Introduction

Intestinal polyps are known as benign neoplasms and slowly overgrow in mucosa. Polyposis is generally more common reported in the lower gastrointestinal tract, especially in colon, in humans than in animals. Among animal species, they are more frequently seen in dogs [3, 6].

In human medicine, the colorectal polyps, which usually called due to their common localization, are classified into hyperplastic, hamartomatous and adenomatous polyps. Among hamartomatous and adenomatous polyps, several subtypes are identified, such as Peutz-Jeghers Syndrome and Juvenile polyps. Furthermore, because of the arborescent organisation of the muscle cells from the lamina muscularis to the lamina propria and the resultant polyp invasion, these lesions would correspond to the Peutz-Jeghers type described in human medicine. These results emphasize the interest to couple histological and immunohistochemical examinations of colorectal polyps in order to classify polyps in dog with more accuracy.

Keywords: Dog, rectum, hamartomatous polyp, muscle cells, histology, immunohistochemistry.
HISTOLOGICAL AND IMMUNOHISTOCHEMICAL INVESTIGATIONS

A biopsy from the injured rectum (4 cm length) was removed surgically and sent to the Department of Pathology, Faculty of Veterinary Medicine, Ankara, Turkey for diagnosis. The tissue samples were fixed in buffered 10% formalin and embedded in paraffin wax by routine method. The sections were cut at 5 μm thickness and mounted on glass slides. They were firstly stained routinely with haematoxylin-eosin and then, the same sections were stained with Masson’s trichrome and Mayer’s mucicarmine methods.

The sections were also examined by immunohistochemistry using the Avidin-Biotin Complex Peroxidase (ABC-P) method (Dako, Carpinteria, USA) for immune complex revelation. After blocking endogenous peroxidase activities by immersing the sections in 0.3% hydrogen peroxide in absolute methanol for 30 minutes, tissues were digested with 0.1% trypsin. Subsequently, incubations with the different primary antibodies (Table I) were performed. The revelation step was performed according to the manufacturer’s instructions and the 3-Amino-Ethyl Carbazole (AEC, Dako) was used as chromogene. The sections were counterstained with the Mayer’s haematoxylin stain and observed under light microscopy (Leica, DM 4000 M light microscope and Leica DFC-280 camera attachment). The intensity of the immunolabelling was semi-quantified according to the mean number of positive cells counted by microscopic field from 10 fields examined at X 100 magnification. The following scores were defined: (+) slight or inconspicuous positivity (5 - 10% of positive cells), (2+) mild positivity (10 - 20% of positive cells), (3+) strong positivity (more than 20% of positive cells).

**Results**

The injured part of rectum was covered with numerous yellowish-brown polyps (figure 1) which progressed into lumina and exhibited pedunculated structures. The polyp diameters were comprised between 0.5 and 1.5 cm. Histopathologically, cuboid or spindle shaped epithelial cells covered the polyp surface and the epithelium was desquamated in some areas. The goblet cells were hyperplasic and enlarged (figure 2). Fibrocytes, fibroblasts and proliferated capillary vessels were found in the lamina propria (figure 3). Numerous dilated glands containing abundant deposits were found in the polyp stroma (figure 4) and their epithelia were flattened or desquamated into the gland lumen. Metaplastic changes were occasionally seen in the polyp stroma (figure 5). The mucin nature of deposits into the goblet cells and into the dilated or cystic glands was confirmed by the red colouration of the substance with the Mayer’s mucicarmine staining method (figure 6). Some spindle shaped cells in bundles located on the ground of few polyps arose from the lamina muscularis into the lamina propria. As they were red coloured with the Masson’s trichrome staining method, they were considered as derivate muscle cells (figure 7).

The Table II summarizes the immunohistochemical properties of the various cells that have composed rectum polyps in the 15 year old Pekinese dog. Using immunohistochemistry, the epithelial cells on the polyp surface and the epithelial cells from crypts and glands were strongly stained by the primary anti-pancytokeratin antibody (Table II, figure 8). Muscle cells in the lamina muscularis and their branches in the lamina propria, fibrocytes / fibroblasts and capillaries were positively labelled by the monoclonal anti-α actin (figure 9) and anti-desmine (figure 10) primary antibodies but contrary to the muscle cells, fibrocytes / fibroblasts and capillary structures were strongly immunoreactive with the anti-vimentin antibody (figure 11) as well as the cytoplasm of macrophages, lymphocytes and few neutrophils. Furthermore, the immunoreactivity with anti-α actin and anti-desmine antibodies was markedly more intense in the muscle cells than in fibrocytes and in capillaries structures (Table II).

**Discussion**

Polyps are mostly localized on colon and rectum and consist more often in benign epithelial neoplasia, the malignancy frequency remaining below 1% [3, 9]. Most polyps are originated from epithelial cells of the intestinal tract and slowly expanded into lumina [3]. These types of cases are known in humans and frequently encountered in animals, mainly in dogs.

The reported case concerns an old (15 year old) male Pekinese dog. Polyps are most commonly found in Collie breeds and are also frequently reported in Poodle, Airedale Terrier, German Shepherd, Golden Retriever, Beagle, Corgi, Cocker hitherto [4, 5, 8]. In Pekinese dog, no colon or rectum polyps are not yet described in the literature. No sex predisposition in colorectal polyps was mentioned, their frequencies being nearly identical in both genders [5, 8]. These polyps were encountered in 1.5 year to 12 year old dogs, with an average of 6.4 year old [5, 6] and compared to other animal species or to humans, their prevalence is higher in older dogs [4].

<table>
<thead>
<tr>
<th>Primary antibody</th>
<th>Laboratory reference</th>
<th>Dilution / incubation conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>pancytokeratin AE1/AE3</td>
<td>clone MS-343-P1, Neomarkers</td>
<td>1:50 / 1 hour at 37°C</td>
</tr>
<tr>
<td>monoclonal mouse anti-vimentin</td>
<td>cloneviment3B4, Dako</td>
<td>1:100 / 1 hour at 37°C</td>
</tr>
<tr>
<td>monoclonal anti-α muscle actin</td>
<td>clone 1A4, Sigma</td>
<td>1:200 / 1 hour at 37°C</td>
</tr>
<tr>
<td>monoclonal anti-desmin</td>
<td>clone DE-4-10-D-1033, Sigma</td>
<td>1:100 / 1 hour at 37°C</td>
</tr>
</tbody>
</table>

*Table I: Primary antibodies used for immunohistochemistry in the present study.*
FIGURE 1: Macroscopic appearance of polyposis (arrows) in the rectum from the 15 year old male Pekinese dog.

FIGURE 2: Invasive growth of polyp into the rectum lumina. Note the epithelium (arrow) covering the polyp structure and the enlarged goblet cells (arrowhead), Haematoxylin-eosin, X40.

FIGURE 3: Fibrocytes, fibroblasts (arrow) and proliferated capillary vessels (arrowhead) in the lamina propria, Haematoxylin-eosin, X40.

FIGURE 4: Dilated glands (arrows) with abundant deposits in the polyp stroma, Haematoxylin-eosin, X40.

FIGURE 5: Metaplastic change (arrow) in the polyp stroma, Haematoxylin-eosin, X100.

FIGURE 6: Mucin deposits in the dilated and cystic glands (arrows) and in the goblet cells (arrowheads) appearing red coloured with the Mayer’s mucicarmine method, X 100.
Primary antibody | Localization in the polyp and cell types | Positivity intensity
--- | --- | ---
Anti-pancytokeratin | Lamina epithelialis / lamina propria |  
Epithelial cells from the surface | 3+  
Epithelial cells from the crypts and glands | 2+
Anti-α actin | Lamina propria / Lamina muscularis |  
Fibrocytes / Fibroblasts | +  
Capillaries | +  
Muscle cells | 3+
Anti-desmine | Lamina propria / Lamina muscularis |  
Fibrocytes / Fibroblasts | +  
Capillaries | +  
Muscle cells | 3+
Anti-vimentin | Lamina propria |  
Fibrocytes / Fibroblasts | 3+  
Capillaries | 3+  
Inflammatory cells | +

Scores of positivity intensity: (+) slight or inconspicuous positivity (5 - 10% of positive cells), (2+) mild positivity (10 - 20% of positive cells), (3+) strong positivity (more than 20% of positive cells).

**Table II:** Immunopositive reactions towards the anti-cytokeratin, anti-α actin, anti-desmine and anti-vimentin primary antibodies according to the localization and cell types from the rectum polyps in the 15 year old Pekinese dog.

![Figure 7: Proliferated capillaries (white arrows) and muscle cells (black arrows) in polyps, Masson’s trichrome method, X 400.](image)

![Figure 8: Positive immunolabelling (arrows) of crypt and gland epithelial cells and of epithelial cells lining the polyp surface using the anti-pancytokeratin AE1/AE3 primary antibody, Avidin-biotin complex peroxidase (ABC-P) method, X 100.](image)

![Figure 9: Positive immunolabelling (arrows) of muscle cells in the polyp stroma using the monoclonal anti-α actin primary antibody, Avidin-biotin complex peroxidase (ABC-P) method, X 100.](image)

![Figure 10: Positive immunolabelling (arrows) of muscle cells in the polyp stroma using the monoclonal anti-desmine primary antibody, Avidin-biotin complex peroxidase (ABC-P) method, X 100.](image)
Macroskopically, polyps are easily recognizable neoplastic changes, growing slowly and forming persistent structures that progressively invade the lumen of the intestinal tract [3]. Polyps, seen pigmented or not pigmented, can be overgrowth of mucosa in different sizes, numbers and distribution [3, 9]. However, they exhibit different histopathological traits leading to their classification as hyperplastic, hamartomatous and adenomatous types [1, 3, 6, 9] in human oncology. In the hamartomatous type, pathological changes simultaneously involved epithelial and mesenchymal tissues [3, 9]. In addition, normally epithelial and mesenchymal components are encountered in areas adjacent to neoplastic structures [9]. Furthermore, not only the goblet cells, glands and proliferated capillary vessels, but also some muscle cells, which are originated from the lamina muscularis and reached into the lamina propria, are found in the hamartomatous type polyps [7, 10]. The progressive polyp invasion by muscle cells is a phenomenon called “arborisation” and is occasionally seen on some human polyp types [11]. These specific hamartomatous type polyps are also known as Peutz-Jeghers type polyps [3, 7, 10]. In the present case, the rectal polyps were formed by epithelial cells and mesenchymal structures such as goblet cells, dilated and cystic glands and proliferated capillaries and were considered as a hamartomatous type polyp. In addition, the presence of muscle cells in the lamina propria characterized a Peutz-Jeghers type like in human medicine.

Although the main different histological structures (muscle cells in propria, glands and capillaries) can be determined with haematoxylin-eosin or Masson’s trichrome stains, immunohistochemistry using anti-pancytokeratin, actin, desmine and vimentin primary antibodies was performed in order to undoubtedly identify the various cell origins. Muscle cells and some arborescent organisations between the lamina muscularis and the lamina propria were evidenced throughout specific staining and immunolabelling with anti-α actin and anti-desmine antibodies although the 2 methods have not always given exactly superimposed patterns. Moreover, some polyps have not exhibited any muscle cell specific immunoreactivity. Additionally, the occurrence of metaplasia changes metaplastic changes may be associated to the progressive development of polyps.

As a conclusion, the rectal polyps observed in the 15 year old male Pekinese dog can be considered as hamartomatous type according to the histopathological and immunohistochemical findings, and with more accuracy as a Peutz-Jeghers type because of the polyp invasion by muscular arborescent structures. However, polyps or polypoid changes need to be investigated in different parts of the intestinal tract and particularly in the colon. The histological and immunohistochemical examinations of all lesions would help to better characterize the tumours and to improve the polyp classification in dogs.

Acknowledgement

The authors greatly thanks Prof.Dr.Omer Besalti for providing specimen. In addition, the study was study presented as oral presentation in 20th Anniversary International Scientific Conference, 3-4th June 2010, Stara Zagora, Bulgaria.

References