Dermal perifollicular changes in Poodles are not always associated with mineralization.

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Short running title: Hair follicle changes in poodles

Abstract

Background - It is commonly accepted that canine dystrophic mineralization of the hair follicle glassy membrane can be seen in hyperadrenocorticism and as a senile change of poodles. Pathology textbooks define this change as deposition of calcium salts in form of basophilic, amorphous, granular material along collagen fibrils. The aim was to evaluate whether the incidence of the lesion is specific of poodles and if it is always associated with calcium deposition.

Animals - One-hundred-forty-seven dogs divided into three groups: 1) 91 normal poodles; 2) 40 dogs of other breeds; 3) 16 dogs with clinical/histopathological diagnosis of hyperadrenocorticism.

Methods – Retrospective study; Haematohylin and Eosin and Von Kossa staining.

Results - Our finding demonstrate that perifollicular changes of the hair follicle glassy membrane of poodles are not always associated with mineralization.

Conclusions and clinical importance – A specific staining is needed to differentiate true mineralization when examining poodles skin.

Introduction

Dermal perifollicular mineralization was first reported as a normal finding of miniature poodles in 1984.[1] As reported, the lesion consists of deeply basophilic deposits in dermal collagen immediately surrounding occasional hair follicles. More specifically, the deposits are located in the glassy membrane which surrounds the external root sheath. The mentioned work reported that the mineralized material detected in 22 out of 29 (76%) female toy poodles, stained black with the von Kossa staining procedure for calcium and bright blue with the Prussian blue stain for iron. X-ray microanalysis of the mineral deposits indicated that they were composed of calcium, iron and phosphorus. Authors reported that in the examined tissues mineralization prevailed in telogen follicles and in only one instance it was recorded in the anagen phase. The mentioned data arose from a larger study whose aim was to determine safety and efficacy of mibolerone (a non progestational androgenic steroid), in 227 dogs of seven different breeds that lasted 9.6 years.[2] Since dermal perifollicular mineralization was recorded only in poodles, the conclusion was that this is a normal finding in elderly animals belonging to this breed.[1] Since then, dermal perifollicular mineralization was never investigated and the reference texts for veterinary dermatology report this lesion (as dystrophic mineralization) as a breed specific finding related to ageing.[3,4] Dermal perifollicular mineralization is also a common finding in dogs with naturally occurring or iatrogenic Cushing syndrome.[4]
To evaluate the incidence of this lesion we performed a retrospective study in normal poodles, poodles with clinical/histopathological diagnosis of hyperadrenocorticism and normal dogs of different breeds.

Materials and methods

One-hundred-forty-seven (147) dogs were included in the study; animals were divided into three groups: 1) 91 normal poodles (regardless of their size); 2) 40 dogs of other breeds; 3) 16 poodles with clinical/histopathological diagnosis of hyperadrenocorticism. Skin samples were obtained from paraffin embedded blocks of the department histopathology service. Ethical committee authorization was not necessary since archival samples were used.

Inclusion criteria were: Group 1 (poodles): presence of a skin biopsy presenting an area of normal skin with at least 5 follicular units; Group 2 (other breeds): as for group 1; Group 3: poodles, clinical/histopathological diagnosis of hyperadrenocorticism.

Two serial sections were obtained from each skin block. The first one was stained with Haematoxylin and Eosin (H&E) while the second was stained for calcium deposits with the Von Kossa (VK) staining procedure, using a commercial kit in order to assure standardization and reproducibility of data (cat. 04-170801, Bio-Optica Milano SpA, Milan, Italy). Samples were thus evaluated for the presence/absence of dermal perifollicular mineralization in the glassy membrane of the hair follicle surrounding the external root sheath, and classified as either positive or negative, respectively. Results obtained by two independent investigators were compared and conflicts were resolved by re-examination of the slide.

For statistical purposes, animals from group 1 were arbitrarily divided in two sub-groups to evaluate the incidence of dermal perifollicular mineralization with aging: A) animals younger than 6 years (n=17); B) animals older than 6 years (n=74). Animals belonging to group 2 and 3 aged between 6 and 14 years old and were thus comparable to sub-group 1B. Data obtained within the two staining methods were compared using the Fisher’s exact test.

Results

H&E stained samples showed the presence of perifollicular changes ranging from a slight thickening of basophilic appearance (figure 1a) to evident basophilic granular deposits (figure 1b) to mineralized spicules (figure 1c) within or surrounding the hair follicle glassy membrane; its presence was different among experimental groups. In group 1A, 59% of animals (10/17) were classified as positive while the remaining (7/17) were devoid of changes. Elderly animals, belonging to group 1B showed a significant increase of the presence of the lesion (88%, 65/74) with only 9/74 animals (12%) being negative. Group 2 (animals of other breeds) did not show a significant presence of the lesion since only 7/40 animals (17%) were recorded as positive. Group 3 showed a significant presence of deposits (75% of positives, 12/16). Results are summarized in figure 1d.

The VK staining showed the same distribution pattern observed with H&E, appearing as brown/black deposits of calcium (Figure 2a, b, c). Group 1A showed 24% of positive cases (4/17) while 1B showed 30% of positives (22/74). Group 3 showed instead 94% of animals recorded as positives (15/16). VK results are summarized in figure 2d.
H&E staining showed statistical differences between group 1A and 1B (young vs elderly poodles); group 2 (animals of other breeds) was statistically different from group 1B and not from 1A. The VK stain showed that groups 1A and 1B are not different for calcium deposits. An high statistical difference was observed between groups 1B and 3, this latter composed of dogs with confirmed diagnosis of hyperadrenocorticism.

Discussion

H&E staining showed group 2 to be different from group 1b but not from group 1a: from these findings it is thus possible to conclude that the described lesion observed in poodles is related to the breed and increases with age. Results emanating from the VK staining showed that groups 1A and 1B are not different for calcium deposits thus leading to the conclusion that the lesion commonly regarded as "mineralization" is not always confirmed by a specific staining.

The sensibility of the VK staining in this setting is confirmed by the high statistical difference observed between groups 1B (young poodles) and 3 (poodles with hyperadrenocorticism).

Our results demonstrate that dermal perifollicular changes observed histologically in biopsy samples from poodles are not always associated with mineralization. The basophilic material may thus account for a different alteration of for a precocious lesion where calcium deposits are not yet visible by VK stain.

A similar lesion affecting vibrissae was considered as an early biomarker of disease in ABCC6$^{-/-}$ mice,[5] this mentioned gene being involved in aberrant ectopic mineralization.[5-7] ABCC6 appears among a number of genes associated with human photoreceptor degeneration, a disease that may have a counterpart in poodles, namely progressive rod and cone dystrophy.[8,9] Further studies are thus needed both to characterize the lesion and to evaluate whether a genetic background is present.

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Legends for figures

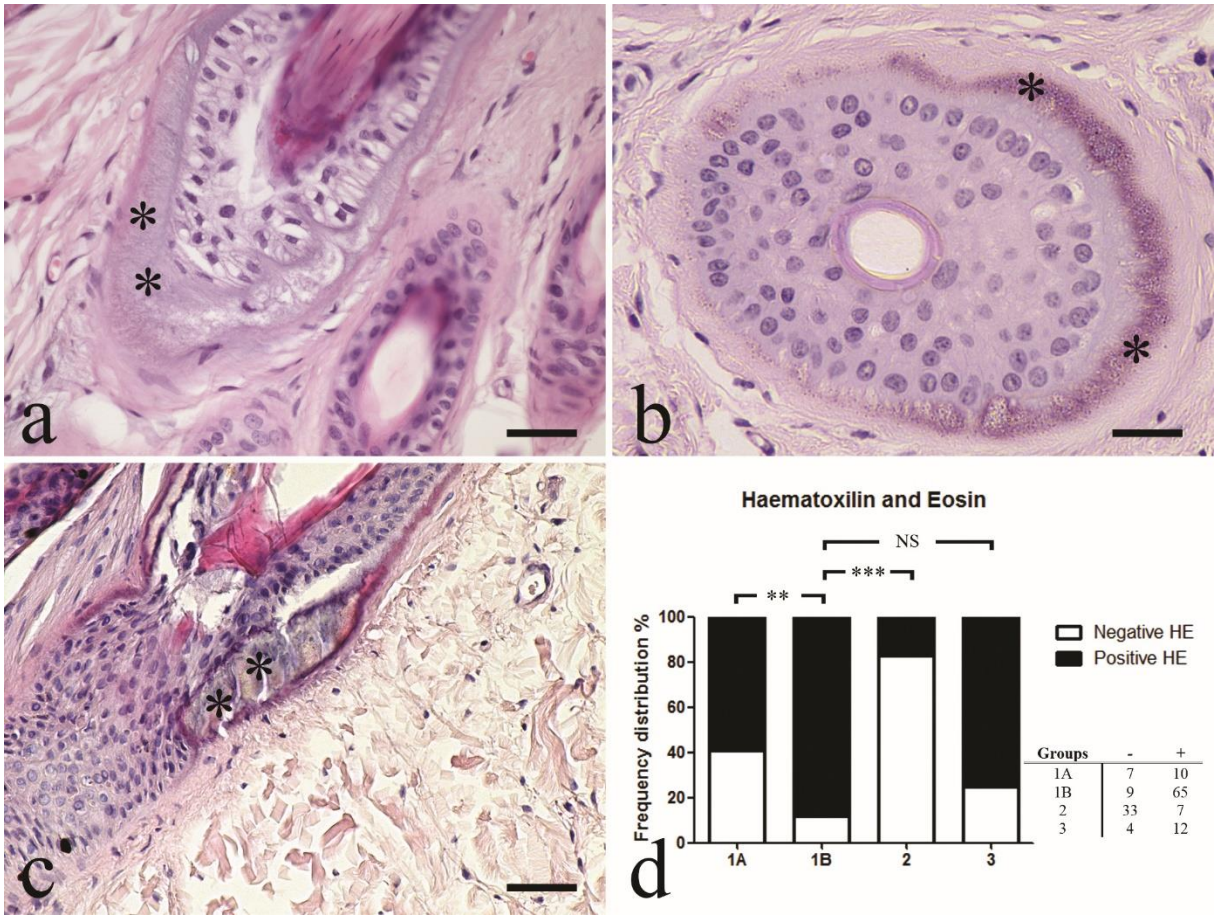

Figure 1: photomicrographs of Haematoxylin and Eosin stained samples to show different appearance of the perifollicular lesion, indicated with asterisks: a) basophilic thickening of the follicular glassy membrane; b) basophilic granular deposits; c) amorphic material in form of spiculae; d) frequency distribution of the lesion: **= $p<0.001$, ***= $p<0.0001$, NS= non-significant. Scale bars are equal to 25 μm in a and b and to 50 μm in c.

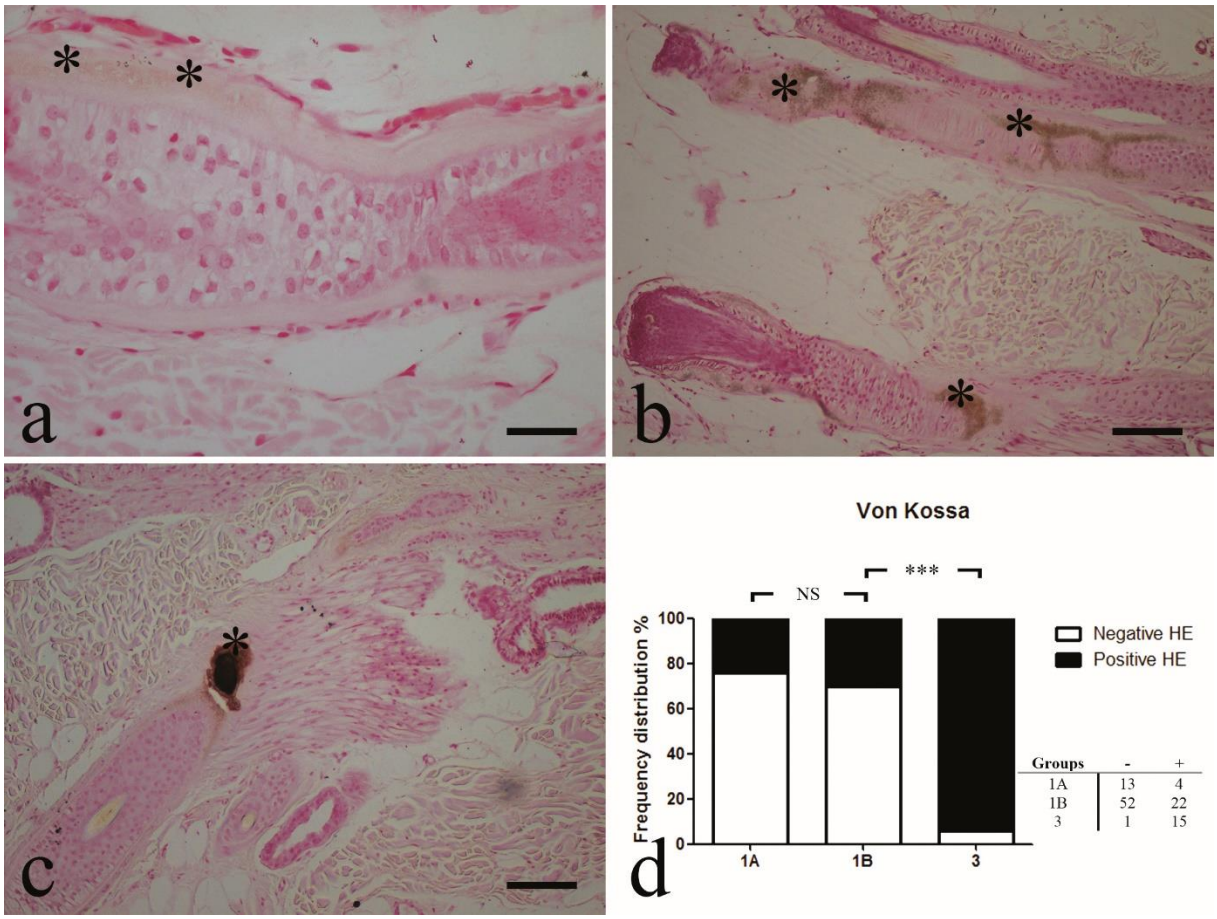

Figure 2: photomicrographs of Von Kossa stained samples to show different appearance of the calcium deposits, indicated with asterisks: a) minimal calcium deposition; b) moderate calcium deposition; c) evident mineralization of a lesion appearing as a spicula; d) frequency distribution of calcium deposits: ***= $p<0.0001$, NS= non-significant. Scale bars are equal to 25 μm in a and 100 μm in b and c.