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# Spontaneous mixed type trichoblastoma in a pet lop rabbit: immunohistochemical application of CK7, CK20 and CD10 in animal trichoblastoma

Amir Amniattalab[1*] and Amin Rezazadeh[2]

[1] Associate Professor, Department of Pathology, Faculty of Veterinary Medicine, Urmia Branch, Islamic Azad University, Urmia, Iran
[2] DVSc Student of Veterinary Surgery, Department of Clinical Sciences, Faculty of Veterinary Medicine, Urmia Branch, Islamic Azad University, Urmia, Iran

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## Abstract

Trichoblastomas are the most common benign cutaneous tumors in rabbits. Since, these neoplasms have various sub-types; therefore they may be confused with other similar tumors having basaloid epithelial cells constituents. A male five-year-old mixed lop-eared rabbit had a palpable two-lobulated firm and motile oval mass located under the right ear between the mandible and neck measuring 3×1.5×1 cm without ulceration. The general condition of the rabbit was good and there was no fever or abnormality in the leukogram. A grayish-white mass was fixed in 10% buffered formalin after surgical excision. Pathologic observations of Hematoxylin and Eosin, Periodic Acid Schiff and Masson's trichrome stained sections from the mass confirmed a tumor consisting of typical basaloid epithelial cells in both ribbon and trabecular (mixed) features with stromal connective tissue. Moreover, immunolabeling of the tumor was performed by CK AE1/AE3, CK7, CK20, CD10, Vimentin, S100 and Ki67. Based on pathology and immunohistochemistry, a diagnosis of mixed type trichoblastoma was made for the mass. Immunohistochemically, there was a strong CK AE1/AE3 expression (>50% of basaloid tumor cells), strong immunolabelling (>50%) of tumor-associated stromal trabecular connective tissue against vimentin and weak positive immunoreaction (1-10% of tumor stromal cells) against S100. Also, the tumor was confirmed as a weak proliferative by Ki67. On the other hand, the expression of CK7, CK20 and CD10 was 30-40% (moderate), 20-30% (moderate) and <10% (weak) in tumor cells respectively. Accordingly, using CK7, CK20, and CD10 with other markers can be useful in differentiating animal trichoblastoma from similar tumors such as basal cell carcinoma, trichoepithelioma or other tumors that originate from basaloid epithelial cells of hair follicles.

**Key words**: Rabbit, Trichoblastoma, CK7, CK20, CD10

## Introduction

Rabbits are popular animals that kept as companion pets (Von Bomhard et al., 2007). Up to now, epithelial non-viral induced skin tumors have been reported as sporadic and limited cases in rabbits (Martino et al., 2017). In fact, the most reported cutaneous tumors were related to those kept under laboratory conditions in rabbits (Von Bomhard et al., 2007). Today some families may have one rabbit as a pet animal. Since their owners are emotionally bond to their pets, the owners are very interested in fully caring for them. Therefore, the diseases of pet rabbits especially oncology are important for the veterinarians who work in exotic pet

[*] **Corresponding Author**: Amir Amniattalab, Associate Professor, Department of Pathology, Faculty of Veterinary Medicine, Urmia Branch, Islamic Azad University, Urmia, Iran
E-mail: a.amniattalab@iaurmia.ac.ir

medicine (Kanfer and Reavill, 2013). According to the increasing popularity of rabbits to keep as pets occurrence of tumors has had an upward trend in the animal's life span (Hammer et al., 2011). Until now most publications of cutaneous tumors have been associated with spontaneous or virally induced neoplasia in laboratory rabbits (Von Bomhard et al., 2007). Since keeping the rabbits as companion pets have been increased these integumentary neoplastic diseases more happened because of getting longer life span (Hammer et al., 2011). Skin diseases of rabbits can be classified as infectious, traumatic, behavioral, parasitic, and neoplastic (Jenkins, 2001). On the other hand, the main integumentary-related neoplasms are virally induced (such as shope fibroma and papilloma) and non-viral tumors in rabbits (Von Bomhard et al., 2007). In this respect, cutaneous non-viral neoplasms can be classified as epithelial and mesenchymal tumors. Trichoblastoma is considered a tumor that originates from primitive epidermal germ cells of hair follicles in embryonic development. This is the most common benign skin tumor in the rabbit but due to having various sub-types, it is confused with other tumors of basal cell origin in the skin. Therefore, it seems necessary to be differentiated by immunohistochemistry from other similar tumors. Hair follicle tumors especially trichoblastoma are prevalent in the companion animals such as dogs, cats and rabbits whereas basal cell carcinoma is more common in human. Indeed, in rabbits, similar to dogs and cats, trichoblastoma are observed in three types including ribbon, trabecular and mixed. This classification is based on the proliferation rate of stromal elements and connective tissue in the tumor. For example, in ribbon type due to increasing stromal elements and limited tumor cell proliferation, the cord-like pattern will appear in the tumor cell arrangement. As increased tumor cell proliferation and their nodular, lobular, or fascicular formation will be observed trabecular type. In connection with the pathogenesis of rabbit's trichoblastoma dysregulation in embryonic trichogenesis has been postulated as the most possible hypothesis (Kok et al., 2017). Although trichoepithelioma has been described as a common benign tumor in rabbits few reports of this tumor are in the veterinary literature. Until now in Iran, only one case of cutaneous trichoblastoma has been reported in rabbits without immunohistochemical confirmation (Ashrafihelan et al., 2005). To the authors' knowledge, the present case is the first rabbit trichoblastoma that has been diagnosed immunohistochemically in Iran. Indeed, the markers CK7, CK20 and CD10 were used first time for differentiating rabbit trichoblasoma.

**Case history**

A male, intact five-year-old mixed lop-eared rabbit weighing 2.4 kg was referred to Veterinary Hospital of the University on February 2021. The animal's owner complained of a skin mass that has been formed about eight months ago so that mass growth was slow and gradual in this period. The rabbit also had not any health abnormality during this time. In the physical examination, a flappy nonpedunculated, firm, and movable mass measuring 3×1.5×1cm was found in the skin under the right ear between the mandible and neck (Fig 1). Furthermore, in clinical examination, rectal temperature (38.4°C) was in a normal range and discoloration of mucosal tissues (anemia) was not observed. According to the owner's declaration, the rabbit was in normal condition and the animal has not been impressed by another disease for five months before the formation of the mass. A complete blood count (CBC) test and differential white blood cell count were taken from the rabbit. The results of the blood test are presented in Table 1. Based on gross examination and absence of swelling or inflammation in local lymph

nodes (lymphadenitis) as well as a normal pattern of the leukogram was confirmed possible benign nature of the mass. Thus, surgical excision of the mass was suggested to the rabbit owner. After obtaining the consent of the pet owner, anesthesia was performed to remove the cutaneous mass. For this purpose, pre-anesthesia was done by Medetomidine (0.1mg/kg, iv, Dorbene vet®, Laboratorios Syva, S.A., Spain) and also ketamine (5mg/kg, iv, Alfasan, Netherland) used to induce anesthesia (Kim et al., 2004). Subsequently, anesthetic maintenance was continued with inhalation anesthesia (vapored 2% Isoflurane + 98% Oxygen) (Drager Sulla 808v, Germany). An incision was made by a scalpel on the skin where the mass located. Then, the mass was separated from the surrounding tissues (2 cm) by electrocautery. The wound was closed using simple interrupted 3-0 nylon sutures. Topical ointments 2% mupirocin (DarouPakhsh Pharmaceutical MFG co, Iran) and %1phenytoin (Kishmedipharm, Iran) were used simultaneously for postoperative care of the cutaneous wound during 7 days. The solitary well-circumscribed intradermal mass had a two-lobulated structure and 3×1.5×1 cm in size grossly. Also, the consistency of the mass was firm and its color was white-grey on the cut surface. The excised mass was placed in 10% buffered formalin for fixation. Next, the fixed tissue processed stepwise which constitutes dehydration, clearing, impregnation and paraffin embedding. The tissue sections were prepared in 5µm of thickness and stained by Hematoxylin & Eosin (H&E), Periodic Acid Schiff (PAS) and Masson's trichrome staining methods based on a routine protocol. On the other hand we used some immunohistochemical antibodies including CK AE1/AE3 (Dako, Glostrup, Denmark; 1:50), CK7 (Dako, Glostrup, Denmark; 1:500), CK20 (Dako, Glostrup, Denmark; RTU), VIM (Dako, Glostrup, Denmark; 1:150), CD10 (Dako, Glostrup, Denmark; RTU), S100 (Dako, Glostrup, Denmark; 1:800) and Ki67 (Dako, Glostrup, Denmark; 1:150). EnVision$^{+}$ Dual Link System-HRP staining protocol was used for differential diagnosis of the mass from other basaloid cell type cutaneous tumors. In summary, the dewaxed sections with 5µm of thickness was rehydrated by ethanol. Then, the sections were incubated in hydrogen peroxide for 45 min for inhibiting endogenous peroxidase activation. Subsequently, antigen retrieval was performed by 0.01M citrate buffer for 5 min. Also, %5 bovine serum albumin was considered to block non-specific binding. After washing the sections by water and Tris-buffered saline (TBS) they incubated in a dark humid room. The sections were incubated with the primary antibodies CK AE1/AE3, CK7, CK20, VIM, CD10, S100 and Ki67. Incubation by the primary antibodies was followed by rinsing the slides with phosphate-buffered saline (PBS) and treating by streptavidin horseradish peroxidase for 20 min. Thereafter, the sections rinsed with PBS and covered by diaminobenzidine for 10 min at room temperature. Finally, the slides were counterstained by Hariss's hematoxylin for 1 min. Normal portion of the rabbit skin was used as internal positive control and each primary antibody which was replaced by non-immune IgG considered as negative control as well. Immunohistochemical scoring was performed according to Kok et al. as -, negative; + (1-10%), weak positive; ++ (11-50%), moderate positive; +++ (>50%), strong positive (Kok et al., 2017). The microscopic photos were taken by an Axis camera (Model P1347, Sweden) connected to a light microscope (Olympus CX31RBSF, Philippines).

**Results and Discussion**

Histopathological and immunohistochemical results for the mass have been gathered in Figures 1 and 2 and also in Table 2.

**Table 1: Leukogram of the rabbit with trichoblastoma**

| Test | Result | Reference range | Test | Result | Reference range |
|---|---|---|---|---|---|
| HCT | 40.2 | 33-50% | WBC | 10.5 | 5.2-12.5×$10^3$/µl |
| HGB | 13.3 | 10-17.4 g/dl | Heterophils | 59 | 20%-75% |
| RBC | 5.07 | 5.1-7.9 ×$10^6$/µl | Lymphocytes | 37 | 30%-85% |
| MCV | 62.3 | 57.8-66.5 *f*l | Monocytes | 2 | 1%-4% |
| MCHC | 32.7 | 29-37 g/dl | Eosinophils | 1 | 1%-4% |
| PLT | 252 | 250-260×$10^3$/µl | Basophils | 1 | 1%-7% |


**Figure 1: Gross appearance of the rabbit's cutaneous trichoblastoma as a pedunculated, movable and oval shape mass measuring 3×1.5×1 cm located between the mandible and neck.**

**Table 2: Immunohistochemical scoring of rabbit trichoblastoma as -, negative; + (1-10%), weak positive; ++ (11-50%), moderate positive; +++ (>50%), strong positive.**

| Antibody | Clone | Dilution | Immunoreaction scoring | Immunolabbeled cells |
|---|---|---|---|---|
| CK | AE1 and AE3 | 1:50 | +++ | Basaloid cells |
| VIM | V9 | 1:150 | +++<br>++ | Fibroblasts in trabecular connective tissue<br>PMBs |
| CK7 | OV-TL | 1:500 | + | Basaloid cells |
| CK20 | Ks20.8 | RTU | ++ | Basaloid cells |
| CD10 | 56C6, Code IR648 | RTU | +<br>+ | Fibroblasts in trabecular connective tissue<br>PMBs |
| S100 | GA50461–2 J | 1:800 | + | Nonbasaloid stromal cells |
| Ki67 | MIB-1 | 1:150 | + | Basaloid cells |

Our microscopical examination showed that the tumorous mass predominantly consisted of typical basal cells arranged in ribbon and trabecular (lobular) features

(mixed type) interacting variable connective tissue. This stromal connective tissue also featured in trabecular and papillary mesenchymal bodies (PMBs) forms which were visible in ribbon areas and lobular ones respectively (Fig 2A). Regarding this, the PMBs, fibroblast clusters similar to papillary dermal cells, were more observed in the periphery of lobular areas. Papillary mesenchymal bodies (PMBs) have been described as predominant histopathologic characteristic features in rabbit's cutaneous trichoblastomas while these cellular populations (PMBs) just reported in some cases of human and canine trichoblastomas (Kok et al., 2017). These cells seem which originate from dermal papilla changes of hair follicles during their development in embryonic period (Kok et al., 2017). Masson's trichrome staining was utilized to differentiate tumor-stromal connective tissue and collagenous materials. Indeed, the distribution of connective tissue trabecular demarcations thoroughly existed in the stromal tissue. Although the existence of collagenous depositions in trabecular areas was more especially in PMBs than in ribbon ones (Fig 2B). On the other hand, the tonality and intensity of blue staining in PMBs were lesser than in trabecular connective tissue due to the lower quantity of existence of collagens in PMBs (Kok et al., 2017). In PAS stained section, a moderate positive reaction was observed in the tumor-cell structure. Herein, Peripheral basal cells of tumor cell cluster in trabecular areas and scattered cells in ribbon parts mostly had cytoplasmic glycogen and were PAS-positive while, cell clusters in PMBs, were negative against this staining method (Figs 2C and D). In this respect, the PAS-positive tumor cells may differentiate from outer root sheath of hair follicles (Kok et al., 2017).

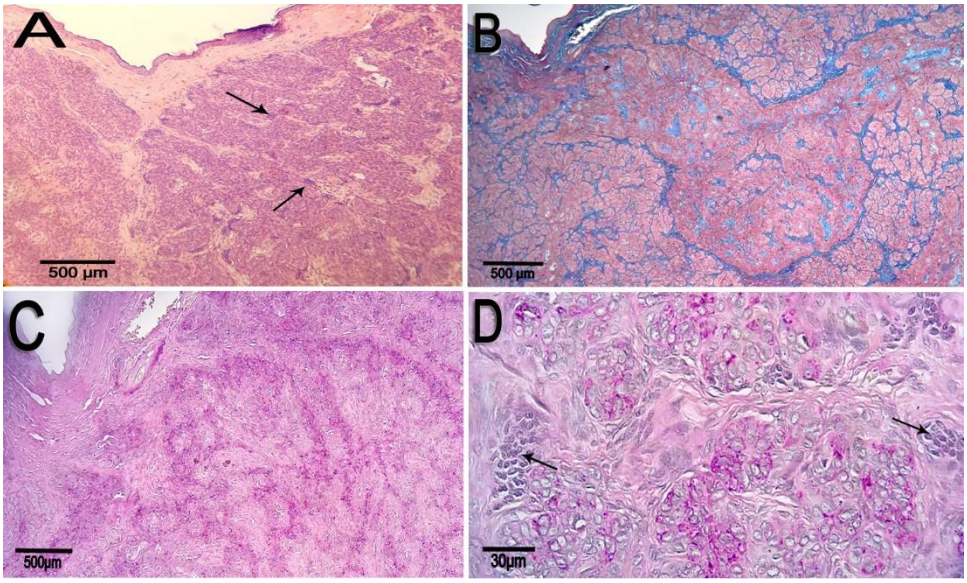


**Figure 2: Microscopic features of the trichoblastoma in the pathologic sections. A) Encapsulated dermal tumor formed in both ribbon (left side) and trabecular (right side) types as a mixed pattern of trichoblastoma. PMBs (arrow) are visible in different areas of the tumor (H&E). B) Stromal connective tissues (blue colored) representing collagenous materials in both trabecular and PMBs areas (Masson's trichrome). C) Cytoplasmic glycogen accumulation (magneta-colored particles) in the peripheral basal cells of tumor cell cluster which are more amount in trabecular areas than ribbon parts (PAS). D) Lack of presence of cytoplasmic glycogen in PMBs (arrow) against these accumulations in the basal cells of the tumor cell cluster (PAS).**

Immunohistochemical evaluation of CKs (CK, CK5/6, CK14, CK17, CK18 and CK19) were previously performed for rabbits' trichoblastomas (Kok et al., 2017). Knowing that CK7, CK20 and CD10 were used for differential diagnosis of human trichoblastomas at the prior time (Kneitz et al., 2018; Wang et al., 2015), thus we decided to utilize the antibodies CK7 and CK20 in addition to the antibodies CK, CD10, S100 and Ki67 for differentiating rabbit trichoblastoma from other similar cutaneous tumors. Diffuse strong expression of CK was notable (80-90%) in basaloid epithelial cells of the tumor (Fig 3A and Table 2). CK is a very useful marker to differentiate and determine the cell origin of trichoepithelial tumors (Kok et al., 2017). However, trichoepithelial origin of basaloid cells was confirmed due to their strong immunoreaction. CK20 immunoreaction in the mass was %20-%30 (Fig 3B and Table 2) and however, regarding differential diagnosis, CK20 immunonopositivity was visible for Merkel cells similar trichoblastoma while, this marker was negative in syringoma (Wang et al., 2015). On the other hand, immunopositive basaloid cells against CK7 were 30-40% (Fig 3C and Table 2) while PMBs and trabecular fibroblasts were negative in the current case. In connection with using CD10, most of the trabecular fibroblasts and also PMBs had positive immunoreaction but basaloid epithelial cells were negative in the present tumor (Fig 3D and Table 2). Our result is in relation to CD10 consistent with Cordoba et al. (2009) for differential diagnosis of trichoblastoma and basal cell carcinoma (BCC) that they declared CD10 positivity of epithelial basaloid cells in BCC, unlike trichoblastoma (Córdoba et al., 2009). About vimentin usage in the present case, trabecular fibroblasts had in the tumor a strong reaction and also PMBs were positive against vimentin but not the same as trabecular-fibroblasts intensity (Fig 3E and Table 2). Up to now, ribbon and trabecular types of rabbit trichoblastoma have predominantly been reported with strong positive immunoreaction to vimentin, however, the existence of variable positive immunoreaction declared for this marker (Kok et al., 2017). We also utilized S100 for our tumor to differentiate it from those tumors which originate from melanocytes, dendritic and Langerhans cells (Tacha, 2003). So, the basaloid tumor cells had no reaction to S100 and only a few stromal cells had very weak immunoreactivity (<5%) (Fig 3F and Table 2). Ki67, as a cell proliferation nuclear marker, was performed to evaluate our tumor-cell proliferating activity although cellular pleomorphism or notable mitotic figures were not observed in tumor-mass cells. Indeed, the mitotic index (MI) of this trichoblastoma was 0.7 (mean number of mitoses in 10 microscopic fields ×400). Accordingly, Ki67 positive tumor cells were found in very small numbers (<5%) (Fig 3G and Table 2) and thus confirmed benign and non-proliferative nature of the tumor. Apparently, the Ki67 expression rate in trichoblastomas varies between different species, so that, the high and low expression rate of this marker has been reported in dog and sheep trichoblastoma (about 20%) respectively (Mineshige et al., 2014; Polinas et al., 2020). On the other hand, Ki67 nuclear expression is different based on the type of constituent cellular populations of trichoblastomas. For instance, a high rate of Ki67 expression has been reported in both basaloid epithelial cells and PMBs of canine trichoblastoma (Mineshige et al., 2014) while, we observed the expression only in tumor basaloid epithelial cells.

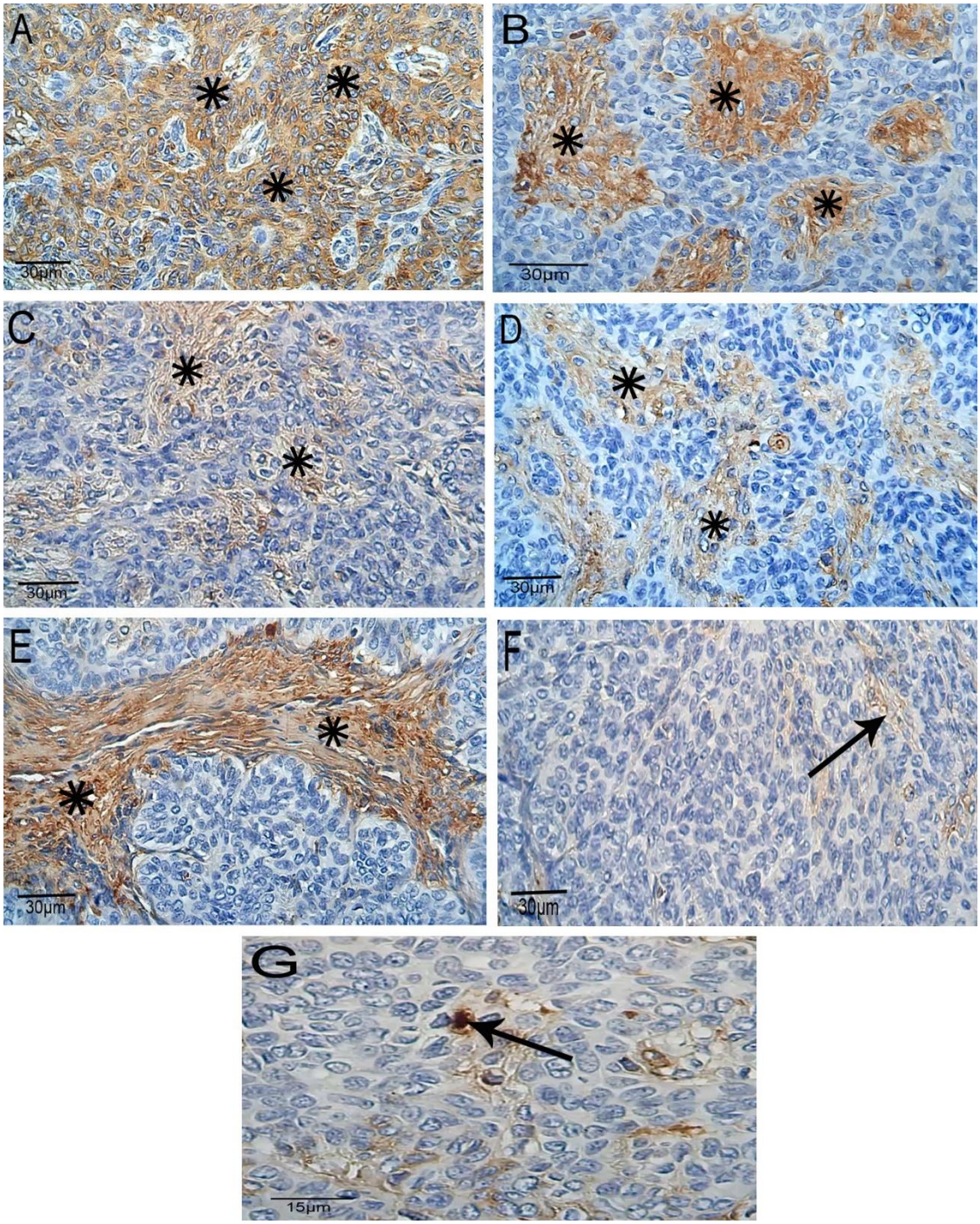


**Figure 3: Immunohistochemical labeling of rabbit trichoblastoma. A) Strong positive (>50% of tumor cells or +++) immunoreaction to CK diffusely visible in tumor cells (asterisk). B) Focal scattered immunoreaction to CK7 in basaloid epithelial cells (asterisk) of tumor. C) Moderate diffuse immunolabelling of basaloid tumor cells (asterisk) against CK20. D) Weak expression of CD10 in stromal connective tissue (asterisk) of the tumor. E) Strong vimentin expression in trabecular fibroblasts of the tumor (asterisk). F) Very weak expression of S100 in some stromal cells (arrow) of trichoblastoma. H) Very weak nuclear immunolabbeling of tumor basaloid cells (arrow) by Ki67 proliferation marker.**

There is no sex or breed susceptibility to affect rabbits to cutaneous trichoepithelioma (Kok et al., 2017). Also, surgical excision is the suggested treatment method for trichoblastoma. Post operating recurrence has rarely been reported for this

tumor. Indeed, incomplete removal of the tumor or remaining the tumor cells in the marginal tissue of the tumor can be considered a possible recurrence cause (Freitas et al., 2019). In the present case, there was no evidence of recurrence or metastasis of cutaneous tumor according to two months postoperative follow-up.

In conclusion, it seems that using immunohistochemical markers CK7, CK20 and CD10 can be useful to differentiate rabbit trichoblastoma from similar benign and malignant cutaneous tumors same as human cases.

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**Conflict of interest**

The authors have no conflict of interest to declare.

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# تریکوبلاستوم خود به خودی نوع مختلط در یک خرگوش لوپ خانگی: کاربرد ایمونوهیستوشیمیایی CK7، CK20 و CD10 در تریکوبلاستوم حیوانی

امیر امنیت‌طلب[1*] و امین رضازاده[2]

[1] دانشیار، گروه آسیب‌شناسی، دانشکده دامپزشکی، واحد ارومیه، دانشگاه آزاد اسلامی، ارومیه، ایران

[2] دانشجوی دکترای تخصصی جراحی دامپزشکی، دانشکده دامپزشکی، واحد ارومیه، دانشگاه آزاد اسلامی، ارومیه، ایران

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## چکیده

تریکوبلاستوم‌ها معمول‌ترین تومورهای جلدی خوش‌خیم در خرگوش‌ها هستند. از آن جا که این نئوپلاسم‌ها زیر گونه‌های متنوع دارند، بنابراین ممکن است آن‌ها با تومورهای مشابه دیگری که دارای ترکیب سلول‌های پوشش بازالوئید هستند، مورد اشتباه واقع شوند. یک خرگوش لوپ نر پنج ساله نژاد مخلوط، دارای یک توده قابل لمس دو لوبی سفت متحرک بیضی شکل بود که در زیر گوش راست مابین فک پایین و گردن با ابعاد ۱×۱/۵×۳ بدون ایجاد زخم واقع شده بود. وضعیت عمومی خرگوش خوب بود و تب یا حالت غیرطبیعی در لوکوگرام وجود نداشت. پس از جراحی، یک توده سفید خاکستری در مجلول بافر قرمالین ۱۰ درصد تثبیت شد. مشاهدات آسیب‌شناسی مقاطع توده رنگ‌آمیزی شده با روش‌های هماتوکسیلین و ائوزین، پریودیک اسید شیف و تری کروم ماسون، یک تومور متشکل از سلول-های معمولی پوششی بازالوئید با هر دو الگوی ریبون و ترابکولی (مختلط) همراه با بافت همبندی زمینه‌ای را تایید کرد. علاوه بر این، ایمونولیبلینگ تومور توسط CK AE1/AE3، CK7، CK20، CD10، وایمنتین (Vimentin)، S100 و Ki67 انجام شد. بر اساس آسیب-شناسی و ایمونوهیستوشیمی، تشخیص تریکوبلاستوم نوع مختلط برای توده انجام شد. از لحاظ ایمونوهیستوشیمی، بیان قوی CK AE1/AE3(بیشتر از ۵۰ درصد سلول‌های توموری بازالوئید)، ایمونولیبلینگ قوی (بیش‌تر از ۵۰ درصد) بافت همبندی ترابکولی زمینه‌ای مربوط به تومور در برابر وایمنتین و واکنش ایمنی مثبت ضعیف (۱۰-۱ درصد از سلول‌های زمینه‌ای تومور) وجود داشت. از طرف دیگر، بیان مارکرهای CK7، CK20 و CD10 در سلول‌های توموری به ترتیب ۴۰-۳۰ درصد (متوسط)، ۳۰-۲۰ درصد (متوسط) و <۱۰ درصد (ضعیف) بود. بر این اساس، استفاده از CK7، CK20 و CD10 همراه مارکرهای دیگر می‌تواند در تفریق دقیق‌تر تریکوبلاستوم حیوانی از تومورهای مشابه مانند کارسینوم سلول بازال، تریکواپیتلیوما یا سایر تومورهایی که از سلول‌های پوششی بازالوئید فولیکول‌های مو منشا می‌گیرند، مفید باشد.

کلمات کلیدی: خرگوش، تریکوبلاستوم، CK7، CK20، CD10

*نویسنده مسئول : امیر امنیت‌طلب، دانشیار، گروه آسیب‌شناسی، دانشکده دامپزشکی، واحد ارومیه، دانشگاه آزاد اسلامی، ارومیه، ایران
E-mail: a.amniattalab@iaurmia.ac.ir