

Calcium deposits in the cornea: histopathological study

Depósitos de cálcio na córnea: estudo histopatológico

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ABSTRACT

Objective: To study, through histopathological examination, calcium deposits in the cornea and its different presentation forms. **Methods:** Observational, transversal, retrospective and descriptive study of corneal buttons from penetrating keratoplasty from 2006 to 2015. Routine staining was performed with Hematoxylin-eosin, and in some cases additional staining was performed: Masson, PAS (Periodic Acid Of Schiff reaction) and reticulín. The tissues were examined with an optical microscope by the authors. We selected only the cases in which calcium deposits were identified in the histopathological examination. After the cases were selected, a chart review was carried out looking for information about sex, age and transplantation etiology. **Results:** Twelve cases were included. The different forms of corneal calcium deposits presentation found in the histopathological examination were granules, plaques and oval formations or their associations. The main form of deposition were isolated or associated granules in 9 (75%) cases, followed by isolated or associated plaques in 8 (66.66% cases). The most frequent association was granule + plaque in 5 (41.66%) cases, followed by granules + oval formations in 2 (16.66%) cases. From the findings in the histopathological examination, a classification and a possible evolution of the corneal calcium deposits was proposed. **Conclusion:** Through histopathological examination we demonstrate the different forms of calcium deposits presentation in the cornea and propose a possible evolution for them. Oval formations were described as a new presentation form for these deposits, which remain demanding a better understanding.

Keywords: Calcium deposits; Cornea/histopathology; Keratoplasty

RESUMO

Objetivo: Estudar, através do exame histopatológico, os depósitos de cálcio na córnea e suas diferentes formas de apresentação. **Metodos:** Estudo observacional, transversal, retrospectivo e descritivo de botões corneanos provenientes de ceratoplastia penetrante no período de 2006 a 2015. Coloração de rotina foi realizada com Hematoxilina-eosina, tendo sido realizadas em alguns casos as colorações adicionais: Masson, PAS (Periodic Acid of Schiff reaction) e reticulina. Os tecidos foram examinados com microscópio óptico pelos autores. Selecionamos apenas os casos em que foram identificados depósitos de cálcio no exame histopatológico. Após selecionados os casos, foi realizada revisão de prontuários em busca de informações sobre sexo, idade e etiologia do transplante. **Resultados:** Foram, então, incluídos 12 casos. As diferentes formas de apresentação dos depósitos de cálcio corneanos encontradas no exame histopatológico foram grânulos, placas e formações ovaladas ou suas associações. A principal forma de depósito foram os grânulos isolados ou associados em 9 (75%) casos, seguido pelas placas isoladas ou associadas em 8 (66,66% casos). A associação mais frequente encontrada foi de grânulos + placa em 5 (41,66%) casos, seguida por grânulos + formações ovaladas em 2 (16,66%) casos. A partir dos achados no exame histopatológico, foi proposta uma classificação e uma possível evolução dos depósitos de cálcio corneanos. **Conclusão:** Através do exame histopatológico, demonstramos as diferentes formas de apresentação dos depósitos de cálcio na córnea e, para eles, sugerimos uma possível evolução. Formação ovaladas foram descritas como uma nova forma de apresentação desses depósitos, que permanecem, porém, necessitando de uma melhor compreensão.

Descritores: Depósitos de cálcio; Córnea/histopatologia; Ceratoplastia

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The authors declare no conflicts of interests.

Received for publication 02/08/2017 - Accepted for publication 02/10/2017.

INTRODUCTION

Corneal calcifications have traditionally been divided into two distinct types: keratopathy in the calcified range, and calcareous degeneration of the cornea.⁽¹⁾ Band keratopathy, on the other hand, may be calcified or spheroidal. The calcified type usually occurs secondary to chronic ocular disease or abnormal metabolism of calcium or phosphorus.⁽²⁾ Non-calcified band keratopathy, as well as advanced spheroidal degeneration or urate keratopathy,⁽³⁾ was identified as collagenous elastomeric degeneration, and is still known by several alternative terms,⁽⁴⁾ but we will not go into details about it, since our study deals with calcium deposits in the cornea.

Band keratopathy, calcium salts on Bowman's membrane, is described as the most classic form of corneal calcification seen in clinical practice. Calcareous degeneration of the cornea, deposition of calcium involving total corneal thickness, is considered rare. Histopathological examinations of corneal buds have also shown that calcareous degeneration and band keratopathy may coincide in some cases.⁽⁵⁾ Calcaneal degeneration of the cornea is generally seen in association with neoplasms or phthisis, but has been described in patients with persistent epithelial defects and dry eye⁽⁵⁻⁸⁾, patients with AIDS,⁽⁹⁾ graft disease versus host,⁽¹⁰⁾ patients treated with topical retinoic acid⁽¹¹⁾, and phosphate-containing eye drops.⁽¹²⁾

Several stages of what in this work is called corneal calcification⁽¹³⁾ seem to be described. Calcifications may consist of small concentrically laminated spheres that may merge and form areas of solid plaques. Calcification here refers to the mineralization of the Bowman's layer and of the entire corneal stroma.⁽¹³⁾ Histopathologically, in band keratopathy, thin basophilic granules are first seen at Bowman's level, and these granules can coalesce.⁽⁴⁾

A possible division of the calcifications in stages, according to our understanding, would seem an attempt to classify the calcium deposits in the cornea. The term corneal calcification was found to be used as a synonym for calcium deposit in the cornea,⁽¹⁾ as well as to refer to the mineralization of the Bowman's layer and all corneal stroma.⁽¹³⁾ As previously mentioned, corneal calcifications are traditionally divided into two distinct types: keratopathy in the calcified range, and calcareous degeneration of the cornea.⁽¹⁾ Calcified band keratopathy has been reported for calcium deposits at the Bowman's layer^(3,5) and calcareous degeneration levels, for calcium deposits involving all corneal thickness.⁽⁵⁾ A work on primary calcareous degeneration of the cornea, however, reports that, in calcareous degeneration of the cornea, calcium carbonate and phosphate are deposited as granules in the superficial layers of the cornea.⁽¹⁴⁾ Thus, in our view, a clearer nomenclature and classification of calcium deposits in the cornea seem necessary.

We carried out a study on calcium deposits in the cornea through histopathological examination, describing their different forms of presentation and possible developments. From there, we will try to present a possible new classification.

METHODS

We carried out an observational, cross-sectional, retrospective and descriptive study of corneal buds from penetrating keratoplasty in the period between 2006 and 2015, sent for histopathological examination at Banco de Olhos do Hospital Geral

de Fortaleza. During this period, 5651 transplants were performed, and the corneal buds coming from emergency keratoplasty are received for histopathological examination as routine at Bancos de Olhos. Cases in need of further clarification on the diagnosis by the surgeons are also received. Thus, blades of 943 cases were examined.

The tissues had first been sent to the pathology department where they were fixed in neutral formol 10% and trimmed. The inclusion was done in paraffin, with subsequent cuts of 2µm, and routine staining was performed with Hematoxylin-eosin. For better evaluation and comparison, in some cases additional staining was performed: Masson trichome, PAS (Periodic Acid of Schiff reaction) and reticulin. After preparation, the authors examined the tissues under an optical microscope.

We selected only the cases in which calcium deposits on the cornea could be identified by histopathological examination. After selecting the cases, the medical records were reviewed in search of information about gender, age and transplantation etiology.

As a retrospective and descriptive study was performed, we did not use probability statistics. The different forms of presentation of calcium deposits in the cornea in the histopathological examination are described, as well as their possible development. A new classification is suggested based on the findings.

RESULTS

We included 12 cases of calcium deposits in the cornea, of which 5 were from female patients and 7 from male ones. The ages ranged from 27 to 83 years, with the average being 58.67 ± 17.12 (average $\pm$ SD). The etiologies of the transplants of each case can be found in table 1.

Table 1
Distribution of the cases of corneal calcium deposits studied by the authors according to the transplant etiology

Transplant etiology	Number of cases
Perforation	2
Bullous keratopathy	1
Dystrophy	1
Leukoma	2
Primary failure	1
Band keratopathy	1
Descemetocoele	2
Ulcer without response to treatment	1
Others	1
Total	12

The different forms of presentation of the corneal calcium deposits found in the histopathological examination were granules (Figure 1), plaques (Figure 2), and oval formations or their associations (granules + plaque - Figure 3, granules + oval formations - Figure 4, and plaque + oval formations - Figure 5). We call plaque the consolidated basophilic formation of irregular margins. Oval formations are the roundish consolidated formations of well-defined margins with staining ranging from basophilic to slightly eosinophilic. The distribution of these findings in the cases is found in figure 6 and in table 2.

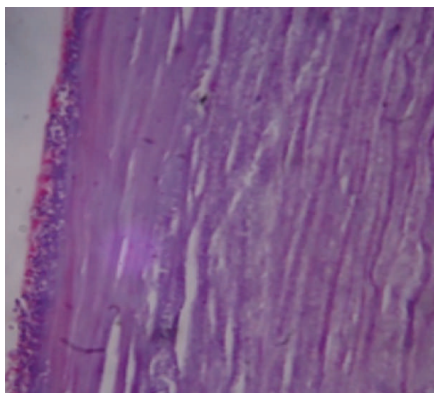


Figure 1: Presence of basophilic granules of superficial calcium and in middle stroma. (Ximenes, Vasconcelos and Monte – parafin, H.E. – 100x).

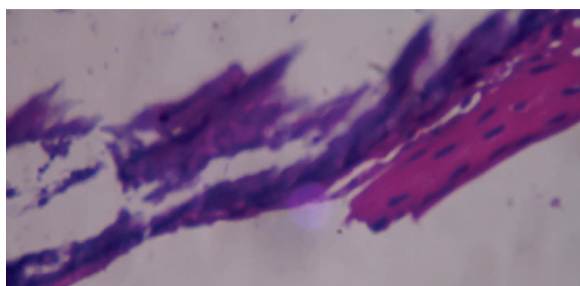


Figure 2: Presence of calcium plaque below the epithelium and in de-epithelialized area. (Ximenes, Vasconcelos and Monte – parafin, PAS – 400x).

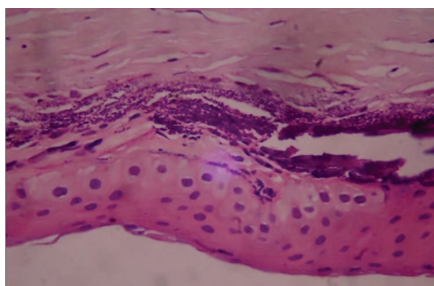


Figure 3: Presence of plaque above and granules below, both on the surface and cornea. (Ximenes, Vasconcelos and Monte – parafin, H.E. – 400x).

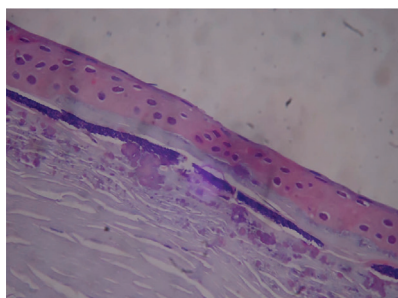


Figure 4: Presence of granules above and oval formations below, both on the surface and cornea studied. (Ximenes, Vasconcelos and Monte – parafin, H.E. – 400x)

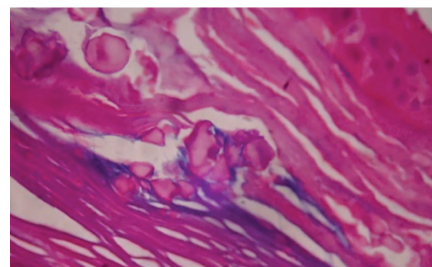


Figure 5: Presence of oval formations associated with calcification plaque in the same case. (Ximenes, Vasconcelos and Monte – parafin, PAS – 400x).

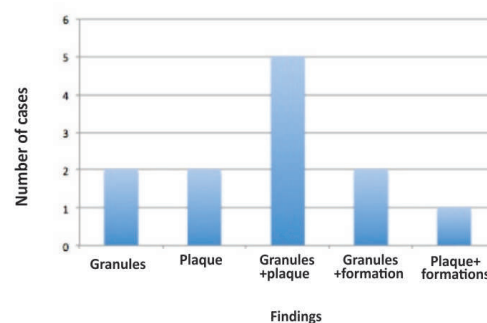


Figure 6: Distribution of the cases studied according to the different forms of presentation of corneal calcium deposits found in the histopathological examination.

Table 2
Distribution of the different forms of corneal deposits found in the histopathological examination in the cases studied.

	Case											
	1	2	3	4	5	6	7	8	9	10	11	12
Forms of Deposit												
Granules	x	x	-	x	x	x	x	x	x	x	-	-
Plaque	x	-	x	-	-	x	x	x	x	-	x	x
Oval formations	-	-	x	x	x	-	-	-	-	-	-	-

(x) = presence of the form of corneal deposit; (-) = absence of form of corneal deposit

The cases were classified according to the different forms of calcium deposits found in the histopathological examination. The cases where we found only granules were called keratopathy. Band keratopathy associated to calcification was the cases having granules associated to plaques, and band keratopathy associated to oval formations presented granules associated to these formations. Cases of pure calcification had only plaques, and plaques associated with these formations were found in calcifications associated to oval formations. The distribution of cases according to the classification proposed is found in table 3.

Table 3
Classification of the cases studied according to the form of calcium deposits found in the histopathological examination

Classification proposed	Number of cases
Pure band keratopathy	2
Band keratopathy + Calcification	5
Band keratopathy + oval formations	2
Pure calcification	2
Calcification + oval formations	1
Total	12

Some of the forms of calcium deposit found were presented differently. Some granules were larger, lighter and less basophilic, differently from what is shown in Figures 1, 3 and 4. Some plaques were smaller and lighter than those shown in Figures 2, 3, and 5. We call these smaller block plaques. The variations in the presentations of the calcium deposits observed in the histopathological examination are found in figure 7.

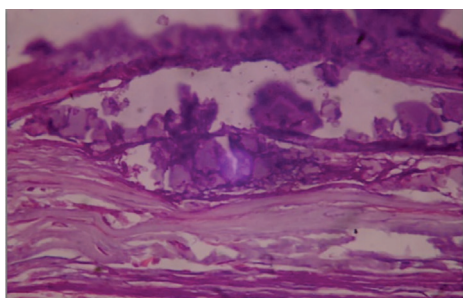


Figure 7: Variations in the presentation of calcium deposits in the cornea. Below, we have larger, lighter and less basophilic granules. Above the granules and below the consolidated plaque, we can see smaller and less stained plaques called blocks. (Ximenes, Vasconcelos and Monte – parafin, PAS – 400x).

The presentations of the calcium deposits in the form of granules, plaques or oval formations were separated in each case according to the depth / location in the cornea, as shown in Tables 4, 5 and 6. Based on that, we classified the cases as superficial and deep, as shown in Figure 8. We highlighted some locations of granules on the cornea by means of photographs (Figures 9 and 10).

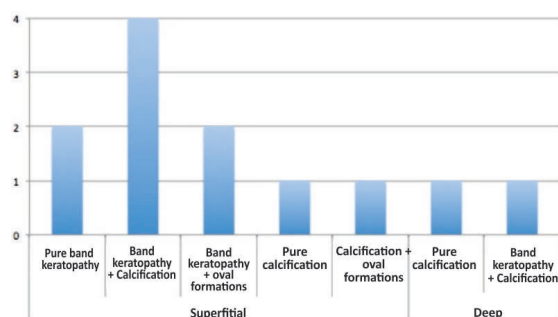


Figure 8: Distribution of the cases studied between superficial and deep according to the location of the calcium deposits found in the histopathological examination.

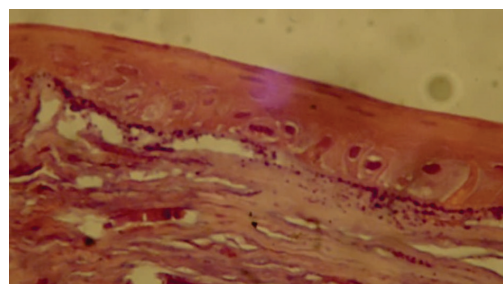


Figure 9: Granules under the basement epithelium and anterior stroma. (Ximenes, Vasconcelos and Monte – parafin, H.E. – 400x).

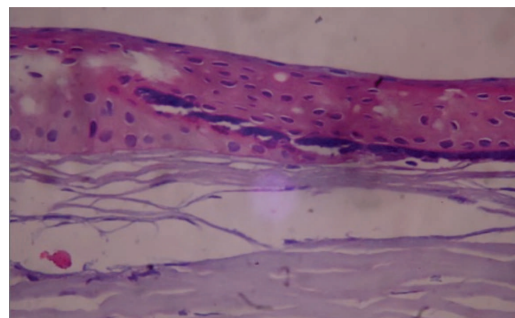


Figure 10: Granules in the corneal epithelium. (Ximenes, Vasconcelos and Monte – parafin, PAS – 400x).

Table 4
Distribution of cases that had granules in histopathological examination according to their location on the cornea.

	Case											
	1	2	3	4	5	6	7	8	9	10	11	12
Location												
Under basement	x	-	-	-	x	-	-	-	-	-	-	-
Epithelium	x	-	-	-	x	-	-	-	-	-	-	-
Bowman	-	x	-	-	x	-	-	-	x	-	-	-
Anterior stroma	x	-	-	x	x	x	x	x	x	x	-	-
Middle stroma	-	-	-	x	-	-	-	-	-	x	-	-
Posterior stroma	-	-	-	-	-	-	-	-	-	-	-	-

(x) = presence of the form of corneal deposit; (-) = absence of form of corneal deposit

Table 5
Distribution of cases of plaques in histopathological examination according to their location on the cornea

	Case											
	1	2	3	4	5	6	7	8	9	10	11	12
Location												
Under basement	x	-	-	-	-	-	-	x	x	-	-	-
Epithelium	-	-	-	-	-	-	-	x	-	-	-	-
Bowman	-	-	-	-	-	-	-	-	-	-	-	-
Anterior stroma	x	-	x	-	-	x	x	x	x	-	x	-
Middle stroma	-	-	-	-	-	-	-	-	-	-	x	-
Posterior stroma	-	-	-	-	-	-	-	-	-	-	x	-

(x) = presence of the form of corneal deposit; (-) = absence of form of corneal deposit

Table 6
Distribution of cases of oval formation
in histopathological examination according
to their location on the cornea

Location	Case											
	1	2	3	4	5	6	7	8	9	10	11	12
Under basement	-	-	-	-	-	-	-	-	-	-	-	-
Epithelium	-	-	-	-	-	-	-	-	-	-	-	-
Bowman	-	-	-	-	-	-	-	-	-	-	-	-
Anterior stroma	-	-	x	x	x	-	-	-	-	-	-	-
Middle stroma	-	-	-	x	-	-	-	-	-	-	-	-
Posterior stroma	-	-	-	-	-	-	-	-	-	-	-	-

(x) = presence of the form of corneal deposit; (-) = absence of form of corneal deposit

DISCUSSION

We studied the different forms of presentation of calcium deposits in the cornea in the histopathological examination. According to the etiology of the transplants, as shown in table 1, there was a certain equivalence between cases occurring in inflammatory diseases of the eye (perforation, descemetocoele and ulcer without response to treatment) in 5 (41.66%) cases and non-inflammatory changes (bullous keratopathy, dystrophy, leukoma, primary failure, band keratopathy) in 6 (50%) cases. Our findings are in accordance with the literature describing corneal calcifications in patients with ocular inflammatory diseases, with high serum calcium, following ocular procedures⁽¹⁾, in addition to the primary⁽¹⁴⁾ and family⁽²⁾ forms. In cases secondary to local disease, calcium deposits are extracellular. In cases of systemic diseases such as hyperparathyroidism, hypophosphatemia or Fanconi's syndrome, the deposits shall be intracellular.^(2,15) In our study, we did not have access to information on the presence of systemic diseases, since we used information of medical records from Banco de Olhos.

The main forms of deposit found in the histopathological examination were isolated or associated granules in 9 (75%) cases, followed by isolated or associated plaques in 8 (66.66% cases), as shown in table 2 and figure 6. The same table and figure also show isolated granules and plaques found in the same number of cases (2 cases). The most frequent association was granules + plaque in 5 (41.66%) cases, followed by granules + oval formations in 2 (16.66%) cases. Oval formations were found only in association with granules (2 cases) or plaque (1 case). According to the findings in the histopathological examination, we created a classification. The presence of granules or plaque determined what we call respectively band keratopathy or calcification and which, when isolated, shows the pure form. For oval formations we did not give a specific name, since we did not find reports in the literature about this finding. Most of the cases corresponded to the association between band keratopathy + calcification, as shown in table 3. When considering pure forms or associations, band keratopathy was slightly more frequent in 9 (75%) cases in relation to calcification in 8 (66.66 %) cases, which could be inferred when we previously mentioned the findings. Literature also mentions that band keratopathy is the most classic form of

calcification of the cornea seen in clinical practice⁽⁵⁾. We emphasize, however, that our classification differs from that usually found in other works. As previously mentioned, corneal calcifications have traditionally been divided into two distinct types: keratopathy in the calcified range, and calcaneous degeneration of the cornea.

⁽¹⁾ The term corneal calcification was found to be used as a synonym for calcium deposit in the cornea,⁽¹⁾ as well as to refer to the mineralization of the Bowman's layer and all corneal stroma. ⁽¹³⁾ Calcified band keratopathy has been reported for calcium deposits at the Bowman's layer^(3,5) and calcareous degeneration levels, for calcium deposits involving all corneal thickness.⁽⁵⁾ Arora et al. mention that in band keratopathy the calcium deposits are located beyond the Bowman's layer, in the basal epithelial membrane and superficial stroma.⁽²⁾ In our classification we did not use the criterion of deposit depth, but rather the different forms of presentation of these deposits in the cornea.

We have also previously mentioned possible stages of what is called corneal calcification in a study, where it is mentioned that calcifications may consist of concentrically-laminated small spheres which may converge and form areas of solid plaques. Calcification here refers to the mineralization of the Bowman's layer and the entire corneal stroma.⁽¹³⁾ Band keratopathy also proposes that the basophilic granules first seen at the Bowman's level may coalesce.⁽⁴⁾ At this moment, our findings also allows us to propose a possible evolution for the different forms of deposit: from the earliest (granules), which would consolidate into plaques, and which would arrange better to create the oval formations (Figure 11). The development of granules into plaques would be in accordance with the literature previously mentioned in this paragraph. The finding in our study of various forms of association of calcium deposits in the cornea would also strengthen our proposal. In order to better explain the oval formations, we suggest that it could become a later level of organization of the plaques, which would become more uniform and with a less basophilic staining (Figures 4 and 5). The aspect found in the oval formations would still remember the ossification found in other ocular tissues. Some examples of intraocular ossification are in the crystalline,^(16,17) choroid,⁽¹⁸⁾ retina, all parts of the posterior segment, and the most frequent site is the retinal pigment epithelial region and the inner surface of the choroid. ⁽¹⁷⁾ To assert the existence of corneal ossification, however, further, more detailed studies would be required. We also note that in none of our cases have we found the three stages we have proposed. Cases in which we found the association granules + plaque may not yet have evolved to oval formations; those of granules + formations may already have their plaques evolved to oval formations; and those of formations + plaques could have had granules already consolidating plaques. This would be a possible explanation, and another would be development without passing through the three stages proposed.



Figure 11: Suggested development for different forms of corneal deposit.

CONCLUSION

Depósitos de cálcio na córnea foram estudados através do exame histopatológico. Suas diferentes formas de apresentação foram demonstradas e uma possível evolução sugerida. Formação ovaladas foram descritas como uma nova forma de apresentação dos depósitos de cálcio na córnea, mas permanecem necessitando de estudos posteriores para uma melhor compreensão.

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