

## Haematological, Biochemical and Immunomorphologic Changes in dogs with Melanoma and Melanocytoma

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The purpose of this study is to analyze the disease incidence in dogs with melanoma and melanocytoma living in the territory of Uralsk. Dogs with neoplastic diseases of the skin were objects of research. Predominant localization of tumors in dogs, were observed in the mouth cavity. Mostly male dogs aged 9 to 12 years came down with melanoma and melanocytoma. Among the diseased purebred dogs the golden retrievers were encountered most of all.

**Key words:** Melanoma, tumor, melanocytoma, dogs.

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Melanoma is a malignant tumor that develops from melanocytes - pigment cells that produce melanin. Usually it is a dark spot or new formation of a round shape with irregular edges. It is characterized by dark color, glossy surface and a tendency to Degradation(MacEwen E.G. *et al*, 1999).

As reported by (Barker B. F. *et al*, 1997), (Ramos-Vara J. A. *et al*, 2000), (Rapidis A. D. *et al*, 2003), (Eisen D., Voorhees J. J., 1991), (Umeda M. *et al*, 2002), (Pour H., 2008), (Tanaka N. *et al*, 2004), (Patrick R. J., Fenske N. A., Messina J. L., 2007), (Ebenezer J. *et al*, 2006), (Sanchez J. *et al*, 2007) melanoma in dogs is typically localized in tunica mucosa of mouth.

According to (Vinores S. A. *et al*, 1995), (Singh A. D., Bergman L., Seregard S., 2005) melanoma is less localized on the eye retina.

As noted by (Withrow S. J., David M. Vail, 2007) the etiology of melanocytic tumors in animals is insufficiently studied. Since melanoma occurs mainly on the skin coated with hair or in the mouth cavity, many authors assert that solar radiation is not the leading element in the development of tumors in dogs, whereas in humans, ultraviolet irradiation is one of the main predisposing factors. Melanoma is most often encountered in males, comprising from 2 to 9% of all malignant neoplasms. As a rule, it occurs in dogs older than 10 years. Skin melanoma is more common in dogs of small breeds, especially in Cocker Spaniels, poodles, Scottish terriers, cockers, dachshunds and the dogs of other breeds with a strong pigmentation of the tunica mucosa of mouth and skin covering. Some discrepancy in the data for pedigree predisposition of dogs to the emergence of melanoma can be relative to the pronouncement of the population of

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a particular breed in a country where the study was conducted (Mitin V.N., Kozlovsky N.G., 2005). As noted by (Folkman J., Cole P., Zimmerman S., 1966), (Williams L. E., Packer R. A., 2003), (Dewhirst M. W. *et al.*, 1985), (Hyman J. A., Koch S. A., Wilcock B. P., 2002), (Minami T., Patnaik A. K., 1992) melanoma is characterized by violent and early metastasis. The two main ways metastasizing are lymphatic and hematogenous. Regional lymph nodes, less distant ones are affected most often. There are often metastases in the skin. They have the form of multiple small, rashes of brown or black color slightly raised above the skin. Hematogenous metastases may occur in any organ, but usually he lungs, liver, brain and adrenals are affected.

## METHODS

Diagnosis of melanoma in dogs was conducted on the basis of physical examination of animals, hematological, cytological and histological studies.

For cytological examination smears obtained by applying the glass slide to ulcerated tumor surface were taken, then the surplus fluid were removed using a paper towel. The smears were colored by the Leishman's method.

For histological examinations the material were obtained by incisional and excisional biopsy. The obtained material was fixed in 10% neutral formalin aqueous solution. To obtain histological sections paraffin embedding is used. The method of coloring sections with hematoxylin and eosin was used. Microscopic examination was carried out using a microscope of Nikon Eclipse E100 brand.

Haematological and biochemical examinations of blood of ill animals subject to finish treatment were carried out using a Abacus junior hematology analyzer and Stat-Fax-1904 biochemical analyzer, for the following parameters: number of erythrocytes, leukocytes, platelets, lymphocytes, hemoglobin, erythrocyte sedimentation rate, total protein, glucose, cholesterol, creatinine, total bilirubin.

It was established that by differential counting of leukocytes and lymphocytes in neoplasms of the skin in dogs the arithmetical average data exceed compared to the norm.

## RESULTS AND DISCUSSION

### Haematological examination of blood

We examined 78 dogs with suspected tumors of the skin in the "Talap" veterinary clinics from September 2013 to December 2014.

Skin melanoma was found in 16 of 78 dogs (20.5% of total number examined dogs) and melanocytoma in 8 dogs (10.2% of the total number of the examined dogs). 24 dogs were subjected to complete examination, including hematological, cytological and histological study.

As a result of hematological examinations of the dogs blood deviations were detected in all respects compared with the norm.

**Table 1.** Occurrence of melanoma and melanocytoma in dogs

| Localization of a tumor          | Number |           |           |
|----------------------------------|--------|-----------|-----------|
|                                  | Total  | Malignant | Benignant |
| Mouth cavity                     | 15     | 10        | 5         |
| Lips                             | 7      | 5         | 2         |
| Eyes                             | 2      | 1         | 1         |
| Total number of dogs with tumors | 24     | 16        | 8         |

**Table 2.** Determination of melanoma and melanocytoma incidence in dogs depending on age

| Localization of a tumor and age of an animal | Number |           |           |
|--|--------|-----------|-----------|
|  | Total  | Malignant | Benignant |
| 0-3 years old                                | 3      | 3         | 0         |
| 3-6 years old                                | 1      | 1         | 0         |
| 6-9 years old                                | 5      | 2         | 3         |
| 9-12 years old                               | 8      | 4         | 4         |
| 12 years old and over                        | 7      | 6         | 1         |
| Total number of dogs with tumors             | 24     | 16        | 8         |

0-3 years old - 3 cases (12.5% of all dogs with spontaneous tumors), 3 of them are malignant (100% of cases in this age group), 0 - benignant (0%);

3-6 years old - 1 case (4%), 1 of them is malignant (100%) 0 - benignant (0%);

6-9 years old - 5 cases (20%), 2 of them are malignant (40%), 3 - benignant (60%);

9-12 years old - 8 cases (33.3%), 4 of them are malignant (50%), 4 - benignant (50%);

12 years old and over - 7 cases (29%), 6 of them are malignant (85%) 1 - benignant (14.3%).

**Table 3.** Distribution of the incidence of melanoma and melanocytoma in dogs depending on the breed

| Breed                     | Melanoma |      | Melanocytoma |      |
|---------------------------|----------|------|--------------|------|
|                           | Number   | %    | Number       | %    |
| Golden Retriever          | 4        | 25   | 0            | 0    |
| Half-breed                | 4        | 25   | 4            | 50   |
| Poodle                    | 2        | 12.5 | 0            | 0    |
| Labrador                  | 2        | 12.5 | 0            | 0    |
| Tibetan Terrier           | 1        | 6.25 | 0            | 0    |
| Pekinese                  | 1        | 6.25 | 0            | 0    |
| Bernese Mountain Dog      | 1        | 6.25 | 0            | 0    |
| Rottweiler                | 1        | 6.25 | 1            | 12.5 |
| Briard                    | 0        | 0    | 1            | 12.5 |
| Soft-coated Irish Terrier | 0        | 0    | 1            | 12.5 |
| Fox terrier               | 0        | 0    | 1            | 12.5 |
| Total                     | 16       | 100  | 8            | 100  |

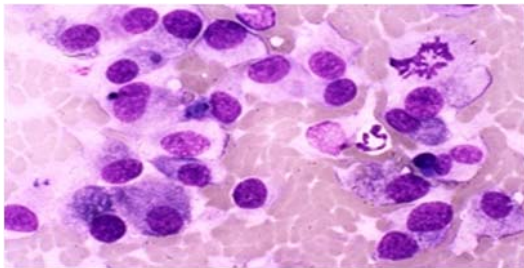
In the study of hemoglobin in the blood of animals of the examined groups its decline by 18%, respectively with  $P < 0.05$  was noted.

Besides that the decrease of the number of erythrocytes by 9.3% was also noted. Our examinations show that the reduction of these indicators is clinically significant and corresponds to a low level of acceptance probability ( $P < 0.05$ ).

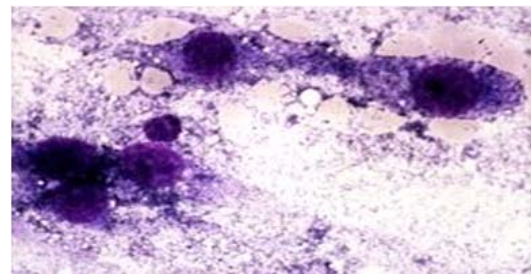
Normochromic anemia, which arose due

to neoplastic processes occurring in the skin, hemolysis of erythrocytes and hemoglobin synthesis abnormality is observed in the tested animals.

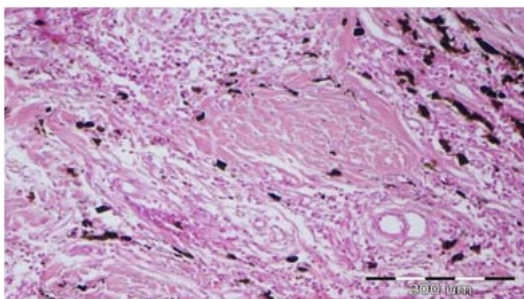
Our assumption confirms the presence of thrombocytopenia in animals, where the number of platelets is decreased by 12.5% compared with the norm.



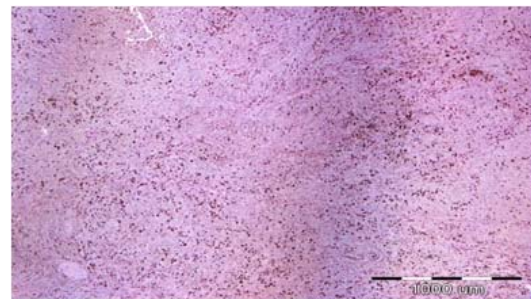
**Fig. 1.** Epithelioid melanoma. Cytogram, Leishman staining.



**Fig. 2.** Spindle-shaped cells melanoma. Cytogram, Leishman staining



**Fig. 3.** Weakly pigmented melanocytoma: (x 20; staining by hematoxylin - eosin)



**Fig. 4.** Melanoma of mixed-cell-type (x 100; staining by hematoxylin - eosin)

Erythrocyte sedimentation rate (ESR) for neoplasms on the dogs' skin is higher in comparison with the norm by 19.0%.

The obtained data suggest that the increase in erythrocyte sedimentation rate is one of the valuable indicators for cancer, because it gives an opportunity to make out the differential diagnosis between inflammation process and a neoplasm.

It was established that the differential counting of leukocytes and lymphocytes in neoplasms of the skin in dogs the arithmetical average data exceed compared to the norm.

So the difference in percentage of leukocytes number in dogs was 25.8% with a high level acceptance probability ( $P < 0.001$ ). The increase in digital indicators shows the initial stage of leukocytosis.

In the study of biochemical parameters of all animals' blood the level of glucose increased by 8.4%, respectively, at a high level of acceptance probability ( $P < 0.001$ ). At the same time the increase in the amount of total bilirubin by 16.2% was revealed, which indicates abnormality of the energy function and liver pigmental metabolism.

Abnormal liver function is observed in dogs with melanoma, which indicates the increase of the level of cholesterol in the blood serum and this figure was above the normal range of 5.8%.

Despite the changes in the biochemical blood picture in ill animals, the amount of total protein remained within the normal range, indicating that the preservation of protein - synthesizing function of the liver.

All registered changes in the morphological and biochemical indicators of blood in ill dogs with melanoma show the development of normochromic anemia, increase of erythrocyte sedimentation rate, hypoglycemia, hyperbilirubinemia, increased amount of cholesterol.

### **Results of cytological and histological examinations**

The materials of study of drugs of pathological conditions of the skin are presented below. Every form of tumor is characterized taking into account the data obtained from the microscopic (cytological) study. As it can be seen in the micrograph (Figure 1) tumor cells in smears are of round, incorrectly rounded and oval form, located

separately in form of friable aggregates that bear a resemblance with epithelial cells.

The cell nucleuses are blade, bean-shaped, have irregular contours. Nucleus chromatin structure is relatively uniform, fine-grained. Cytoplasm of cells is abundant, with smooth contours.

As it can be seen in the micrograph (Figure 2), tumor cells have an elongated spindle-shaped and located separately, by clusters, intertwined by cytoplasm appendages in the form of beams.

The nuclei are round and oval. Cytoplasm is colored basophilously, it has appendages of different lengths. Dust-like melanin granules are mostly concentrated in the appendages of the cytoplasm, which gives them a grainy appearance.

The results of histological examinations are presented in the figures 3, 4.

As it can be seen in the photomicrograph (Figure 3), infiltrate of mononuclear cells, some of which contain large amount and some minimal amount of melanin, is observed in soft tissues. The cells found in humus tissue are extremely edematous, and dilatation of apocrine glands and strong pigmentation of the epidermis is observed in subepidermal tissue.

As it can be seen in the micrograph (Figure 4), hyperplasia of melanocytes is observed in tumor tissue. The cells are well pigmented by melanin. In some cells cell structure cannot be even seen due to pigmentation. Less pigmented cells are mostly round with large round nuclei. The cells borders are not distinguishable. In some spots the spindle-shaped cells which in most cases are also pigmented are seen. Strong pleomorphism is pronounced. In some melanocytes cytoplasm granularity can be seen, also dual-core and giant cells are revealed.

### **Analysis of data concerning melanoma and benign(ant) incidence**

From the data on the incidence of melanoma and benign(ant) 16 of 24 tumors were malignant, 8 - benignant.

Thus, 66.6% of all tumors accounted for malignant neoplasms, and 33.3% - benignant. (Table 1).

The tumors of surface localizations were most accessible for examination during outpatient examination.

Thus, tumors more frequently affect the mouth cavity (62.5), then the lips (29.2%) and the eyes (8.3%) of dogs.

Melanoma and melanocytoma in dogs were diagnosed in different age groups (Table 2).

Thus, in the first place the dogs aged 9 to 12 years develop melanoma and melanocytoma most often. The second place is taken by the age group of from 12 years and older. The average age of the dogs suffering from spontaneous tumors is approximately 9.4 years. At the same time in the most adult age group of dogs (12 years and older) the incidence of malignant tumors is significantly higher than the number of benign tumors. In other age groups, the number of dogs with malignant and benign tumors is about the same.

Among the dogs affected by melanoma, the percentage of females was 25% and males 75% (12 males and 4 females).

Among the dogs affected by melanocytoma, the percentage of females was 37.5%, the percentage of males -62% (5 males and 3 females).

Thus, preferably males develop melanoma and melanocytoma.

During outpatient examination we recorded neoplasms in dogs of different breeds, as well as in half-breed and outbred dogs (Table 3).

The relative share of purebred dogs, suffering from melanoma patients and melanocytoma, was 67%, half-breed dogs - 33.3%.

Most often among the diseased purebred dogs there are: Golden Retrievers (25%), poodles and Labradors (12.5%). Less frequently tumor diseases were diagnosed in such breeds as: Tibetan Terrier, Bernese Mountain Dog, Rottweiler, briard, Soft-coated Irish Terrier, Fox Terrier and Pekinese (6.25%).

## CONCLUSIONS

Thus, during examination of the blood of the dogs with skin melanoma there are observed various deviations of hematological parameters of blood and erythrocyte sedimentation rate, glucose, cholesterol, creatinine, and total bilirubin, the emergence of anemia processes, affecting the intensity of natural organism protection. Also it was found that the pathological characteristics of spontaneous melanoma in dogs are very similar to

human skin melanoma according to morphological pattern and the clinical course.

We found that among total number of examined dogs, 16 of 24 neoplasms were malignant and 8 - benign. Thus, 66.6% of all tumors accounted for malignant neoplasms, and 33.3% - benign.

At the outpatient examination of dogs preferential localization of tumors was determined. According to our data neoplasms affect the oral cavity (62.5), then mouth (29.2%) and eyes (8.3%).

As for the age the dogs aged 9 to 12 years suffer from melanoma melanocytoma most often. The average age of the dogs suffering from spontaneous tumors is approximately 9.4 years.

The percentage of males affected by melanoma and melanocytoma was 70.8% and the proportion of females 29.2%, respectively.

Golden retrievers and half-breed dogs are susceptible to melanoma and melanocytoma most of all breeds of dogs.

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