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RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

Cellular evaluation of hair follicle and epidermal tumours in dogs and cats: a histopathological and immunohistochemical study

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Abstract

The aim of this study was to identify patterns of epidermal and hair follicular tumours in dogs and cats. A total of 50 samples were collected from skin lesions. Hair follicular tumours were examined with routine histological method but the malignant epidermal tumours were examined with immunohistochemical technique. Grading for all of the mentioned markers was as follows: not stained (-), stained less than 10% (1+), stained 10-50% (2+), and stained more than 50% (3+). According to the immunohistochemical staining results, regardless of the type of animal, P53 expression levels in samples of basal cell carcinoma were (1+) in one sample and (2+) in three, while all cutaneous lymphosarcoma samples were (2+). In addition, CK8 expression levels in samples of basal cell carcinoma were (1+) in three samples and (3+) in one sample. Ki67 expression levels in the cutaneous lymphosarcoma samples were (1+) in two samples and (2+) in one sample. Finally, CD99 expression levels in all cutaneous lymphosarcoma samples were (2+). Combined diagnostic panels such as CK8/P53 markers for basal cell carcinoma and P53/Ki67/CD99 markers for cutaneous lymphosarcoma diagnosis are useful diagnostic methods in dogs and cats.

Keywords: Dogs; cats; epidermal tumors; hair follicular tumors; immunohistochemistry

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Introduction

Skin is continuously exposed to a wide variety of chemical and physical insults and other environmental factors, and therefore, it is prone to neoplastic proliferation. In dogs, approximately 30% of all neoplasms are reported to arise in the skin. Skin tumours are generally classified histologically according to the tissue of origin (epithelial cell and mesenchymal cell), along with individual cells of origin (round and spindle cells) if sufficient differentiation is present. Tumours are further classified in terms of the degree of malignancy based on several histological characteristics such as the mitotic index and degree of cellular or nuclear atypia (Janes et al., 2002; Goldschmidt et al., 2005; Bidur et al., 2007). Adnexal tumours of the skin are very common in dogs, whereas they are relatively rare in cats and humans. The World Health Organization (WHO) classifies canine tumours with adnexal differentiations into follicular tumours, nail-bed tumours, sebaceous and modified sebaceous gland tumours, apocrine and modified apocrine gland tumours, and eccrine (atrichial) tumours (Goldschmidt et al., 1998; Janes and Hutter, 2002; Rezaie and Tavassoli, 2012). The development of adnexa is a result of an intimate interaction between basal and mesenchymal cells. Basal cells become the germinative cells of the hair follicle and mesenchymal cells become follicular papilla. Follicular stem cells are considered to be predominantly located in a specific region of the

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external root sheath, especially the bulge region, and they are capable of giving rise to hair follicles, epidermis, sebaceous glands, and apocrine sweat glands (Goldschmidt et al., 2005). Follicular tumours show differentiation to hair follicular structures. There is marked variability in the histopathologic appearance of these tumours, and there may be some variability within a single tumour when examined by light microscopy. This variability is due to the complex histology of the normal hair which is mimicked by these tumours showing hair follicular differentiation. During embryologic development of the skin and adnexal structures, the pillar germ emerges at an angle from the underside of the epidermis. Under the influence of inductive mesenchymal cells, which will form the papillae of the hair, the cells extend into the deep dermis (Haghdoost, 1997). Current diagnostic techniques, like special and differential tissue staining, accurate molecular tests, immunohistochemistry and other tests could be applied effectively to achieve an exact diagnosis. The goal of this survey was the identification of common epidermal and follicular tumour patterns in pets (dogs and cats). In the present study, histomorphological features and the expression of different monoclonal and polyclonal antibodies in adnexal tumours (follicular tumours) and epidermal tumours in dogs and cats were investigated. In addition, the usefulness/efficacy of different antibodies in the evaluation of histogenesis and characteristic features of adnexal tumour differentiation were determined.

Materials and Methods

A total, 50 skin lesions suspected of being tumours or pseudotumor lesions from 47 dogs (94% of total samples) and 3 cats (6% of total samples) were collected from "Parsa pet clinic/laboratory section/No.2, Bistochaharmetri Blvd., Sa`adatabaad, Tehran/1998699116-IRAN" which included divers species and mix-breeds of both sexes and all ages from April 2008 to July 2010. This sample size is high given current conditions in our country, because of the small population of pets. Samples, after a gross and clinical evaluation, were collected at the margin between the lesion and intact region from different locations such as the head and neck, trunk, and limb extremities. With regards to the visual form of the cutaneous lesion, the full thickness was collected in 10% neutral buffered formaldehyde containers. After fixation, the samples were dehydrated by mean of an automatic tissue processor and embedded in paraffin, before cutting into 5 micrometer slices with a rotary microtome, in addition the samples were stained with a routine Hematoxylin and Eosin method. Malignancies or suspected cases of malignancy were examined with the immunohistochemical method for P53, Ki67, CK8 and CD99 (DakoCytomation®, Denmark). Semi-quantitative evaluation for IHC markers was conducted as follows: P53 had a brown nucleus (uniform nucleus staining), Ki67 base with diffuse nuclear staining (nuclear stained non-uniform with diffuse spots), CK8 base on perinuclear staining (space around nuclear membrane in the cytoplasm), and CD99 base on perinuclear staining (space around nuclear membrane in the cytoplasm), and more cytoplasmic membrane stained. Grading for all of the mentioned markers was as follows: not stained (-), stained less than 10% (1+), stained 10-50% (2+), and stained more than 50% (3+). Graphs were designed with Excel and means of age factors were calculated. The present study was based on sample collection (case series), a descriptive study-therefore quantitative statistical analysis was unattainable.

Results

In the present study, 50 skin samples for the detection of hair follicle tumours and epidermal tumours were evaluated through histopathology from 47 dogs and 3 cats. Eight samples (16% of total samples) corresponded to eight different patterns, from 12 follicle tumour patterns as follow: infundibular keratinizing acanthoma (IKA), tricholemmoma-bulb type (TLB), trichoblastoma-trabecular type (TBT), trichoblastoma-ribbon type (TBR), trichoblastoma-granular cell type (TBG), trichoblastoma-medusoid type (TBM), trichoepithelioma (TE), malignant trichoepithelioma (MTE). Of the samples, only TBT were present in both cat and dog. In the present study, each pattern raised 6.66% of the total tumours. Mean age of the affected dogs was 6.5±3.06 years. There were no samples obtained from tricholemmoma-infundibular (TLI), trichoblastoma-spindle (TBS), pilomatricoma (PM) and malignant pilomatricoma (MPM) patterns. The remaining seven tumours were basal cell carcinoma (BCC) from four samples (8% of total samples) {1 cat and 3 dogs}, and cutaneous lymphosarcoma (CL) from three samples (6% total samples) {1 cat and 2 dogs}. These results have been expressed as pie histograms (Diagrams 1, 2, 3 and 4). In most of the sampled cases, a series of non-specific clinical signs like focal alopecia combined with pruritus and redness were observed. In addition, other non-specific alterations like thickness and hyper-pigmentation, wound and hemorrhage, oedema, ulceration and necrosis, nodular forms of skin were also observed.

Characteristics of tumour in hair follicle

Infundibular keratinizing acanthoma (IKA) type tumour had a central pore towards the skin surface from a previously existing follicular infundibulum. The pore

was filled with hard keratinized material and accumulation in the central lesion area, creating the red-brown area seen in the dermal and hypodermal regions. In a microscopic view, the tumour infundibulum was covered with stratified keratinized squamous epithelium and the cytoplasm of these cells contained keratohyalin granules. Under the keratin layer, large keratinocytes with pale cytoplasm occasionally contained basophilic keratohyalin granules. Compact fibres of connective fibrils and islands of squamous epithelium with central keratin accumulation and accumulations of fibrovascular stroma surrounded the tumour with penetration to the compact tumour wall were observed (Fig 1a). Tricholemmoma-bulb (TLB) type tumours constituted islands of epithelial cells surrounded by a fibrocollagenous stroma, and these cells had a central nucleus with medium eosinophilic cytoplasm, with peripheral epithelial cells arranged like a fence on the thick eosinophilic basal lamina (Fig 1b). In a microscopic view of the trichoblastoma-trabecular (TBT) type, a multilobar pattern of neoplastic cells was demonstrated which was surrounded by delicate bonds of inter-lobular collagen stroma, and perilobular fence like cells, although the central lobular cells had an oval to elongated nucleus with a large amount of eosinophilic cytoplasm (Fig 1c). The trichoblastoma-ribbon (TBR) type was composed of elongated bonds strongly attached to each other and scattered with 2 or 3 cell thick branches that exhibited a fence like appearance. Tumour cell nucleus tumours were prominent with scarce cytoplasm. Nucleus with normochromatic or hyperchromatic and nucleolus were unclear (Fig 1d). Trichoblastoma-granular cell (TBG) type tumours; the neoplastic cell islands were little dense with obvious cytoplasmic membranes and large amounts of eosinophilic granular cytoplasm and small, hyperchromatic nuclei. Few cells were observed in the mitosis phases (Fig. 1.e). One prominent specification of the TBM pattern is the scattered cellular fibres from the central cellular accumulation with large eosinophilic cytoplasm scattered peripherally, and this pattern was in a medusoid form. A dense fibre eosinophilic stroma with low cellularity filled spaces was seen around these cellular fibres, and the scattered cellular fibres in comparison to the peripheral stroma, appeared basophilic (Fig. 1.f). Histological alterations were observed in trichoepithelioma (TE) created islands of neoplastic cells that were surrounded with collagen or in some regions mucinous stroma. In the centre of these islands, keratin and ghost cell accumulation were observed, and the presence of these cells is evidence of tumour differentiation to the hair matrix. Outer epithelial cells constitute heterogeneous populations of: cells with pale eosinophilic cytoplasm and vesicular nuclei (e.g., cells at the lower part of the outer root sheet), small cells with hyperchromatic nuclei and

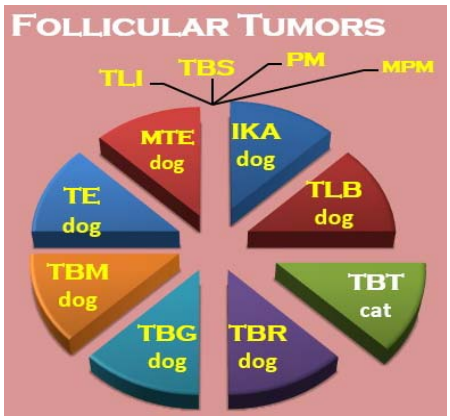

Diagram 1: Frequency of follicular tumours in this study

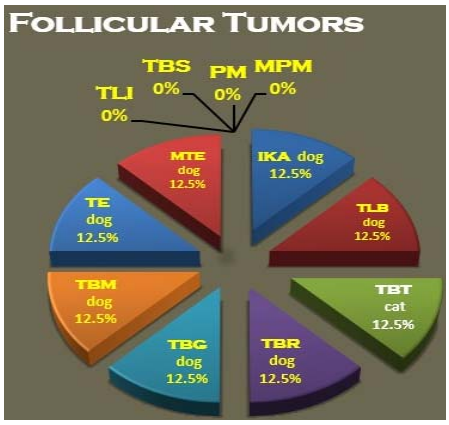

Diagram 2: Relative frequency of follicular tumours in this study

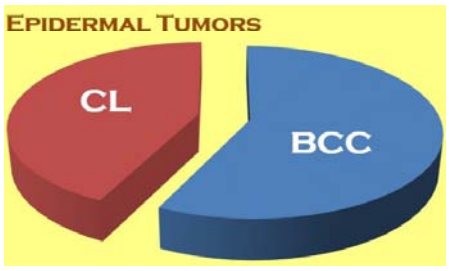

Diagram 3: Frequency of epidermal tumours in this study

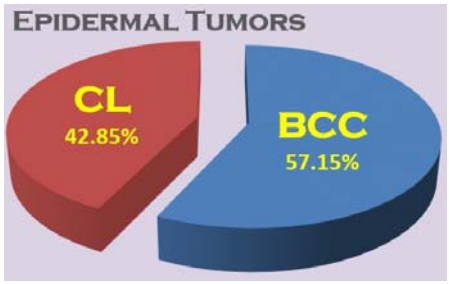

Diagram 4: Relative frequency of epidermal tumours in this study

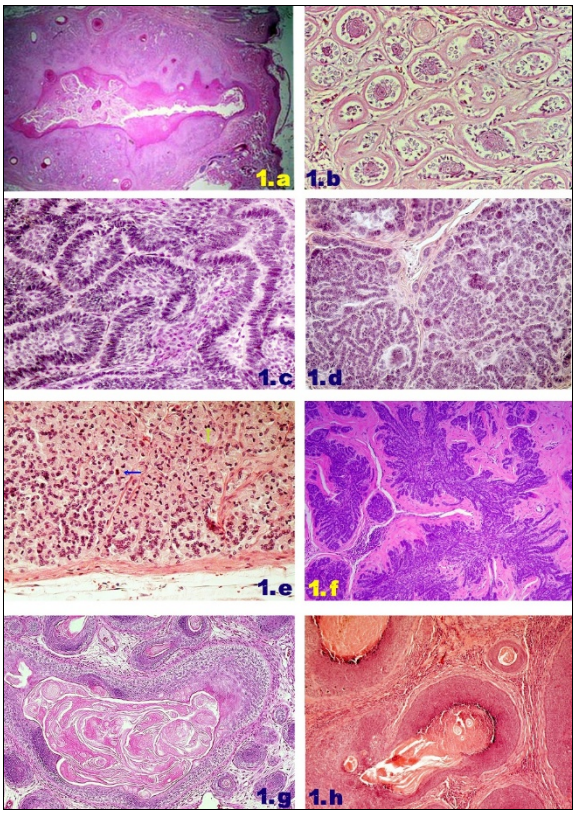

Fig. 1: 1a. IKA in a female 4.5 years-old mix-breed dog (H&E, X40); 1b. TLB in a male 4 years-old terrier dog (H&E, X100); 1c. TBT in a male 3.5 years-old mix-breed cat (H&E, X100); 1d. TBR in a male 2 years-old terrier dog (H&E, X100); 1e. TBG in a male 11 years-old terrier dog. Blue arrow shows metaphase and green arrow shows completed mitosis. (H&E, X100); 1f. TBM in a male 6 years-old German-Shepherd dog (H&E, X40); 1g. TE in a female 10 years-old mix-breed dog (H&E, X100); 1h. MTE in a female 8 years-old mix-breed dog (H&E, X40)

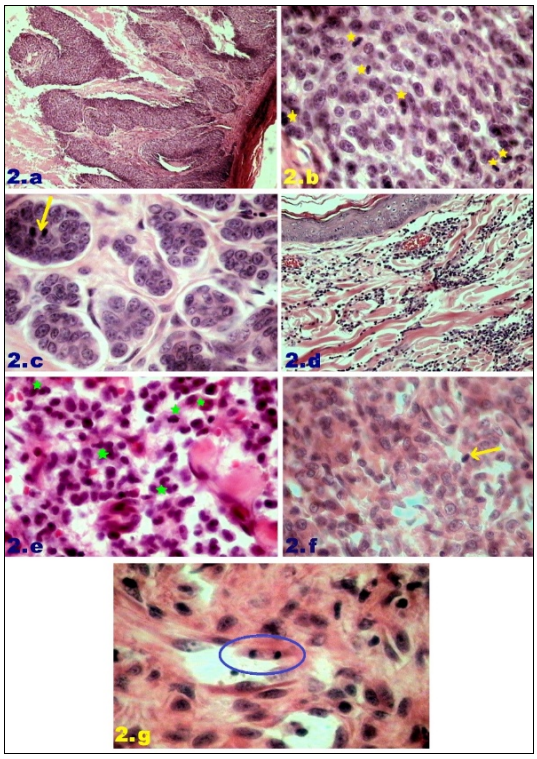

Fig. 2: 2a. BCC in a male 6 years-old terrier dog (H&E, X40) /2b. BCC in a female 2 years-old Persian cat. Pleomorphism with several mitotic figures (yellow pluses). (H&E, X400) / 2c. BCC in a male 8 years-old collie dog. Pleomorphism with a telophase (yellow arrow). (H&E, X400) / 2d. CL in a male 5 years-old terrier dog. Leukocyte infiltration into epiderm. (H&E, X40) / 2e. CL in a male 4 years-old cat. Pleomorphism with several mitotic figures (green stars). (H&E, X400) / 2f. CL in a male 10 years-old terrier dog. Pleomorphism with a metaphase (yellow arrow). (H&E, X400) / 2g. CL in a male 5 years-old terrier dog. Pleomorphism with a telophase (H&E, X100).

sparse cytoplasm (e.g., hair bulb undifferentiated cells), and cells containing cytoplasmic keratohyalin granules (e.g., inner root sheet cells) (Fig. 1.g). Malignant trichoepithelioma (MTE) with nodular infiltrated accumulation to the dermis and hypodermis were observed. Bundles and islands of basophilic cells with connection to the epidermal covering and follicular infundibulum, penetrated into the dermis. In the centre of larger tumour cell islands, accumulations of ghost cells with hyperchromatic nucleus and pale eosinophilic cytoplasm which are a sign of matrix keratinization were apparent (Fig 1.h).

Characteristics of epidermal tumors in our study

In total, four samples from a total of 50 samples corresponded to BCC (1 cat & 3 dogs) and three samples to CL (1 cat & 2 dogs). Mean age of the affected dogs with epidermal tumors in the present study were 6.7±4.08 years old and the mean age of the affected dogs with BCC was 6.16±2.05 years old, moreover all cases was male. The only affected cat was a 2 year-old female Persian cat. Mean age of the affected dogs with lymphosarcoma was 7.5 years and all cases were male. The only affected cat was a male 4-year-old domestic cat. Major changes in the BCC samples in this present study were generally the same as the common BCC pattern, and consisted of large cells with hyperchromatic nucleus in a striated pattern that were surrounded by a foundation ofthick fibrous connective tissue. Some cases had multifocal compact cells with fewer cells than in the margins of the cell foci, multiple rows of cells in a ladder shape arrangement and inner cells located in an irregular pattern. In some cases, several necrotic regions were

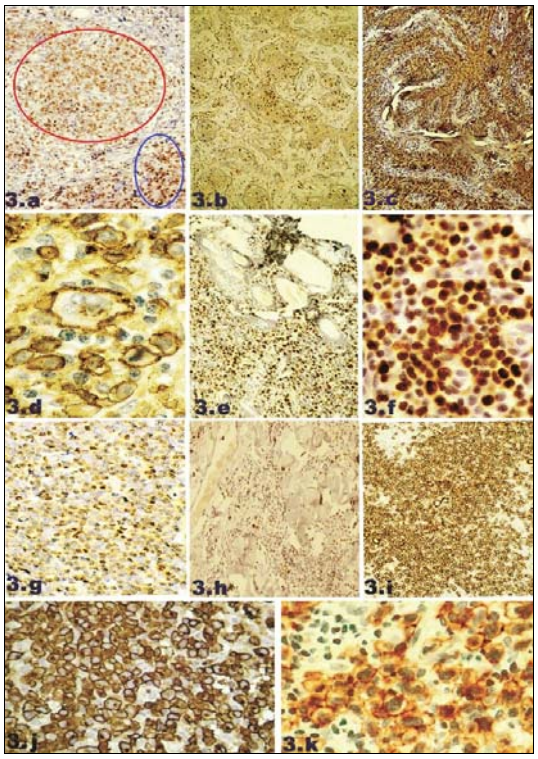

Fig. 3: 3a. BCC in dog. P53 (2+). Some nuclei (region showed by red line) are low density staining in comparation with other areas (region showed by blue line) (X100) / 3b. BCC in dog. P53 (1+). Lower nuclear density staining in comparation with previous sample (X40) / 3c. BCC in cat. CK8 (3+). High cytoplasmic density staining (X40)3d. BCC in dog. CK8 (2+). Moderate cytoplasmic density staining (X400) / 3e. CL in dog. P53 (2+). Moderate nuclear density staining (X100) / 3f. CL in cat. P53 (2+). Moderate nuclear density staining (X400) / 3g. CL in cat. Ki67 (1+). Diffuse nuclear density staining (X100) / 3h. CL in dog. Ki67 (1+). Moderate diffuse nuclear density staining (X40) / 3i. CL in dog. CD99 (2+). High cell membrane density staining (X40) / 3j. CL in dog. CD99 (2+). High cell membrane density staining (X400) / 3k. CL in cat. CD99 (2+). High cell membrane density staining (X400).

observed in the lower dermis. With high magnification, pleomorphism and mitotic forms were also observed (Figs 2.a, 2.b and 2.c). Major microscopic findings in the lymphosarcoma samples were neoplastic lymphocytes that consisted of differentiated small cells, including large histiocytoids that were diffused or in small groups which had invaded into the dermis and epidermis (Fig 2.d). The epitheliotropic nature of lesions was the criterion for diagnosis of the tumor epitheliotropic forms in two cases, as mitosis is very delicate and in some microscopic fields, mitosis was not observed. In these samples, at higher magnification, uniform diffusion of basophilic cells was the only finding. However, with more precise observation, the presence of lymphocyte populations with large numbers of mitotic forms was observed (Fig 2.e). In one of the previously mentioned samples, neoplastic lymphocytes were mixed in considerable numbers with normal lymphocytes, plasma cells and histiocytes, thus making the real neoplastic nature of the lesion difficult to determine, therefore the existing pleomorphisms (Figs 2.e, 2.f and 2.g) and large number of mitotic forms were used to make an exact diagnosis (Figs 2.e and 2.f).

Based on immunohistochemical staining results - regardless of the animal species - expression of P53 in BCCs were (1+) in one case, and (2+) in three cases, although in all cutaneous lymphosarcoma they were (2+). In addition, expression of CK8 were (1+) in three cases of BCC samples, and (3+) in one case. Expression of Ki67 in cutaneous lymphosarcomas were (1+) in two cases and (2+) in one case. Finally, expression of CD99 in cutaneous lymphosarcomas were (2+) in all samples. Results of the immunohistochemistry with regard to sex and species of animals are shown in Figure 3 with Tables 1-4.

Discussion

Results of an epidemiological study reported by Goldschmidt et al. (2005) were based on tumours that had differentiated from hair follicles. They focused their attention on the age and sex of the animals (healthy males and females, and sterile females), location and the different breeds of affected dogs (Goldschmidt et al., 2005). Scott et al. (2008) in a retrospective study on 80 hair follicle neoplastic samples from 1986 to 1987, stated that 5.3% of all skin tumours in dogs are comprised of these types of hair follicle tumours (Scott et al., 2008). In the present study, the IKA pattern was found in a 4.5 year-old female dog. Only dogs suffer from this kind of tumour pattern (Goldschmidt et al., 2005). Bidur et al. (2007) reported 21 cases (2.9%) of IKA pattern from the overall skin tumour samples of 748 dogs (Bidur et al., 2007). Akhtardanesh et al. (2009) reported an irregular diffuse hyperkeratinized acanthoma pattern in a cat that was similar to IKA (Akhtardanesh et al., 2009). Concerning trichoepithelioma (TE), there are no complete studies and few reports or retrospective studies existing. In this study, the TE pattern was seen in one 10 year-old mix-breed female dog. TE is a benign tumour that shows differentiation to all three parts of the hair follicle and incomplete or failed trichogenesis can be seen. TE has a high prevalence in dogs, it is not that common in cats and rarely diagnosed in other species. This pattern is most often seen in dogs in the 1-15 year-old range, but the greatest prevalence is seen between 5-9 years of age (Goldschmidt et al.,

Table 1: P53 and Ck8 expression in BCC samples

	Ck8	P53
dog	2+	1+
dog	2+	2+
dog	2+	2+
cat	3+	2+

Table 2: P53, Ki67 and CD99 expression in CL samples

	CD99	Ki67	P53
dog	2+	2+	2+
dog	2+	1+	2+
cat	2+	1+	2+

Table 3: Expression Level of P53 and Ck8 in BCC samples

Expression level marker	(3+)	(2+)	(1+)	**(-)**
P53	-	3	1	-
Ck8	1	3	-	-

Table 4: Expression Level of P53, Ki67 and CD99 in CL samples

Expression level marker	(3+)	(2+)	(1+)	**(-)**
P53	-	3	-	-
Ki67	-	1	2	-
CD99	-	3	-	-

2005). Scott et al. (2008) reported a 78.8% TE prevalence, which is a very high result (Scott et al., 2008). However, it is notable that there was no differentiation between benign or invasive patterns of TE tumours in Scott's study. Trichoblastoma is a benign tumour which origins from a growing hair root or differentiates from it. This tumour previously known as a basal cell tumour; is now called a trichoblastoma in most of the scientific literature. This tumour is prevalent in dogs and cats, not common in horses, and rare in other species. Occurrence of this tumour is most often seen in dogs 4-9 years-of-age (Goldschmidt et al., 2005). The trichoblastoma-ribbon type (TBR) in this study was related to a 2 year-old male terrier. Ashrafi Halan (2005) reported TBR in the one rabbit case (Ashrafi Halan, 2005). The only case of a trichoblastoma-medusoid (TBM) type in this study related to one 6 year-old German-Shepherd dog. There is a great deal of similarity between TBM and TBR, by way of the cellular columns from the central cellular mass, with large areas of eosinophilic cytoplasm scattered out to peripheral areas. The highest incidence of this type of tumour is observed in dogs (Goldschmidt et al., 2005). The granular trichoblastoma sample in this study belonged to an 11 year-old male terrier. The trabecular trichoblastoma pattern in this study related to one 3.5 year-old mix-breed domestic male cat. This type of tumour pattern has a high incidence in cats (Goldschmidt et al., 2005). Bidur et al. (2007) in their study, without specifying this particular form of tumour, reported 15 cases (2.01%) overall in 748 skin tumours cases in dogs (Bidur et al., 2007). The tricholemmoma-bulb type (TLB) sample in this study belonged to a 4 year-old male terrier. This type of tumour is uncommon in dogs and rare or not reported in other species (Goldschmidt et al., 2005). Bidur et al. (2007) without identifying the particular form of TLB, reported two TLB (0.2%) cases from an overall sample of 748 skin tumours in dogs (Bidur et al., 2007). Diters and Goldschmidt (1983) reported tumour cases with a high resemblance to tricholemoma in six dogs. Low prevalence (1.2%) of tricholemoma was reported in a retrospective study by Scott et al. (2008) on 80 dogs without differentiation between the various forms of TLB (Scott et al., 2008). Malignant trichoepithelioma in this study related to one 8 year-old mix-breed female dog. This irregular skin tumour type has only been described in dogs, and an association between age, sex, or species, with the occurrence of this tumour has not been recorded (Goldschmidt et al., 2005). Bidur et al. (2007) reported one (0.13%) malignant trichoepithelioma case from 748 skin tumor cases in dogs. In a 42-month retrospective survey by Bidur et al. (2007) carried out on cutaneous tumours found in dogs in South-Korea, from a total of 2 952 biopsies of tumour samples in different dog species, a total of 748 cases of diverse skin tumours were identified, and 74 cases were epidermal with follicular tumours (9.9%). The average age of the dogs in the Bidur survey was 6.5±3.06 years (Bidur et al., 2007). In total, seven cases from the 50 samples in present study related to epidermal tumours and the average age between the affected dogs was 6.44±2.74 years. Basal cell carcinoma is one of the most common skin tumours found in dogs and humans, and while it is relatively uncommon in cats, it is even rarer in other domestic animals. On the basis of previous retrospective researches, the average age of BCC occurrence in dogs is almost seven years old and there is a higher prevalence in males than females (Hargris and Ginn, 2007). BCC lacks differentiation to the epidermal and adnexa of the epidermis, and morphologically it resemble normal epidermal basal cells. Cats and dogs within the 3-14 year-old range are more likely to be affected (Goldschmidt et al., 2005). From a total of four BCC samples in this present study, one case concerned a cat and three were related to dogs. The average age of the dogs affected with BCC in this study was 5.86 ± 3.36 years-of-age and it had a higher prevalence in females than in males. Lymphomas/lymphosarcomas are compact, multicellular masses that are usually located in the lymphatic organs (Ashrafi Halan, 2005; Hargris and Ginn, 2007). Out of three cutaneous lymphosarcomas discovered in this study, one case concerned a cat and two others related to dogs. Average age of the affected dogs with cutaneous lymphosarcoma

in this study was 7.5 ± 0.29 year. Investigations involving mutations of the P53 gene in more than 10, 000 tumours revealed that the possibility of mutation in P53 was substantially related to the tissue that the tumour originated from. For example, in lung cancer, the prevalence of a mutation of the P53 gene is more than 75%, although in mammary tumours it is 30%, and in leukemia the mutation is less than 5%. The relationship of the P53 gene is being investigated in a great range of human tumours. In some cancers 50% of tumors had a mutation of the P53 gene with 87% of mutations appearing in exons 5 to 8 (Rezaie and Tavassoli, 2012; Sasani et al., 2013). Mutation of the P53 gene in various animal tumours is reported to be similar in cutaneous squamous cell tumours, mammary tumours in dogs, lymphoma, colon and lung cancer, osteosarcoma, and mast cell tumours in cats. A study on the palpebral SCC of dog cases revealed a 68% expression of P53 in the samples. Although a study on the 15 palpebral SCC tumors showed that 10 tumours were positive for P53. Increased expression of P53 in conjunctival SCC tumours of cats, cattle and horses has a direct correlation with ultraviolet radiation from the sun (Sasani et al., 2013). Rezaie et al. (2012) reported the expression of P53 protein in one mix-breed Labrador dog affected with subcutaneous lymphoma (Rezaie and Tavassoli, 2012). Pena et al. (1998) investigated the correlation between expression levels of P53 and the mitotic index in dogs with mammary cancer. Results revealed that a low mitotic index has a direct correlation with lower expression of the Ki67 marker in the nucleus of the tumor cells and tumor prognosis was much better (Penna et al., 1998). Carvalho et al. (2005) investigated the expression of Ki67 in cattle eye SCC and it showed that Ki67 has a significant relationship with the histological pattern and grade of tumour cellular proliferation. With regard to these results, increased levels of the mentioned marker and cellular proliferation occurred in the tumour, differentiation of the tumour is brittle, though a significant correlation was not found between expression of this marker and the grade of tumour malignancy (Carvalho et al., 2005). Rezaie et al. (2009) demonstrated expression of Ki67, P53, c-erB2 and CD31 in mammary carcinoma in dogs (Rezaie, 2009). In the present study, simultaneous expression of P53 and Ki67 in cutaneous lymphosarcoma was detected. Apaydin et al. (2005) stated that CK1-8 is definitive for BCC occurrence (Kooy et al., 1995; Apaydin et al., 2005). Kooy et al. (1995) highlighted the special role of CK8 in the detection of 91% of BCC in samples sent to the laboratory (Kooy et al., 1995). Kooy et al. (1996) through results obtained with electron microscopy techniques confirmed the role of CK8 in situ carcinoma (Kooy et al., 1996). Yamamoto and Asahi (1999) investigated several cytokeratins including CK8, and they found that expression of these markers was useful for differentiating BCC from trichoblastic fibroma and trichoepithelioma. CD99 is a beneficial marker for differentiating neuroblastoma from small round cell tumors. A survey of human lymphoma and lymphosarcoma showed a clear reaction with CD99. Moreover, CD99 is useful for the detection of leukocytes. This marker is recommended for differentiating lymphocytes from other mononuclear cells including plasma cells in round cell tumours (Dabbs, 2010). A review of the literature was conducted to search for comparative information about immunohistochemistry and simultaneous use of CK8 and P53 markers, singularly for BCC detection, and P53, Ki67 and CD99 together, as well as for cutaneous lymphosarcoma detection in dog and cat, however, relevant literature was not found.

Final results of the present study showed that P53 and CK8 in BCC and P53, Ki67 and CD99 in cutaneous lymphosarcoma detection in dogs and cats is a beneficial diagnostic panel, and can be used for future studies. Numerous skin lesions, especially tumours associated with the current study, may possibly occur in diverse animal species, but these animals often do not receive accurate examination or samples sent to pathology laboratories. It is important to determine the type and exact name for these tumours using accurate methods in order to achieve a base for the discipline that can be recorded in the literature (Goldschmidt et al., 2005). This kind of discipline makes the results of this study and other similar studies more useful. Briefly, the total number of tumours, their causes, and prevalence rates, are not known, and many direct or indirect factors result in tumours, on the other hand some factors cause differentiation of the histologic patterns. Finally with regard to diversity in species susceptibility such as that found in cats and dogs, we recommend that future broad surveys be done, elimination of extraneous factors, and taking into account factors like age, sex and species. The present study was constrained in regard to the time frame and local limitations; in addition it also had economic limitations, and unfortunately lacked the flexibility to produce more complete results.

Our research team recommended that by means of immunohistochemical techniques and markers, including animal antibodies in particular and improved investigation design, it would be possible to discover the exact prevalence, aetiology and possibly therapeutic methods for cutaneous tumours. Further comprehensive and accurate investigations on tumour patterns in the future with regard to certain variables and elimination of bias factors, in combination with clinical and visual findings, would produce a more comprehensive view of the aetiology, along with the total and relative prevalence of these lesions.

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