Histopathology and Immunohistochemistry of tumors in animals attending veterinary teaching hospital

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Abstract

We aim in the current study to investigate the pathology and incidence of tumors that are excised surgically in Veterinary Teaching Hospital. This study collected the tissue samples from animals from 1 October 2020 until 1 April 2021. These samples were collected from animals that undergo surgical procedures to remove neoplastic growths in their body. After tissue grossing, samples were collected from tumor mass, then fixed in neutral buffered 10% formalin for 72 hours, then processed to embedded in paraffin wax. A routine Harris’s hematoxylin and eosin stain were also used, histochemical stains such as Masson’s trichrome and PAS techniques were used as needed. In exceptional cases, IHC protocol was used for diagnostic steps to some types of tumors included in the current study. The antibodies that were used are Vimentin for canine osteosarcoma, P53 for squamous cell carcinoma, Mdm2 for ovine pulmonary adenocarcinoma, and CD10 for neoplastic lymphocytes. The result of the current study showed that the prevalence of tumor recorded was Marek’s diseases 22.8%, squamous cell carcinoma 22.8%, ovine pulmonary adenocarcinoma 15.4%, third eyelid adenoma 15.4%, canine osteosarcomas 7.4%, venereal transmitting tumors 7.4%, and feline mammary gland tumor 7.4%. In conclusion, the incidence of tumor in animals that attending Veterinary Teaching Hospital belong to the College of Veterinary Medicine, the University of Mosul was in general high in comparison to other studies in Iraq and other countries, this high incidence should pay attention to the causes of these cancerous conditions and their relation to environmental aetiology.

Keywords: Tumor, P53, Mdm2, Vimentin, CD10

Introduction

The tumor is an abnormal mass that contains cells that result from the abnormal growth of normal cells in the body; also, it is termed neoplasia (1). The word tumor describes the actual swelling or abnormal physical appearance of this type of cell. The word cancer is mostly confused with neoplasia (2). All cancer tumors were considered malignant, tumors can be roughly classified into two groups; malignant and benign (3). The malignant one has a remarkable ability to invade the nearing tissues by invasion and spread to another organ by metastasis and ultimately kill the animal by interfering with normal body physiology (4). On the other hand, benign tumors do not invade surrounding tissue and do not spread to other organs; thus, they rarely cause the death of affected animals. Tumor cell can spread to a newly anatomical location by many mean, the most common way by using blood and lymphatic vessels, and this called metastatic invasion; also, the tumor mass has an excellent affinity for recurrences that means this tumor mass will have reestablished after surgical excision, commonly this tumor of tumors can be aggressive and cause death due to loss of condition to the affected animals (5). In Mosul city, they were more observed and recorded in long-living animal species, most commonly recorded in cats and dogs, and less recorded in poultry and rarely recorded in ruminant (6). We aim in the current study to investigate the pathology of tumors that excised surgically in Veterinary Teaching Hospital.
Hospital, belong to College of Veterinary Medicine, University of Mosul.

Materials and methods

Sample collection
In this study, we collected the tissue samples from animals attending Veterinary Teaching Hospital belong to the College of Veterinary Medicine, University of Mosul. These samples were collected from animals that undergo surgical procedures to remove neoplastic growths in their body.

Tissue grossing
After receiving the tissue samples, the grossing process was conducted by external examination of tissue samples with recording the gross appearance appears with choosing a histological sample (7).

Tissue fixation
Representative histological samples were having been collected with 1.5*1.5*0.5 cm size and labeled according to sample recorded; the tissue put in cassettes and fixed in 10% neutral buffered formalin (10 ml of 37% formaldehyde, 90 ml of distilled water, 6.3 grams’ sodium monobasic, 3.9 grams’ sodium dibasic) for at least 27 hours to conduct the perfect fixation (7,8).

Tissue processing
After fixation, these samples were processed to be embedded in paraffin. The first step was dehydration, and this conduct by ascending concentrations of ethyl alcohol to withdraw the water from tissue samples, then cleared by xylenes in order to facilitate the paraffin wax to be infiltrate the samples, the samples rinsed in hot paraffin wax at 55-58ºC for three changes (on hour for each change) in order to infiltrate the paraffin wax into tissue samples and withdraw the xylene. The samples were embedded in paraffin mold and left to dry at room temperature (8).

Table 1: Type and number of tumor in the current study

<table>
<thead>
<tr>
<th>Species</th>
<th>Tumor type</th>
<th>Organ</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cat</td>
<td>Mammary gland tumor</td>
<td>Mammary gland</td>
<td>7.4 (1/13)</td>
</tr>
<tr>
<td>Cat</td>
<td>Third eye lid tumor</td>
<td>Third eye lid</td>
<td>7.4 (1/13)</td>
</tr>
<tr>
<td>Cat</td>
<td>Venereal transmitting tumor</td>
<td>Vagina</td>
<td>7.4 (1/13)</td>
</tr>
<tr>
<td>Dog</td>
<td>Third eye lid tumor</td>
<td>Third eye lid</td>
<td>7.4 (1/13)</td>
</tr>
<tr>
<td>Dog</td>
<td>Osteosarcoma</td>
<td>Femur bone</td>
<td>7.4 (1/13)</td>
</tr>
<tr>
<td>Sheep</td>
<td>Pulmonary adenocarcinoma</td>
<td>Lung</td>
<td>15.4 (2/13)</td>
</tr>
<tr>
<td>Cow</td>
<td>Squamous cell carcinoma</td>
<td>Skin</td>
<td>15.4 (2/13)</td>
</tr>
<tr>
<td>Cow</td>
<td>Squamous cell carcinoma</td>
<td>Vagina</td>
<td>7.4 (1/13)</td>
</tr>
<tr>
<td>Chicken</td>
<td>Marek’s disease</td>
<td>Different organs</td>
<td>22.8 (3/13)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>100 (13/13)</td>
</tr>
</tbody>
</table>

Tissue sectioning and routine staining
The paraffin cassettes section using rotary microtome at 6µm thickness and the paraffin ribbon floated on a water bath at 40ºC then lifted on the glass slide.

The slides dried using a hot plate at 60ºC for 15 minutes, then stained using routine Harris hematoxylin and eosin stain (7). A histochemical stain was applied as needed; these stains included PAS stain and Masson’s trichrome stain (7).

Immunohistochemistry Staining
In exceptional cases, IHC protocol was used for diagnostic steps to some types of tumors included in the current study.

The antibodies that were used are Vimentin for canine osteosarcoma, P53 for squamous cell carcinoma, Mdm2 for ovine pulmonary adenocarcinoma, and CD10 for neoplastic lymphocytes, all immunohistochemistry producers was applied manually.

Results

Tumor incidence
A total of 13 tumor samples included in the current study were collected from animals that attended Veterinary Teaching Hospital during six months included in the current study. The result showed that the highest number of tumors were recorded in a cow with squamous cell carcinoma in the skin of the cow, pulmonary adenocarcinomas in sheep, and Marek’s diseases in chickens with incidence reach up to 15.4 (2/13). In contrast, all other tumor types were recorded with an incidence of about 7.4 (1/13) (Table 1).

Mammary gland tumor in a cat
The histopathological lesion of this type of tumor composed from server infiltration of neoplastic cells, these neoplastic cells identified as lymphocytes which arrange either in clusters or individually in fibrous stroma, the IHC for CD10 showed the presence of neoplastic lymphocytes that their cytoplasm has been reacted positively with CD10 antibodies (Figure 1).
Third eyelid tumor in cat and dog

This type of tumor composed from well-demarcated nodules contains neoplastic sebaceous cells that present in glandular form, and combined by hyperplasia of epidermis that extended inside neoplastic mass, these neoplastic sebaceous cells were characterized by large cuboidal or columnar shape with large dense nucleus present near the basement membrane of the tubules (Figure 2).

Venereal transmitting tumor in a cat

This type of tumor comprises cells with a round to oval shape, and the cytoplasm is poorly defined by routine stain. These tumor cells are separated on some occasions with dense fibrous tissue. The IHC showed that the cytoplasm of these cells was positive for CD10 antibodies (Figure 3).

Osteosarcoma in dog

This type of tumor has a pleomorphic nature with an anaplastic form composed of islands of osteoid materials. The tumor comprises three types of cells: osteoblast, chondroblast, and fibroblast. The IHC staining showed that these cells, especially fibroblasts, were stained positively with vimentin antibody (Figures 4).

Pulmonary adenocarcinoma in sheep

The tumor lesions composed from a nodular formation that contain dense collagen fiber deposition with the transformation of cells lining alveoli into cuboidal or columnar cells with also can be observed in other air passages; these cells were projected in papillary or acinar form, these neoplastic cells were found to be originated from alveolar cell type II and Clara cells. The IHC staining showed that the cytoplasm of neoplastic cells was stained positively with Mdm2 antibodies (Figure 5).

Squamous cell carcinomas

This type of tumor primarily found in skin lesions associated with the formation of pathognomonic lesions known as keratin pearls; this lesion composed of an acentric layer of keratin produced by neoplastic keratinocytes, that characterized by hypertrophic cells with large nucleus with high mitotic figure, these keratin pearls contain collagen fibers deposition either in mixed with the keratin of present
surrounding these lesions. The IHC of squamous cell carcinoma showed a positive staining reaction to P53 antibodies (Figure 6).

Figure 5: Histological section of sheep’s lung with adenocarcinoma. [A] showed nodular formation that contains dense collagen fiber deposition (arrow), with the transformation of cells lining alveoli into cuboidal or columnar cells were project in papillary or acinar form (arrow). 40x, H&E. [B] showed cuboidal or columnar cells were projected in papillary or acinar form (arrow), with dense collagen deposition as extracellular matrix (arrow). 40x, Masson’s trichrome. [C] showed positive reaction to Mdm2 (arrow). 40x, IHC-DAB stain. [D] showed positive reaction to Mdm2 (arrow). 400x, IHC-DAB stain.

Figure 6: Histological section of cow’s skin with squamous cell carcinoma. [A] showed multiple keratin pearls (arrow). 40x, H&E. [B] showed keratin pearls composed from an acentric layer of keratin (arrow), produced by neoplastic keratinocytes (arrow). 400x, H&E. [C] showed collagen fibers deposition in keratin peals (arrow) or surrounding these pearls (arrow). 40x, Masson’s trichrome. [D] showed positive reaction to P53 (arrow). 400x, IHC-DAB stain.

Marek’s disease
This type of tumor was observed in visceral organs such as the liver, lung, kidney, intestines, and spleen composed from pleomorphic lymphocytes which diffusely proliferating small-to-medium size of lymphoblast. The IHC staining showed that the neoplastic cells were stained positively with CD10 antibodies (Figure 7).

Figure 7: Histological section of chicken’s liver with Marek’s disease. [A] showed neoplastic lymphocytes (arrow), with few normal hepatocytes (arrow). 40x, H&E. [B] showed neoplastic lymphocytes (arrow), with few normal hepatocytes (arrow). 100x, H&E. [C] neoplastic lymphocytes (arrow) in lung tissue. 40x, H&E. [D] showed neoplastic lymphocytes (arrow) in kidney tissue. 400x, H&E. [E] showed neoplastic lymphocytes (arrow) in intestinal tissue. 40x, H&E. [F] showed positive reaction to CD10 (arrow). 40x, IHC-DAB stain.

Discussion
The results of the current study showed that the highest type of tumor incidence was recorded in squamous cell carcinomas and Marek’s diseases at 22.8% (313) to each type of tumor; these results were merely similar to that recorded by Al-Ajeli et al. (2) were they recorded about 22.2% of squamous cell carcinomas in Mosul city, this indicates that the prevalence of this type of tumor was stable in Mosul city since both studies were applied in VTH belong to the college of Veterinary Medicine, University of Mosul. While the prevalence of Marek’s diseases in Karbala reached up to
30.6% that recorded by Wajid et al. (9), which is higher than that recorded in our study, this can belong to different geographical location and climate effect, in addition to the endemic status of this diseases in these two cities. The pathognomic lesions for squamous cell carcinomas are keratin pearls that composed from an acanthic layer of keratin produced by hypertrophied neoplastic keratinocytes, which produce this connective tissue out of control of body function as a result of neoplastic status, mainly these lesions can be observed in the dermis and epidermis of skin in different location of the body (5). At the same time, the primary pathognomic lesions in Marek’s diseases in chicken were composed of uniform small to moderate size lymphocytes that infiltrate in different organs of the body depending on the severity of the infection caused by a herpesvirus that infected lymphocytes and induce this neoplastic status (10).

The prevalence of ovine pulmonary adenocarcinomas was found to be at 15.4% in the current study; this percentage was higher than that recorded by Al-Ajeli et al. (6), who recorded this type of tumor in 11.2%, this may be related to the population of animals that included in the current study; they were investigate this lesion during three months in one flock of sheep, another study conducted by Jassim et al. (11) were they recorded 10.1% of this tumor in sheep in Al-Qadisyah city. Ovine pulmonary adenocarcinomas have a pathognomic lesion characterized by a neoplastic cuboidal and columnar cell that project as papillary of acinar form inside alveoli and bronchioles surrounded by dense fibrous connective tissue (5).

Third eyelid tumor in dog and cat were found to be sebaceous cell tumor (adenoma type) in 15.4% in the current study; this result was merely similar to that recorded by Dees et al. (12) were the recoded about 14.4% of adenoma in the third eyelid in dogs and cats. Silva et al. (13) described a similar neoplastic cell characterized by a cuboidal or columnar cells, with a basement nucleus that large with a high mitotic figure and dense fibrous tissue that gives this neoplastic mass a nodular formation. Canine osteosarcoma was recorded 7.4% in the current study, this percentage was found to be 12% in two dog’s families (14), this can be related to a long life for this type of dogs, and their lifestyle was domesticated in houses in Scotland, and their age can reach up to 13 years’ old which increased the incidence of recording this type of tumors.

The venereal transmitting tumors in cats were recorded in 7.4% in the current study; these results were less than recorded in other countries where they range from 23-40% (15). These can be related to the time included in the previous study where they conducted their work for six years in Europe, the USA, and Canada. Venereal transmitting tumors in a cat can be described as dense infiltration of neoplastic lymphocytes that were poorly differentiated cells with ill define cytoplasmic boarded and highly mitotic figures observed in current work (5). The mammary gland tumor in the cat was recorded in 7.4% in current work, and this prevalence was found to be less than other studies that recorded 17% of mammary gland tumor in feline species (1). Another study conducted by Wypij et al. (16) recorded a 10.4% tumor incidence in feline species in the USA and UK.

Conclusion

The incidence of tumor in animals that attending Veterinary Teaching Hospital belong to the College of Veterinary Medicine, the University of Mosul was in general high in comparison to other studies in Iraq and other countries, this high incidence should pay attention to the causes of this cancerous condition and their relation to environmental-causes.

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Conflict of interest

The authors declare that we did not have any conflict of interest regarding publishing or funding of this article.

References


