and it affects predominantly women, with a 6:1

Diffuse choroidal hemangioma is a benign vascular tumor of large size, with unspecified frequency, associated in 40% of the cases with ipsilateral facial nevus flammeus, as manifestations of the Sturge–Weber syndrome [4]. By contrast, only 1 in 50 patients with facial nevus flammeus is diagnosed with choroidal hemangioma [5], which requires regular eye examination of such patients. In its evolution, diffuse choroidal hemangioma can develop complications such as progressive serous retinal detachment [6], retinal fibrosis, or, very rarely, secondary ossification.

In phthisical globes, scleral calcification is commonly encountered by comparison to scleral ossification or intraocular heterotopic bone formation, which is rare [7]. By contrast, Rohrbach JM et al. [8] conclude that out of 29 phthisical or chronic hypotonic ocular globes, 12 are cases of intraocular ossification (41.3%).

The pathogenesis of intraocular ossification, including the ossification of the choroid, remains unspecified. Recent literature brings to the fore various factors involved in this mechanism. Some of them may become manifest at the level of the choroid, allowing for the detection of a pathogenic model of ossification. Intraocular ossification may be caused by multiple factors, several of which can be identified in the choroid: chronic inflammatory processes, BMPs, growth factors, and, in particular, pericytes and/or circulant MSCs. They are an important source of pluripotent MSCs, capable of differentiation into different cell types, among which osteoprogenitor cells. Osteoprogenitor
cells secrete bone matrix (osteoid), whose regeneration and remodeling lead to the appearance of spicules and subsequently osseous trabeculae, which, by interconnection, generate primary spongy bone, later replaced by lamellar bone. The histopathological aspect of ossified choroidal tissue reveals a spongy type, consisting of osseous lamellae, osteocytes, bone canaliculi and adipose tissue. This (lamellar) bone structure supports the hypothesis of endoconjunctive/desmal ossification, without passing through the cartilage phase.

We report three clinical cases of ossification of the choroid, together with a literature review of the pathogenesis of intraocular ossification, which subsumes the ossification of the choroid.

Patients, Methods and Results

The present study examines three clinical forms of ossification of the choroid, as follows: choroidal osteoma, ossified choroidal hemangioma, and total ossification of the choroid.

Case No. 1

A 31-year-old male, examined for decreased visual acuity of gradual onset in his left eye (visual acuity 20/30), accompanied by phosphenes. Fundus examination showed a yellow-orange plaque, located in the peripapillary and supero-temporal region, with scalloped, well-defined margins (Figure 1A). Fluorescein angiography revealed early, spotted hyperfluorescence with late homogeneity (Figure 1B), and the ultrasound revealed highly reflectivity of the ossified plaque, with the projection of the acoustic wave in the orbital adipose tissue (Figure 1C). Clinical diagnosis: choroidal osteoma.

Case No. 2

A 27-year-old male, with ipsilateral facial nevus flammeus and decreased visual acuity in his left eye (visual acuity 20/30), without other ocular symptoms. Slit-lamp examination showed no changes in the corneal diameter, normal ocular pressure, color change in the pupil, with a bright red-orange hue. Fundus examination showed a red-orange plaque with blurred margins, located in the peripapillary region and associated, in the central area, with yellow-orange spots (Figure 2A). Fluorescein angiography revealed centrally irregular hyperfluorescence (Figure 2B), and ultrasonography showed high reflectivity of the ossified plaque, with the projection of the acoustic wave in the orbital adipose tissue (Figure 2C). T1-weighted MRI showed a hyperintense region around the posterior pole relative to the vitreous body (Figure 2D). Clinical diagnosis: Sturge–Weber syndrome associated with partially ossified choroidal hemangioma.

Case No. 3

A 53-year-old female, examined for atrophy of the left eye caused by an old eye injury. The enucleation of the phthisical eye showed a cup-shaped ossified choroidal structure, at the centre of which the ossified hole of the optic nerve could be noticed (Figure 3A). The
histopathological examination, sampled from an ossified choroidal tissue, revealed a spongy type of osseous structure, consisting of circular osseous lamellae, osteocytes, bone canaliculi, and adipose tissue with microfoci of calcification (Figure 3, B and C). Volumetric and tomographic reconstruction showed an archiform lamellar structure with osseous density (Figure 3D). Clinical diagnosis: total ossification of the choroid.

Figure 3 – Ossification of the choroids: (A) Internal surface of the ossified choroidal tissue: the osseous hole of the optic nerve can be noticed; (B) The histopathological image of circular osseous lamellae, osteocytes, medullary canals and conjunctival adipose tissue with microfoci of calcification (HE staining, ob. ×20); (C) Spongy bone tissue with unequal lamellae with no osteoblasts in periphery; adipose tissue, multiple calcification foci and significant hematic extravasation are between lamellae (HE staining, ob. ×20). (D) Tomodensitometric and volumetric reconstruction image of archiform lamellar structures with osseous density.

Discussion

The ossification of the choroid, the consequence of structured osseous tissue formation controlled by osteoblasts and osteoclasts, encompasses the following types: choroidal osteoma, ossified choroidal haemangiomata and total ossification of the choroid. The frequency of occurrence of choroidal osteoma and ossified choroidal hemangioma is differently assessed. In one of our unpublished studies, carried out on 36 atrophic ocular globes, with antecedent chronic diseases, we have identified the ossification of the choroid in 14 (38.8%) cases. The chronic antecedents include total retina detachment (five cases), trauma (four cases), chronic ocular inflammation (two cases), absolute glaucoma (two cases), and undetermined etiology (one case). The clinical diagnosis of choroidal ossification can differ depending on the date of onset and the examination technique employed. Buchman (1901) describes intraocular ossification in a 10-week child. Most authors, however, agree on a period of 10 to 20 years. Histopathologically, choroidal ossification can be diagnosed one year after ocular trauma, and radiologically between 10 and 20 years [9].

The pathogenesis of intraocular ossification is not specified. Rohrbach JM et al. [8] invoke certain general principles, which involve RPE (retinal pigment epithelium). In this context, the primary lesion plays a minor or non-existent role, conclusion we have also reached based on a diverse range of etiopathogenic aspects observed in the cases of choroidal ossification. Recent research brings into relief particular factors that contribute to the process of intraocular ossification: chronic ocular inflammation, bone morphogenetic proteins, growth factors, and mesenchymal stem cells.

Through the type of cell involved (monocytes, macrophages, chronic intraocular inflammations play a role in the onset of intraocular osseous metaplasia [10]. Evidence of this process lies in the increased frequency of chronic ocular inflammation in atrophic ocular globes, associated with choroidal ossification. In heterotopic bone formation because of trauma, the triggering mechanism is less known, but there is certain involvement of peripheral sensitive nerves, neurogenic inflammation and the bone morphogenetic protein BMP-2 [11, 12]. Bone morphogenetic proteins (BMPs) are morpho-functional cytokines, a subfamily in the transforming growth factor beta superfamily [12]. BMPs are involved in cellular regulation, differentiation and apoptosis [13]. Through a molecular mechanism, they participate in the physiopathology of general or local diseases. At the level of the eye, they may become involved in affections of the ciliary epithelium, the epithelium of the crystalline, the cornea and the retina [14]. Originally named osteoinductive factors, some forms of BMPs (BMP 2–8, 13, 14) have the potential to trigger the transformation of mesenchymal cells into cartilage and the formation of osseous elements [15]. Among them, BMP-7 has a distinct osteoprogenitor capacity, which stimulates ectopic ossification by inducing the differentiation of pluripotent cells into osteoprogenitors [16, 17]. BMP-7 and BMP-6 induce the development of small foci of spongy bone, while BMP-6 and BMP-9 induce the appearance of multiple foci of immature trabecular bone. BMP-9 also has the capacity to induce the mineralization of bone matrix/osteoid. On the other hand, BMP-7 may inhibit the transformation of epithelial cells into fibroblasts [18]. For the transformation of bone metaplasia cells into osteoblasts to occur, BMP-7 has to reach a certain threshold, which explains why not all chronic ocular inflammations lead to ossification.

There are multiple growth factors with a role in intraocular ossification: the tumor necrosis alpha factor (TNF–α), which, together with IL-1, stimulates RPE to produce the transforming growth factor beta 1 [19] and BMP-7 [20, 21]; the transforming growth factor beta1 (TGF–β1) generated by RPE and chronic inflammatory cells is involved in mesenchymal-epithelial transformation [22], a process also observed in the kidney [23]. Bosse A et al. [24] have highlighted the presence of TGF–β1 RNA in the areas of proliferation of mesenchymal tissue, arguing for the role of TGF–β1 as a cell regulator in ectopic ossification. Growth differential factor-5 (GDF-5) also plays a role in bone growth through the differentiation of mesenchymal cells into an osteoblastic line.

Adult mesenchymal stem cells (MSCs), inactive in soft tissues, are involved in intraocular ectopic ossification.
Stimulated by some forms of BMPs (BMP-2 and -6), they have multilineage differentiation potential, which is responsible for the formation of several cell types: osteoblasts, adipocytes, and chondrocytes. The differentiation of mesenchymal stem cells is genetically regulated. It is important to point out that osteogenesis and adipogenesis have 235 genes in common, whereas osteogenesis and chondrogenesis have only three genes in common [26]. MSCs in the form of fibroblast-like cells have been recently identified in the stroma of the corneal limbus. They have self-renewal and plasticity properties, just like embryonic stem cells, even though their source is not embryonic [27]. Polisetty N et al. [27] have demonstrated that mesenchymal cells of the limbus are positive for mesenchymal markers and negative for epithelial and hematopoietic markers, and are capable of multilineage differentiation into osteocytes and adipocytes, which could account for rare cases of intracorneal ossification. Nadri S et al. [28] have identified MSCs derived from human eye conjunctiva stromal cells, and have confirmed their potential to differentiate into osteogenic, adipogenic, chondrogenic, and neurogenic lines, in spite of their origin in an adult source.

Toyran S et al. [29] have carried out immunohistochemical studies to examine the role of some factors involved in intraocular ossification, and, based on both their findings and specialized literature, they have proposed a model for the pathogenesis of intra-ocular ossification. By analyzing the immunoreactivity of GDF-5, TNF-α, and TGF-β1 in normal eyes and in eyes affected by intraocular ossification and bone metaplasia, they have obtained significant findings. They have observed a moderate intensification of the intracytoplasmic immuno-reactivity of BMP-7 and GDF-5 at the level of RPE with fibrous metaplasia, the epithelium adjacent to the bone metaplasia areas. The authors consider that the origin of ectopic ossification may be found in osteoprogenitor stem cells, “dormant” in tissues, which, if stimulated by BMPs, differentiate into osteocytes and produce osteoid, followed by calcification. Toyran S et al. propose a diagram of intraocular ossification. Inflammatory cells (monocytes, macrophages) synthesize IL-1, TNF-α, and TGF-β1. IL-1 and TNF-α stimulate RPE to produce TGF-β1 and BMP-7. TGF-β1 initiates the transformation of mesenchymal epithelium into fibrous metaplasia of RPE cells. BMP-7 inhibits fibroblastic differentiation and induces the transformation of metaphasic RPE cells into osteoblasts. BMP-7 also balances up this process, an excessive increase in BMP-7 producing the trans-differentiation of metaphasic RPE cells into osteoblasts. GDF-5 is co-localized with BMP-7 in zones of fibrous metaplasia of RPE cells and plays the same role of stimulating osseous metaplasia. The process continues with the formation of osteoid, which will subsequently calcify. To sum up, the pathogenic model put forward by Toyran S includes: a process of fibrous metaplasia of RPE cells, in which reprogrammed MSCs are also involved, and a process of trans-differentiation of fibrous cells (emergent from RPE cells) into osteoblasts. Recent literature shows that the first process of fibrous metaplasia takes place by epithelial-mesenchymal transition, a mechanism through which RPE cells change their phenotype and acquire the characteristics of mesenchymal cells [30, 31].

Arguments in favor or against this hypothesis have been advanced. The hypothesis is confirmed by observations made at the level of the non-pigmented ciliary epithelium, cornea or salivary glands. At the level of the non-pigmented ciliary epithelium, the effects of BMP-2 and -4 have been noticed. They could be the consequence of an inflammation of the ciliary body or of certain cytokines (IL-1) in the posterior pole, which, via the vitreous body, lead to the formation of BMP-7 and GDF-5 [31]. In normal corneal keratocytes, in the adult human eye, a moderate reactivity of BMP-7 and GDF-5 has been observed. These proteins may be an important regulator of the proliferation of corneal epithelial cells [33]. BMP-2 and -4 influence corneal fibroblast chemotaxis, being involved in corneal lesion repair. Zhao S et al. [32] point to the existence of a neural potential of isolated cells in the adult corneal limbus. The quality of the neural properties of these cells is regulated by BMP-4 and may originate in trans-differentiated or reprogrammed limbal stem cells. Additionally, GDF-5, together with BMP-7, has been found in salivary glands with pleomorphic adenoma, in association with ectopic osseous formations, which indicates that they have a role in ectopic bone formation [34]. The arguments that question the validity of intraocular ossification invoke the presence of teratoma, medulloepithelioma and choroidal osteoma in the absence of inflammatory processes.

Scientific data on the ossification of the choroid are incomplete and differ according to the clinical form of ossification. The pathogenesis of choroidal osteoma raises a number of hypothetical issues, with arguments for and against: atavistic, inflammatory, choristomatous, traumatic, hormonal, and hereditary ossification [9].

In a similar vein, the pathogenesis of ossified choroidal hemangioma is not clarified. Zolog N [35] performed a histopathological exam on a patient with ossified hemangioma and Sturge–Weber syndrome, and identified a bony structure consisting of circular osseous lamellae and medullary canals. The author places emphasis on the “stagnation of blood” in the cavities of the hemangioma, which, populated with fibroblasts and histiocytes (pluripotent cells), transforms into osteoblasts, followed by calcification and bone tissue formation.

The pathology of the ossification of the choroid has not been elucidated. As previously mentioned, recent data highlight the role of certain factors involved in intraocular ossification. Some of them may take part in the process of choroidal ossification: chronic inflammatory affections, bone morphogenetic proteins, growth factors, and mesenchymal stem cells. A special role in the process of choroidal ossification is played by pericytes and/or circulant mesenchymal stem cells, which may constitute a significant source of pluripotent stem cells, capable of differentiating into certain cell types, among which osteoprogenitor cells [36]. Rare pericytes and a special population of pericyte-like cells, involved in the functional regulation of endothelial cells, have been found in the choriocapillaris [37, 38]. The ratio pericytes–endothelial cells is 1:1 in retinal vessels, while in striated muscles it is 1:100 [39]. Pericytes have multilineage differentiation
potentially, which generates osteoblasts, chondrocytes, adipocytes, fibroblasts, calcifying vascular cells, etc. Initial evidence of the differentiation of pericytes into osteoprogenitor cells was provided by Sato K and Urist MR [40]. The authors demonstrated that BMPs induce the osteogenic differentiation of pericytes conducive to spongy, chondroid bone formation. Pericytes express both osteo-progenetic factors, in particular bone morphogenetic proteins (BMP-1, -2, -4, -7), and factors that inhibit osteogenic differentiation: Gas-6 and its receptor, Axl. At the same time, the differentiation of pericytes into osteoblast-like cells has been associated with the expression of osteogenic markers (osteonecint, osteocalcin and alkaline phosphatase), as well as with the synthesis of macromolecules from bone matrix (collagen type I, IV, laminin, tenascin, thrombospondin-1). During this process, just like osteoblasts, pericytes become capable of generating mineralized bone matrix and express the same type of genes [41]. Pericytes may also be involved in the calcification of arterial walls [42]. In some cases of calcified arterial walls, associated with atherosclerosis, fully formed osseous tissue has been identified, also containing bone marrow, which supports the hypothesis that vascular calcification is similar to osteogenesis. In addition, researchers agree on the involvement of pericytes in angiogenesis, both in its early and late stages, pericytes being localized in angiogenic buds as well as neovessels. The association of choroidal osteoma with choroidal neovascularisation membranes could be taken to illustrate the association of ectopic ossification and angiogenesis. This points to the pathogenetic involvement of only one type of pluripotent cell, the pericyte, which is capable of angiogenic and osteogenic differentiation.

The clinical diagnosis of choroidal osteoma and ossified choroidal hemangioma is accessible to the knowledgeable ophthalmologist and can be confirmed by fundus exam, fluorescein angiography and ultrasonography. The functional symptoms of choroidal osteoma and ossified choroidal hemangioma are unspecified and depend on the localization, size and complications of the ossification of the choroid.

The ophthalmoscopic aspect of choroidal osteoma and ossified choroidal hemangioma has well-defined clinical characteristics. Choroidal osteoma frequently localizes in the juxtapapillary region; it is round or oval in shape, with scalloped, well-defined margins; it can be either yellow-white or red-orange and it has a non-uniform surface, covered with pigmented spots or vascular loops. Ossified choroidal hemangioma may localize in the retroequatorial, peripapillary or macular area. Its surface is relatively flat, with diffuse margins and it is covered, in certain cases, with light spots of pigment; it can be red or bright red, also described as “Tomato Catsup Fundus”.

The dynamic of the fluorescein angiography reveals, in the two clinical types already mentioned, similar characteristics: early, irregular hyperfluorescence, localized in the central area, with a tendency towards diffusion in the late stages.

Ultrasonography confirms the presence of ossified tissue with the same characteristics: hyperreflexia of the ossified plaque with the projection of the acoustic wave in the orbital adipose tissue. Similar characteristics may be found in ossified choroidal melanoma. Magnetic resonance, tomodensitometry and optical coherence tomography contribute to establishing the diagnosis.

The diagnosis of ossification in phthisic eyes is based on ultrasonographic modifications: choroidal thickening and choroidal folds account for the increased, non-uniform hyperreflexia of the osseous plaque. The necessary period for establishing a diagnosis of ossification may vary, in each case, according to the nature of chronic antecedents and the results of histopathological examination (one year after the trauma) or radiological examination (10–20 years).

As far as the ossification of the choroid is concerned, differential diagnosis requires a distinction between choroidal osteoma and ossified choroidal hemangioma and the affections of the posterior pole with a similar clinical aspect; it is also necessary to distinguish them from conditions of high calcium content. The former category includes: metastatic carcinoma,achromatoma melanoma, tumors of the optic nerve with invasion in the posterior pole, pigmented nevus, retinoblastoma, posterior scleritis,circumscribed hemangioma. A special case is that of choroidal melanoma, which, in rare situations, occurs at the onset of ossification. The latter category includes: idiopathic calcifications and sclerochoroidal metastases, calcified inflammatory foci and ossified fundus lesions of the nevus sebaceous of Jadassohn.

Irrespective of the clinical type of ossification of the choroid, the histological examination reveals a spongy structure, similar to the clinical cases we have examined, consisting of trabeculae, circular lamellae, osteocytes, osteoblasts and conjunctival adipose tissue. Depending on the localization and the size of the tumor, the bordering tissues show secondary modifications: disorganization of the choriocapillaris layers, Bruch’s membrane fibrosis, atrophic areas, local proliferations, fibrotic or osseous metaplasia of the retinal pigment epithelium.

In 40% of the cases, choroidal osteoma evolves by increasing over variable periods of time. It may, however, register volume shrinkage or decalcification, osteoclast specific activities. Both choroidal osteoma and ossified choroidal hemangioma may develop complications of the subretinal neovascular membranes, serous retinal detachment, microcystic degeneration, changes in the retinal pigment epithelium of the atrophic type, hyperplasia or osseous metaplasia. All this may trigger reduced visual acuity.

Choroidal osteoma may associate with various types of pathology: labio-palatine clefts, myasthenia, ulcerative colitis, histiocytosis X, nevus sebaceous of Jadassohn, Stargardt’s disease, Rieger’s syndrome. None of these affections has a pathology that can be immediately related to osteogenesis.

Choroidal osteoma and ossified choroidal hemangioma are benign tumors, which do not require special treatment. Such treatment becomes necessary when visual acuity decreases because of secondary complications: choroidal neovascularization and serous retinal detachment. The treatment procedures are part of the well-known therapeutic arsenal: photocoagulation, transpupillary thermotherapy, radiotherapy carried out by different methods, dynamic phototherapy, intravitreal injections with anti-VEGF agents. Functional prognosis is favorable in certain cases. The
possibility of complications requires surveillance of these types of ocular pathology.

Conclusions

The article has examined comparatively three eye diseases that have in common the ossification of the choroid: (1) choroidal osteoma, an osseous tumor, (2) diffuse choroidal hemangioma, a vascular tumor of the choroid with secondary ossification, and (3) a case of total ossification of the choroid in a phthisic eye, caused by an old eye injury. The diagnosis of these diseases involves the use of a large array of tools and methods of investigation, in a context where patients are asymptomatic but risk gradual or sudden vision loss due to retinal or hemorrhagic complications. The mechanisms of choroidal ossification are largely unknown, but specialists agree on the role played by chronic choroidal inflammation, post-traumatic neurogenic inflammation, bone morphogenetic proteins, and adult pluripotent stem cells with potential for multilineage differentiation. Knowledge of the clinical aspects of the ossification of the choroid and its pathogenic mechanisms is necessary for differential diagnosis of affections of the posterior ocular pole and for the prevention and treatment of potentially invalidating complications.

References


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