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Histological and Macroscopical Study of Horse Melanoma

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Abstract. The Equine Melanoma occurs at the level of pilose follicles. Cells have a melanic content; they are either fusiform or dendritic, about 30 microns in diameter. Materials and methods: 11 melanic tumors were diagnosed by H&E. results: cells were spindle in 6 cases and dendritic in 5 cases, mitosis was medium in melanoma and low in melanocytoma, necrosis was more bigger in melanoma than melanocytoma, infiltrated lymphocytes were medium and absent in melanoma, all cases in 4,5 Clark's level. Conclusion: melanic tumors were multiply nodules. The majority malignant melanomas were spindle type cells with some cases were dendritic type cells. The relationship between infiltrated lymphocytes and necrotic zones was inverse legation with mitotic index, it means the aggressive rate increase with reduced infiltrative lymphocytes and extend necrotic zones and inversely.

Keywords. Horse, Histology, Macroscopy, Melanoma

INTRODUCTION

The histological study of melanomas requires the discoloration of the sections, since overloading with melanic pigment makes impossible their examination. Early changes in Eqine Melanoma occur at the level of pilose follicles. Melanic pigment gradually accumulates around the pilose bulb, and extends to the sweat glands, without the participation of the pigmented basal layer of the epidermis or of the outer hair sheath. Cells have a melanic content; they are either fusiform or dendritic, about 30 microns in diameter. With the development of the tumor nodule, polyhedral cells appear on a reticulin and collagen background. According to Levene (1971), during this developmental phase there is a considerable histological similarity to human blue naevus. In older evolution forms, numerous multinucleate cells are noted, with abundant cytoplasm, numerous nuclei (sometimes more than 10 nuclei), usually situated at the periphery of the cell or in the cytoplasmic mass without a precise grouping ***(****Kwieczlnsky et al., 1981, Baba et al., 1981).* Melanocytes appear as dendritic cells with a large, clear nucleus and cytoplasm with melanic granules. Melanophages are large reticuloendothelial cells.

Regional lymph nodes will present pigment cells that have reached this site by afferent lymphatic pathways, inducing tumor embolism at sinus level. Lymphoendothelial cells, dendritic cells and multinucleate cells are found, whose cytoplasm contains melanic pigment. The proportion of these cellular types can vary from one case to another and even from one lymph node to another.

Metastasizing can be achieved by blood vessel route, with the presence of emboli and neoplastic obliterations in the veins, as well as by lymphatic vessel route, when afferent regional lymph nodes will be affected.

In parenchymatous organs, liver, spleen, lung, kidney, digestive tract, genital tract, etc., melanic proliferations have a nodular character, with the described histological structure. However, melanic pigment loading of reticuloendothelial cells, histiocytes and even cells of the white series may be found. The macroscopic aspect of circumscribed nodular tumor is contradicted by the microscopic image, of distant infiltrations with melanocytes and melanophages.*(Baba et al., 1981).*

In macroscopical aspect, the primary locations are almost constant in black skin, with a smooth aspect and scarce fine hairs. The most affected body regions are the inferior side of the tail, the lips, the anus, the vulva, the prepuce, the perineum *(Baba et al., 1981)*, few cases with locations at the level of the ear concha *(Kwieczlnsky et al., 1981)* or in the external auditory canal are reported. Almost without exception, in more advanced cases, external lymph nodes are involved *(Levene et al., 1980).*

The aim of this study was: the extended histological and macroscopical study of equine melanic tumors through study of tumoural localization, tumoural aspect, cell type, mitosis.....etc and use them for comparative between benign and malign tumors.

MATERIALS AND METHODS

9 tissue samples were collected from Alexandria slaughtered horses in the period between 5 -7 may 2010, and from the discipline of pathology, faculty of Veterinary Medicine USAMV Cluj-Napoca, in the period between 1992 to 1998

The samples were taken from the skin of the ventral tail, perineum zone and one case was metastatic melanoma, then were treated by formalin fixed (formalin 10% tamponed, PH7) during of 24 hours, and then paraffin embedded by standard procedures.

These paraffined samples cut to sections 4-5 microns by microtom Leica RM2125 RT The melanin bleaching was performed on all tumoural sections by the Melanin Bleach solution: Kit (polysciences, Inc) that contain two solutions (potassium permanganate solution, oxalic acid) that was applied on 4-6 micron tissue sections of horse melanic tumors. For more details see index nr.1. After tissue sections were bleached, rinse in distilled water and proceed with Hematoxylin & Eosin Stain, for more details see index nr.2, then these samples examined by microscope Olympus BX 51. Imagines were taken by a digital Olympus SP 350 și analized with a program adecvat-Olympus DP soft.

Statistics: Independent group *t*–tests, x2 tests of independence or Fisher's exact test.

RESULTS AND DISSCUSIONS

***Discussion of macroscopic aspect*:** In this study the melanic tumors were nodules in all cases, 6 cases with small nodules (1-2 cm)and 5 cases with big nodules (2-15cm) composed from 1 to 2 nodules, 2 cases were ulcerated in the covered skin of tumor in a tail region (fig 2,4), that conform with specialist literature Johnson who noticed: in the horse they are often flat and firm, may be single or multiple, and can coalesce, creating a cobblestone appearance. Alopecia and ulceration are variable features, in the preferential areas of primary tumors, in the subcutaneous connective tissue; nodules of various sizes appear, sometimes with a rosary appearance, interconnected by fine black striations, which are the lymphatic vessels. Nodules have a black color; they are hard, so that they are very difficult to dissect.

Tumor nodules are encapsulated and bands of fibrous connective tissue can be identified in sections, which traverse the brown blackish mass. Tumor nodules of various sizes (0.5–5–8–10 cm in diameter), (Johnson et al., 1998).

In this study, one metastatic melanoma was multiple nodular formations in variable dimensions, from a wheat bean to a head of man, had a black color with thick-elastic structure, localized in liver mass, kidney, ovary, lung and spleen, it had been grown one over one that conform with specialists literature Baba and Durand that noticed: they are seen in the cavities, which are disseminated over the serous membranes and the organ capsules. Sometimes, huge black tumor formations (3–5 kg), with a bosselated surface appear in the abdominal cavity (on the epiploon and the mesentery). Tumor nodules or large neoplastic masses are found in the pulmonary parenchyma, the myocardium, the hepatic and renal parenchymas. The ovaries and the uterine serous membrane are covered with grape-like melanomas. Cases have been reported in which no organ has been excepted, even (muscular, adipose, connective) tissues being invaded by black neoplasms (Baba AI et al 1981), (Durand MS et al 1975).

Discussion of histological canine melanic tumors*:* in veterinary dermatopathology when evaluating melanocytic neoplasms they may be compound, denoting both an epidermalepithelial and dermal-submucosal component to the neoplasm, or dermal and submucosal with no identifiable epidermal or epithelial component (Goldschmidt et al., 1998). Pagetoid refers to the presence of either individual or small aggregates of neoplastic cells within the upper levels of the epidermis or epithelium (Goldschmidt et al., 1992), and was originally used to describe the carcinoma cells (Paget cells) that infiltrate the epidermis overlying mammary ductal carcinomas in situ (Cotran et al., 1999). In our domestic species, cutaneous, oral, and ocular melanocytic neoplasms are quite variable.

In this study, 8 dog melanomas were formed of spindle type cells in 5 cases and dendritic cells in 3 cases, and the majority cases were in dermal level and intense melanin (fig 3,4). whereas these results were conformed with specialist literature Weiss and Frese that said: the shape of malignant cells in melanoma are epithelioid, fusocellular, mixed of epithelioid and fusiform cells and dendritic and verticillate localized in junctional, dermal and/or hypodermal (BABA et al 2007), and conform with specialist literature Goldschmidt, that said: The cells type is not prognostic significance for melanoma. The tumors cells have variable morphology and include fusiform and epithelioid cells that have a variable degree of cellular and nuclear pleomorphism. An interwoven, whorled, or nested appearance to the tumor may be found (Goldschmidt M H et al., 1998). And were conformed with Levene that said: cells in horse melanoma have a melanic content, they are either fusiform or dendritic, about 30 microns in diameter. With the development of the tumor nodule, polyhedral cells appear on a reticulin and collagen background (Levene et al., 1971).

But horse melanocytoma cases were formed of 3 cases, one case was spindle type cells and two cases were dendritic type cells (fig 1,2), whereas in other report with Weiss and Frese that said: melanocytoma formed of fusiform type cells localization in dermal and/or hypodermal, and junctional level.(Baba et al., 2007)

But in other study with specialist literature Goldschmidt, that said Melanocytoma cell morphology: varies from a small spindle cell with melanic granules to large spindle cells, epithelioid cells, polygonal cells or round cells, which often have a large amount of melanin within the cytoplasm that obscures the nucleus. Localization: The intraepithelial component usually consists of nests of tumor cells in the lower layers of the epidermis or the ERS of the follicle. But in the dermal: the tumor display neuroid differentiation pattern to spindle cells (Goldschmidt M H et al., 1998).

Mitosis: The mitotic index figure in the tumor considers one of the important tools for diagnostic type of tumor whereas three or more mitotic figures per 10 high-power fields indicate malignancy. The identification of mitotic figures in sections must be undertaken with great care to ensure that only true mitoses are counted. Bleached sections should be used for pigmented neoplasms to avoid confusing mitoses with pyknotic nuclei and the small hyperchromatic nuclei of the spindle cells of the supporting stroma. The mitotic count will vary from area to area within the neoplasm, and the count should be performed in areas with the greatest concentration of mitotic figures. Where metastases have been confirmed, the mitotic rate in intraocular melanoma is usually greater than four per 10 high-power fields; less than two mitotic figures per 10 high-power fields is consistent with melanocytoma (Bussanich et al 1987, Wilcock et al., 1986).

In this study, the melanoma was medium grade of mitotic index in all cases, while melanocytoma was low grade of mitotic index figure, according to this classification 1-3 is low grade, 4-14 is over medium grade,15-30 over medium grade and 31and more is high grade of mitotic index figure.

In this study the melanocytoma cases had (1,1,2) mitotic figures per 10 high-power fields that indicate as melanocytoma had less than 3 mitotic index, so these results conform with specialist literature Goldschmidt that said: less than two mitotic figures per 10 high-power fields is consistent with melanocytoma (Goldschmidt et al., 1998).

While the melanoma cases had mitotic index figure more than 3 mitotic figures per 10 high-power fields that conform with specialist literature Goldschmidt M H that said: The important tools for diagnostic type of tumor whereas three or more mitotic figures per 10 high-power fields indicate malignancy (Goldschmidt et al., 1998).

I observed through number of cases and number of mitotic index figure as majority of melanoma cases tend to medium grade of mitotic index figure but melanocytoma cases tend to low grade of mitotic index figure that indicate as number of mitotic index increases in malignant melanic tumor than benign melanic tumors, $P < 0.05$, then I can say that there was a significant difference between melanoma and melanocytoma with mitosis (reject the null hypothesis).

In other part I studied the relationship between cell types and mitotic index figures

Where I found the following: dendritic type cells had numbers of following mitotic index figures (11,1,4,7,2), spindle type cells had number of following mitotic index figures (1,10,7,4,4,5). Whereas, I didn't find any significance between shape cells and mitotic index figures.

Necrosis: the present of extension of necrotic area within the tumor was evaluated. Extensive necrosis indicated that necrotic areas were present diffusely within the tumor. Moderate necrosis indicated that necrotic areas were limited and present sparsely in the tumor. Absence of necrosis was also noted (Millanta et al 2002), whereas the necrosis happen in tumors because speed of growth of cells was bigger than capable of the blood to provide the tissues, as well as tumoural embolus due to obstruct of blood vessels that provide of tissues (Hadad et al., 1998), therefore, tumor necrosis is considered important in cancer progression (Ingeborg et al 2008).

In this study the melanoma cases were extended in 1 case, moderate in 2 cases, reduced in 4 cases and absent in 1 case, whereas the majority of necrotic zones in these tumors were tended moderate to reduced that indicate to moderate of tumoural aggressively (Ingeborg et al 2008). While melanocytoma cases were reduced in all cases to small necrotic zones that indicate to slowly growth in benign tumors (Hadad et al., 1998).

Infiltrated lymphocytes: the intensity of the lymphocytic and mononuclear cell infiltration within the tumor was determined as follows. Intense (lymphocytes and macrophage were present densely throughout the neoplasm; presence of neutrophilic granulocytes, usually associated with necrosis and ulceration, were not considered); moderate (lymphocytes and macrophages present sparsely in and around the tumor); absent (Millanta F et al 2002).

In this study, the melanoma cases were intense in 1case, moderate in 3 cases and absent in 4 cases (see table.2, diagram.4), whereas these cases were tended to aggressively according of Smith that noticed: In both human and swine tumors, regression has been associated with a high number of tumor-infiltrative lymphocytes, so more aggressively of tumors will be in absent and decrease of lymphocytes (Smith et al., 2002). While melanocytoma cases were absent in all cases.

The comparative among necrosis, mitosis and Infiltrate lymphocytes

The classification of mitosis grade was in 4 levels:

The moderate4-14 mitotic index was in (1 extend, 2 moderate, 4 reduced and 1 absent) necrosis, (1 intense, 3 moderate, 0 reduced and 4 absent) infiltrate lymphocytes.

In this study, all melanoma cases were in moderate mitotic index figure whereas the majority cases were tended to reduced necrotic zones, whereas these results indicated as the necrotic zones were small with moderate grade of mitotic index figures, it means the aggressively of these tumors were moderate that conform with specialist literature Ingeborg that said: Tumor necrosis and apoptotic activity are considered important in cancer progression (Ingeborg et al 2008).

The majority moderate and absent of infiltrated lymphocytes were in moderate mitotic index figures, whereas these results indicated to the aggressive of tumors will be moderate with absent to moderate infiltrated lymphocytes that conform with smith that said: in both human and swine tumors, regression has been associated with a high number of tumor-infiltrative lymphocytes (Smith et al 2002).

From these results I can say the relationship between infiltrated lymphocytes and necrotic zones was inverse legation with mitotic index, it means the aggressive rate increase with reduced infiltrative lymphocytes and extend necrotic zones and inversely.

Level of invasion*:* under new guidelines, Clark's levels are now only used for thin melanomas (T1)11 Level I—favorable Level II—reserved Level III—IV—V—unfavorable (BABA et al., 2007).

In this study, 5 melanoma horse cases had 4 grade of Clark's level, and 3 melanoma cases had 5 grade of Clark's level, that consider as these cases were unfavorable. While 2 melanocytoma cases had 5 grade of Clark's level and one melanocytoma case had 4 grade of Clack's level but the Clack's level is not important tool in melanocytoma.

The majority of melanoma cases were in 4,5 grade of Clark's level that indicate as all melanoma cases took a very bad prognosis and unfortunately, the average lifespan of a horse following diagnosis of the most aggressive stage of malignant melanoma is four and five level.

Junctional activity: the junctional activity refers to the proliferation of neoplastic melanocytes at the interface between the epidermis and dermis or epithelium and submucosa (Goldschmidt et al., 1998).

In this study, the junctional activity was in all horse melanoma cases and all horse melanocytoma cases, whereas, it wasn't exist relationship between the junctional activity and tumoural type, that conform with Smith that said: The presence or absence of junctional activity is not specific to melanoma and often occurs in melanoma (Smith et al., 2002*).*

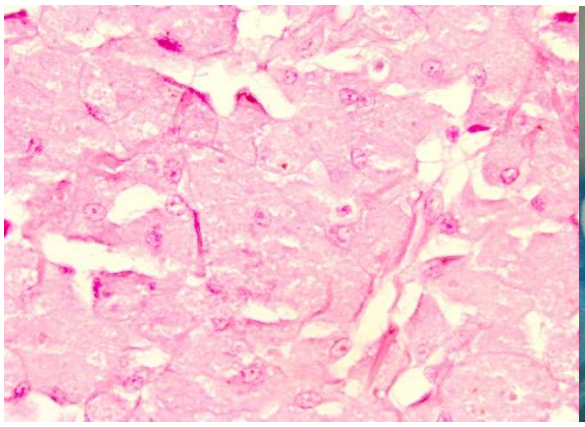

Fig. 1. Perianal region. Horse. Melanocytoma. Microscopic aspect: Dendritic type cells, with round nuclei and big cytoplasm, after bleaching operation, H&E 400X.

Fig 2. Perineal region, sub anus. Horse. Melanocytoma. Macroscopic aspect: Tumoural node appeared in section as smooth dens aspect and black color.

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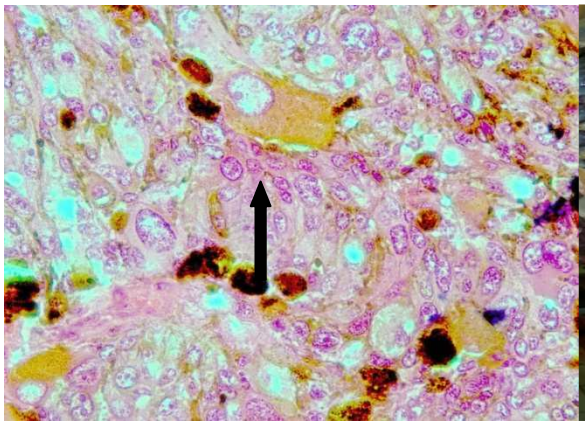

Fig. 3. Tail and anus region. Horse. Melanoma. Microscopic aspect: Spindle type cells with multiply oval to round nuclei, gigant cells were existed in some places (black arrows), H&E 400X.

Fig. 4. Thoracic cavity. Horse. Metastatic melanoma. Macroscopic aspect: metastatic tumoural formations were multiple nodules.

CONCLUSIONS

- The melanic tumors were multiply nodules with big differentiation in size 1 to 15 cm and number of nodules.
- Some cases were ulcerated in the covered skin of tumor in the tail region.
- The metastatic melanoma was multiple nodular formations in variable dimensions, from a wheat bean to a head of man, had a black color with thick-elastic structure, localized in liver mass, kidney, ovary, lung and spleen.

- The majority malignant melanoma cases were spindle type cells with some cases were dendritic type cells.
- The majority melanocytoma cases were dendritic type cells with some cases were spindle type cells.
- Melanoma cases were tended to medium grade of mitotic index figure but melanocytoma cases tended to low grade of mitotic index figure that indicate as number of mitotic index grows with in malignant melanic tumor than benign melanic tumors.
- The majority of necrotic zones in these tumors were tended moderate to reduced that indicate to moderate of tumoural aggressive.
- The melanocytoma cases were reduced necrotic zones in all cases with small necrotic zones that indicate to slowly growth in benign tumors.
- There weren't any clear differentiation between shape cells and mitotic index figures.
- The infiltrated lymphocytes in melanoma were moderate to absent in majority cases that indicate to aggressively of tumors, while in melanocytoma were absent.
- The necrotic zones were small with moderate grade of mitotic index figure.
- The majority moderate and absent of infiltrated lymphocytes were in moderate mitotic index figures.
- The relationship between infiltrated lymphocytes and necrotic zones was inverse legation with mitotic index, it means the aggressive rate increase with reduced infiltrative lymphocytes and extend necrotic zones and inversely.
- All melanoma cases took a very bad prognosis and unfortunately with Clark's level, because the average lifespan of a horse following diagnosis of the most aggressive stage of malignant melanoma was four and five level.
- The melanocytoma cases were in 4, 5 Clark's level but the Clack's level is not important tool in melanocytoma.
- The junctional activity was in all horse melanoma cases and all horse melanocytoma cases**.**

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