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Research article

COMPARISON OF THE SERUM PROTEIN ELECTROPHORETIC PATTERN AND CONCENTRATIONS OF ACUTE PHASE PROTEINS IN BITCHES WITH AND WITHOUT MAMMARY GLAND TUMORS

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Alterations in the serum protein pattern may be associated with many diseases, including neoplastic processes. In veterinary medicine, these changes are poorly understood. Therefore, this study was aimed at the analysis of the distribution of blood serum protein fractions separated by agarose gel electrophoresis, and at the determination of the concentrations of main acute phase proteins in bitches with mammary gland neoplasia. The evaluation was conducted on twelve female dogs with palpable single or multiple nodules in the parenchyma of the mammary gland and on ten tumor-free clinically healthy bitches to compare the possible differences in the obtained results. Blood serum was used to perform agarose gel electrophoresis of the main blood serum protein fractions and to analyze the concentrations of total serum proteins and the following canine acute phase proteins: serum amyloid A, haptoglobin, C-reactive protein and α_1-acid glycoprotein. The concentrations of total serum proteins were slightly higher in bitches with mammary gland tumors. Serum protein electrophoresis showed lower mean concentrations of albumin and α_1-globulins in the affected dogs, while the concentrations of α_2- and β_1-globulins were significantly higher ($P=0.0032$ and $P=0.0021$, respectively) compared to dogs without mammary gland tumors. In the concentrations of acute phase proteins, significantly higher mean concentrations of C-reactive protein and haptoglobin were obtained in dogs with mammary tumors ($P=0.0025$ and $P=0.0002$, respectively). The values of α_1-acid glycoprotein did not vary markedly between the bitches with and without mammary tumors. Presented data suggest that neoplastic processes in the mammary gland may also alter the electrophoretic pattern of blood serum proteins and induce changes in the production of some inflammatory proteins.

Keywords: Acute phase proteins, bitches, electrophoresis, mammary gland neoplasia, serum proteins, protein fractions

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INTRODUCTION

Mammary gland tumors belong to the most frequently occurring neoplastic diseases in bitches, representing more than 50 % of all tumors in female dogs [1,2]. Furthermore, approximately half of all surgically removed tumors of the mammary gland in bitches are classified as malignant by histological examination, and often have a fatal ending [3-5]. The most commonly diagnosed types of mammary tumors in bitches are tubular carcinoma (adenocarcinoma), papillary carcinoma, solid carcinoma, complex carcinoma and carcinosarcoma [6]. Although the pathogenesis of canine mammary tumors has not yet been completely understood, several biological and immunohistochemical characteristics of the mammary masses are comparable in humans and dogs [7,8]. In human medicine, various tumor-associated antigens were introduced as biomarkers to early diagnose and follow-up breast cancer, and to predict the response or resistance to specific therapy and surveillance after primary surgery [9-12]. In addition to these specific markers, acute phase proteins have been intensively studied in women with breast cancer, especially their significance as prognostic indicators [13,14]. Some studies were conducted also in female dogs with mammary tumors, which were oriented to the analysis of main canine acute phase proteins (C-reactive protein, serum amyloid A, haptoglobin) and the magnitude of their increase [15,16]. However, it has not yet been studied whether mammary tumors may cause alterations in the serum protein electrophoretic pattern and distribution of protein fractions.

Electrophoretic separation of blood serum proteins is one the basic laboratory tests used to describe abnormalities in the serum protein profile, evaluate the distribution of protein fractions, and differentiate between the types of hyperproteinemias. Although in the most of cases the results of serum protein electrophoresis are not sufficient to determine a specific disease, they may be helpful in differential diagnosis reducing the list of diseases considered, and in the detection of stimulated humoral immune response [17]. The alterations observed in protein metabolism are usually secondary changes in various diseases and disorders, but in certain conditions serum protein electrophoresis may show some typical morphologic signs suggesting a specific disease [18]. In people, characteristic electrophoretic patterns have been described for some pathological conditions, including patients with malignant tumors, tumors with immunosuppressive properties, as well as multiple myeloma [19]. In veterinary medicine, agarose gel electrophoresis showed in cats with lymphoma bands of markedly increased intensity for α-, β- and γ-globulins, and narrow electrophoretic spikes in the β/γ-globulin zone in some of them [20]. However, the electrophoretic pattern of serum proteins and the distribution of protein fractions in female dogs with malignant masses in the mammary gland is less well studied. Therefore, the purpose of this study was to separate blood serum proteins in bitches with mammary gland tumors, determine the concentrations of main protein fractions, evaluate the changes in their distribution, and to analyze the serum concentrations of the major acute phase proteins.

MATERIAL AND METHODS

Ethical approval

A written informed consent and permission to blood collection and sample analyses were obtained from all dog owners. The blood samples from the dogs were collected as per standard sampling procedure used without any harm to the animals. All procedures with animals in the study were conducted in accordance with the ethical standards and guidelines approved 29 January, 2019 by the Committee of the University of Veterinary Medicine and Pharmacy in Košice on protection of animals used for scientific purposes and complied with the institutional requirements of the Code of Ethics for Scientists (Directive 74/2019/UVLF).

Animals and sample collection

Twelve female dogs with palpable single or multiple nodules in the parenchyma of the mammary gland were included into the study. All animals were clinical patients admitted and cared for at the University Veterinary Hospital of the University of Veterinary Medicine and Pharmacy in Košice (Slovak Republic). They were at the age of 10 to 17 years (median 13.0) and were of mixed breeds. Mammary tumors were diagnosed based on anamnesis, palpation, inspection, and general clinical findings. The standard clinical examination was completed with the evaluation of the characteristics, shape, size, location of the masses in the mammary gland, presence of inflammatory reaction and ulceration, as well as enlargement of inguinal and axillary lymph nodes. Cancer stage was determined using the internationally accepted TNM classification system [21]. Most of the dogs with mammary nodules were brought to the clinic in a later and more progressive stage of the disease. Three thoracic X-ray projections were performed also: dorsoventral or ventrodorsal, and right and left laterolateral projections. No further diseases were detected in the evaluated animals.

The mammary masses were surgically extracted performing mammectomy, regional mastectomy, unilateral or bilateral mastectomy depending on the tumor size and local infiltrative growth according to the surgical oncological protocol designed by Gilson and Stone [22]. Before surgery, animals were subjected to preoperative electrocardiography. All surgical procedures were performed under general anesthesia. Biopsy samples (1 cm^3) were taken from the margins between healthy and diseased tissue of the extirpated neoplasm and submitted to the Department of Pathology for verification and determination of the final diagnosis of mammary neoplasia by histopathological examination. The collected tissue samples were fixed in phosphate buffered 10% neutral formalin solution, processed routinely, embedded in paraffin, stained with hematoxylin and eosin, and coverslipped. The tissue sections were histopathologically evaluated to determine the histologic type and degree of malignancy of the tumors according to WHO guidelines [23]. The size of neoplastic masses and

type of mammary tumors diagnosed according to histopathological examination are shown in Table 1.

Table 1. Clinical and histopathological findings in the evaluated bitches

Patient No.	Age (years)	Clinical findings	Histopathological diagnosis
1.	13	multiple nodules 0.5-2 cm in diameter in the right caudal glands 3-5, and in left gland 1	solid carcinoma
2.	13	single nodule 0.5x0.5 cm in size in the right gland 3	tubulopapillar carcinoma
3.	11	solitary firm nodule 4x2.5 cm in the right gland 5	tubulopapillar carcinoma
4.	11	single nodule 1x2 cm in the right glands 4,5	adenocarcinoma
5.	17	open ulcerative nodule 2 cm in diameter and further small nodules in the left glands 3-5	fibrosarcoma
6.	15	large nodule in the right mammary gland (from gland 4 to 6) with several necrotic changes	cystic papillar adenocarcinoma
7.	11	single firm nodule between the left glands 4-5	benign adenoma
8.	13	single ulcerative nodule 5x3 cm in size in the right gland 2, palpable changes in the right gland 5	adenocarcinoma
9.	10	large single nodule in the left gland 4	fibrosarcoma
10.	10	large single nodule in the left glands 2-3 with signs of inflammation	solid carcinoma
11.	13	multiple nodules in the right glands 3-5	anaplastic carcinoma
12.	16	single nodule 5x4 cm in size in the left glands 1-3	carcinoma

The control group of animals consisted of eighteen clinically healthy female dogs (of mixed breeds at the age of 5 to 10 years) that were clinically examined and laboratory tested during routine preventive clinical examination and vaccination. They were in good general condition, showed no abnormal clinical signs, no mammary lesions and alterations in laboratory results.

Laboratory analyses

Blood samples for laboratory analyses were collected into tubes with clotting activator and gel separator (Sarstedt, Germany) by direct puncture of *v. cephalica antebrachii* or *v. saphena medialis* in both groups of dogs. Blood samples were collected at the time of initial examination, and prior to the surgical intervention in sick animals. The samples were allowed to clot at room temperature for 30 min and then centrifuged at 3,000g for 15 min. The separated serum was placed in Eppendorf tubes and used for the analysis of the concentrations of total serum proteins (TP, g/l), the electrophoretic

evaluation of the serum protein pattern and the determination of the following acute phase proteins: serum amyloid A (SAA, μg/ml), haptoglobin (Hp, mg/ml), C-reactive protein (CRP, μg/ml), and α_1-acid glycoprotein (AGP, mg/ml).

The biuret method was applied to measure the TP concentrations by commercially available diagnostic kits (Randox, Crumlin, United Kingdom) using the automated chemistry analyzer Alizé (Lisabio, Poully en Auxois, France). The separation and distribution of serum protein fractions was performed by zone electrophoresis on agarose gel using an automated electrophoresis system Hydrasys with commercial diagnostic kits Hydragel 7 Proteine (Sebia Corporate, Lisses, Evry Cedex, France) according to the application instructions of the manufacturer. Sebia Normal Control Serum (Ref. 4785) was used for the quality control of electrophoretic quantification. After separation of proteins in the sample, the gels were stained to reveal individual protein fractions and identify them. The width of protein bands and the staining intensity was evaluated using a densitometer Epson Perfection V700 (Epson America Inc., California, USA), specially adapted to scan electrophoretic gels according to light transmission and conversion into optical density curve. The gel images were visualized using the image analysis software Phoresis version 5.50 (Sebia Corporate, France) into characteristic graphs. The identified protein fractions were visually corrected. The protein fractions were expressed as relative values (%) according to the optical density and their absolute concentrations (g/l) were quantified from the TP concentrations. Albumin:globulin ratios (A/G) were calculated as well.

SAA was determined by sandwich type of enzyme-linked immunosorbent assay (ELISA) with TP-802 Multispecies SAA test (Tridelta Development, Kildare, Ireland). Canine CRP was analysed using TP-803 solid-phase CRP ELISA test (Tridelta Development, Kildare, Ireland). Haptoglobin was measured spectrophotometrically with TP-801 colorimetric tests (Tridelta Development, Kildare, Ireland) in microplates. Canine AGP was quantified by commercially available solid phase ELISA kit (AGP-4, Life Diagnostics, Inc., West Chester, PA, USA). The absorbance was read on Opsys MR automatic microplate reader (The Dynex Technologies, USA). The Revelation QuickLink version 4.25 computer software was used for the calculation of results (The Dynex Technologies, USA).

Statistical analyses

The obtained data were analyzed using the statistical computer program GraphPad Prism V5.02 (GraphPad Software Inc., California, USA). Descriptive statistics was applied to calculate mean values and standard deviations. Kolmogorov-Smirnov test was performed to assess normality of data, and the significance of differences between the groups of animals was analyzed by Mann-Whitney U-test. The level of significance was estimated at $P<0.05$.

RESULTS

In bitches with tumors, as well as without tumors six protein fractions were identified, including albumin, α_1-, α_2-, β_1-, β_2- and γ-globulins (Figure 1a-d). Albumin was the most prominent fraction on the serum protein electrophoretogram and was characterized by a high and narrow peak. The α-globulins migrated into α_1- and α_2-subfractions, from which the α_1-globulin fraction was presented as a low peak, while the α_2-globulin fraction as a peak with moderate and sharper electrographic amplitude in bitches with mammary gland tumors (two bitches with markedly higher and narrow electrophoretic spike, see Figure 1b,c). In the β-globulin zone, two subfractions (β_1 and β_2) have been identified with an approximately similar shape, and the β_2-globulin fraction was clearly demarcated from the γ-globulin fraction (see Figure 1d). Beta/gamma bridging was observable only in one sick animal (Figure 1c).

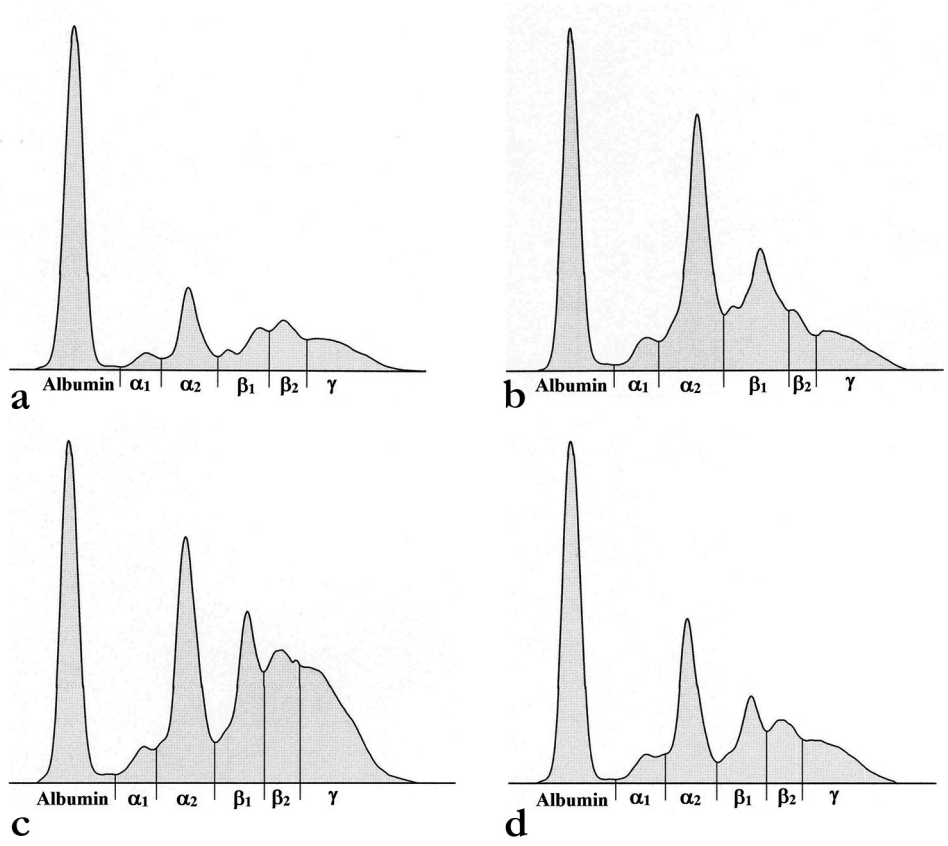

Figure 1. Electrophoretograms of clinically healthy dog (**a**) and dogs with carcinomas of mammary gland (**b** – dog with adenocarcinoma, multiple nodules; **c** – dog with solid carcinoma, large single nodule; **d** – dog with anaplastic carcinoma, multiple nodules).

The descriptive data of the relative concentrations of protein fractions in the serum are demonstrated in Table 2. The presence of mammary tumors significantly affected the relative concentrations of albumin, being significantly lower in bitches with mammary tumors compared to those without tumors (P=0.0149). Significantly lower relative values in bitches with mammary tumors were obtained also for α_1-globulins (P=0.0090). On the other hand, the relative concentrations of α_2- and β_1-globulins were significantly higher (P=0.0199 and P=0.0117, respectively). The concentrations of β_2- and γ-globulins were slightly higher in tumor bearing bitches compared to heathy ones. Significant differences between the groups of dogs were obtained also in the A/G ratios, with significantly lower values in sick dogs (P=0.0149).

Table 2. Differences in the relative concentrations of serum protein fractions (%) and albumin/globulin ratio (A/G) between the evaluated groups of dogs (mean ± SD, median in parenthesis)

Variables	Groups of dogs		P value
	with tumours	healthy	
Albumin	44.5 ± 10.6 (45.4)	53.2 ± 3.6 (51.8)	0.0149
α_1-globulins	4.1 ± 0.5 (3.9)	4.9 ± 0.9 (4.9)	0.0090
α_2-globulins	18.6 ± 4,8 (18.6)	15.0 ± 1.6 (14.8)	0.0199
β_1-globulins	12.8 ± 3.6 (12.6)	9.7 ± 2.0 (9.0)	0.0117
β_2-globulins	9.8 ± 2.3 (10.1)	9.1 ± 1.2 (9.0)	0.2897
γ-globulins	10.2 ± 4.4 (9.5)	8.0 ± 1.6 (8.2)	0.0942
A/G	0.86 ± 0.34 (0.83)	1.15 ± 0.18 (1.08)	0.0149

P value – significance of the differences, A/G – albumin/globulin ratio

The total serum protein concentrations were higher in bitches with mammary tumors (P=0.0987, Table 3). Further analyses of the absolute concentrations of serum protein fractions showed lower values of albumin and significantly lower values of α_1-globulins (P=0.1500 and P=0.0415, respectively) in bitches with mammary tumors than in animals without tumors. An opposite trend of significantly higher concentrations was observed in α_2- and β_1-globulins (P=0.0032 and P=0.0021, respectively). Higher concentrations in bitches with mammary tumors were obtained also for β_2- and γ-globulins (P=0.1271 and P=0.0593, respectively).

Analysis of the concentrations of acute phase proteins showed in bitches with mammary gland tumors significantly higher values of CRP and Hp (P=0.0025 and P=0.0002, respectively). While the mean CRP concentration was more than 7-fold higher in sick dogs compared to healthy animals, the mean value of Hp was approximately 5.5 times higher in bitches with mammary tumors. The mean SAA concentration in sick dogs was about 12-fold higher than in healthy animals. In the concentrations of AGP no marked differences were found between the groups of dogs, its mean value was only slightly higher in female dogs with mammary tumors.

Table 3. Differences in the concentrations of total serum proteins (TP, g/l) and absolute values of protein fractions (g/l) between the evaluated groups of dogs (mean ± SD, median in parenthesis)

Variables	Groups of dogs		P value
	with tumours	healthy	
TP	62.0 ± 4.6 (63.3)	58.9 ± 4.6 (58.8)	0.0987
Albumin	27.7 ± 7.2 (27.8)	31.4 ± 3.1 (30.7)	0.1500
α_1-globulins	2.5 ± 0.3 (2.5)	2.8 ± 0.4 (2.8)	0.0415
α_2-globulins	11.5 ± 2.8 (11.2)	8.9 ± 1.2 (9.1)	0.0032
β_1-globulins	7.9 ± 2.0 (7.9)	5.7 ± 1.4 (5.5)	0.0021
β_2-globulins	6.1 ± 1.5 (6.3)	5.4 ± 1.0 (5.2)	0.1271
γ-globulins	6.4 ± 2.9 (5.9)	4.8 ± 1.0 (4.7)	0.0593

P value – significance of the differences, TP – total proteins

Table 4. Differences in the concentrations of evaluated acute phase proteins between the evaluated groups of dogs (mean ± SD, median in parenthesis)

Variables	Groups of dogs		P value
	with tumours	healthy	
C-reactive protein (ug/ml)	27.8 ± 37.0 (8.7)	3.8 ± 5.3 (1.7)	0.0025
Haptoglobin (mg/ml)	8.1 ± 6.9 (5.4)	1.4 ± 1.5 (0.9)	0.0002
Serum amyloid A (ug/ml)	23.0 ± 33.6 (1.0)	1.9 ± 2.3 (0.7)	0.3343
Alpha-1-acid glycoprotein (mg/ml)	1.8 ± 1.6 (1.1)	1.4 ± 1.2 (1.1)	0.5393

P value – significance of the differences

DISCUSSION

Several types of cancers were associated with abnormal pathophysiological changes at sites distant from primary tumors, altered laboratory findings, as well as systemic responses of the organism with the release of cytokines and consequently increased production of acute phase proteins [24,25]. Tumor growth may directly affect hematological parameters (causing especially anemia and thrombocytopenia) and results also in changes of some biochemical parameters [26]. An increase of total serum protein concentrations was found by Al-Muhtaseb [27] in female patients with breast cancer, as a result of stress, damage, tissue remodeling, and cell death associated with the neoplastic process. The concentrations of total serum proteins were found to be slightly increased in our study in bitches with mammary tumors compared to tumor-free dogs, especially due to the increase of γ-globulins by the

stimulated immune system. These findings are in agreement with those presented by Satilmis et al. [28] in bitches with mammary tumors compared with those from the control group. The highest values were obtained in bitches with a tumor size greater than 5 cm and with tumors in multiple mammary units. Furthermore, Oliveira et al. [29] found differences in the concentrations of total serum proteins according to the clinical staging and progression of mammary tumors, with the highest mean value in bitches in the early clinical stage of the disease and the lowest mean value in animals with inflammatory carcinoma. Among alterations, hypoalbuminemia was a typical finding in the aforementioned study, especially in dogs in advanced clinical staging and with inflammatory carcinoma, reflecting disease progression and prognosis. Lower concentrations of albumin were recorded also in our study in bitches with mammary tumors compared to those without tumors, which was related to the host systemic response to tumor development. Albumin is referred as the major negative acute phase protein with markedly reduced synthesis during inflammatory responses, when the majority of amino acids are used mainly for the synthesis of positive acute phase proteins [30]. Furthermore, albumin belongs to valuable markers for assessing patient's nutritional status in clinical practice, and its lower concentrations recorded in our study may reflect its suppressed synthesis by hepatocytes and inadequate nutritional status in bitches with mammary tumors. In cats with spontaneous malignant mammary tumors, lower albumin concentrations were detected at diagnosis of the disease. The decrease of albumin was significantly associated with neoplastic vascular emboli, metastasis in regional lymph nodes or in distant organs [31]. In our study, the mean concentration of albumin in bitches with mammary tumors was lower compared to animals without tumors, but the standard deviation in the group of sick dogs was relatively higher suggesting different host responses and clinical picture of animals with cancer, as well as heterogeneous pathological features and microenvironment of mammary tumors.

More marked alterations compared to albumin were observed in the distribution of globulin fractions. Although an overall increase of globulins was found previously by Costa-Santos et al. [32] in female dogs with mammary tumors. This was related to the increase in the concentration of hormone binding globulins and it is the first study that investigates the influence of mammary neoplasia on the distribution of globulin fractions. The results showed higher concentrations of α_2-, β_1-, β_2-, as well as γ-globulins resulting from the direct effect of tumor growth, rapid cellular proliferation, and consequent disorders in plasma protein synthesis. While the mean concentration of α_1-globulins in our study was slightly lower in bitches with mammary tumors than in those without tumors, the mean concentration of α_2-globulins was markedly higher and accounted for more than 18% of total serum proteins, thus representing the most dominant fraction from the globulin fractions. The α-globulin fractions are composed of many diagnostically important inflammatory proteins, from which AGP and SAA can be detected in the α_1-zone [19]. Although AGP was described in humans as an important marker of inflammation, as well as different types of cancer [33,34], the data obtained in animals are not uniform. Previous studies have shown that the serum

concentrations of AGP may increase approximately nine-fold or more in dogs during the acute phase response induced by some inflammatory states, including *Ehrlichia canis* infection and pyometra [35,36]. The mean AGP concentration found by Hagman [36] in dogs with pyometra was 1943 ± 913 mg/l, which was significantly higher compared to healthy dogs (495 ± 204 mg/l). Regarding neoplastic diseases, increased AGP concentrations have been reported previously by Ogilvie et al. [37] in dogs with lymphoma, various sarcomas and carcinomas except mammary carcinoma. In the study presented by Thougaard et al. [38], the median concentrations of AGP in dogs with benign, as well as malignant mammary tumors were not significantly different from those in clinically healthy dogs. The aforementioned authors detected only three dogs (diagnosed with papillary carcinoma, complex carcinoma, and infiltrative ductular carcinoma) with very high serum AGP concentrations, while the remaining dogs had AGP concentrations lower than 750 mg/l. In our study, the mean AGP concentration in bitches with mammary tumors was only slightly higher compared to dogs without tumors. Similarly, although the mean SAA concentration was higher in bitches with mammary tumors than in tumor-free dogs, the differences between the groups were statistically not significant. Furthermore, the magnitude of increase obtained in this study in dogs with mammary tumors (approximately 12-fold) was less marked when compared with the increase observed by Szarková et al. [39] in dogs suffering from parvoviral enteritis and pyometra (more than 200-fold). These findings suggest that tumor formation in the mammary tissue evoke less marked alterations in the production of some inflammatory proteins observable also in the serum protein electrophoretogram. However, the wider range of individual values recorded in the evaluated animals (from almost undetectable to 81.20 μg/ml) suggest differences in the reactivity of animals to tumor formation and development in various organs. Furthermore, the animals were brought to the hospital at a different stage of tumor formation, which may also explain the great variability among the measured values. In the study conducted by Tecles et al. [15], significantly higher concentrations of SAA were obtained only in those bitches with mammary tumors that had metastasis or the diameter of tumor was greater than 5 cm and had ulcerations. On the other hand, bitches with mammary tumors in the I-III stages of the disease presented only weak inflammatory response. O´Hanlon et al. [14] recorded similar findings in human patients with breast carcinoma.

More marked and significant magnitude of increase was observed also in the concentrations of Hp, contributing to significantly higher α_2-globulin concentrations in bitches with mammary tumors. Alterations in serum protein electrophoretic pattern with significantly elevated α_2-globulin fraction, as well as increased production of Hp have been observed in dogs with lymphatic neoplastic diseases [40,41]. Mammary gland tumors were found to induce an increase of Hp synthesis also in female dogs and cats with spontaneous malignant mammary tumors [42,31]. However, these studies suggested that mammary tumors in dogs were weaker inducers of inflammatory responses compared to cats, unless if they were of big dimensions, specific histopathological

types or were associated with metastasis, ulceration or secondary inflammation. The magnitude of increase of Hp concentrations recorded in our study in dogs with mammary tumors was comparable to that recorded previously by Szarková et al. [39] in dogs with parvoviral enteritis, acute pancreatitis and pyometra (approximately 5-fold). Among the several other proteins from the α_2-globulin fraction, ceruloplasmin has been found to be an important indicator of tumor development and progression in patients with various forms of cancers, including breast cancer, since it is involved in angiogenesis and neurovascularization [43,44]. In veterinary medicine, the possible alterations in its production associated with neoplastic processes in the mammary gland are poorly understood and therefore, further studies are recommended to find out which proteins are responsible for the aforementioned changes in the distribution of protein fractions.

In the β-globulin region, bitches with mammary gland tumors presented significantly higher concentrations of β_1-globulins, while the β_2-globulins were non-significantly higher. C-reactive protein is an important protein detectable in this serum protein zone. It is not only a reliable marker of inflammation, but its concentrations may increase during regulation of inflammatory processes also in response to cancer [45]. The evaluation of this inflammatory reaction generated in response to tumor development is a critical factor for the assessment of tumor aggressiveness [46]. While Tecles et al. [47] recorded higher CRP concentrations only in bitches with mammary tumors greater than 5 cm and ulceration or in those which had metastasis, Planellas et al. [42] recorded no significant differences in CRP concentrations between dogs based on tumor size or lymph node metastasis. However, the median CRP concentration was significantly higher in dogs with ulcerated skin. Higher mean CRP concentration in bitches with mammary tumors was obtained also in our study suggesting that this tumor type is associated with the inflammatory response. However, the magnitude of CRP increase was less marked (approximately 7-fold) compared to the more than 50-fold increase in dogs with parvoviral enteritis, and to the approximately 30-fold increase in dogs with acute pancreatitis and pyometra [39]. Similarly to the aforementioned acute phase proteins, a wider range of values was observed also in the CRP concentrations, which may be related to its different production according to the heterogeneous pathological features and clinical behavior, formation and development of mammary gland tumors [48]. Although the majority of immunoglobulins are detectable in the γ-globulin zone, some classes of immunoglobulins (IgM and IgG) may migrate into the β-globulin fraction, and their increased production due to the activated immune response to tumor development might contribute to higher concentrations of β_2- and γ-globulins in bitches with mammary gland tumors. Childress [26] and Al-Muhtaseb [27] stated also that hypergammaglobulinemia may develop not only as a result of inflammatory state, but chronic cancer conditions may also increase the concentrations of γ-globulins, especially IgG, IgM and IgA. All the aforementioned changes in the serum protein composition resulted in alterations of the A/G ratio and in its shift from the physiological range in bitches with mammary gland tumors, which were

primarily related to changing globulin pattern due to the formation and proliferation of tumors in mammary tissue.

CONCLUSION

In this study the presented results suggest that the presence and mammary gland tumor development can also induce changes in the serum protein composition and concentrations of some acute phase proteins. Although the concentrations of total serum proteins were only slightly higher in bitches with mammary gland tumors, more marked cancer-related differences were observed in the distribution of serum protein fractions. While the proportion of albumin was lower in the dogs with mammary cancer, the concentrations of globulins, especially α_2-, β_1- and γ-globulins were higher in the affected dogs. In the concentrations of measured acute phase proteins, significantly higher concentrations of CRP and Hp were found in dogs with mammary tumors. A more marked magnitude of increase was observed in the values of SAA, but the increase was not comparable with that obtained in some canine infectious and systemic inflammatory diseases. The concentrations of AGP did not vary significantly between the bitches with and without mammary tumors. Based on the presented results, it is possible to conclude that spontaneous mammary gland malignancies alter serum protein electrophoretic pattern and the production of some inflammatory proteins. However, further studies and larger clinical trials with dogs affected by mammary gland neoplasia are needed to obtain more valid results if the aforementioned variables related to protein metabolism could be useful for a more precise diagnosis of canine mammary cancer, especially for treatment monitoring and to evaluate the continuation or maintaining of inflammatory state in this type of neoplastic processes.

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Authors' contributions

ON conceived and designed the study, revised the manuscript critically. CT performed the laboratory analyses, performed the statistical analyses, drafted the manuscript. AV and LH collected samples and contributed to data collection and analysis. All authors read and approved the final manuscript.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Statement of Informed Consent

The owner understood procedure and agrees that results related to investigation or treatment of their companion animals, could be published in Scientific Journal Acta Veterinaria-Beograd.

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POREĐENJE ELEKTROFORETSKOG OBRASCA SERUMSKIH PROTEINA I KONCENTRACIJA PROTEINA AKUTNE FAZE KOD KUJA SA I BEZ TUMORA MLEČNE ŽLEZDE

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Promene obrasca serumskih proteina mogu biti povezane sa mnogim bolestima, uključujući neoplastične procese. U veterinarskoj medicini ove promene su slabo izučene. Zbog toga je ova studija imala za cilj analizu distribucije frakcija proteina krvnog seruma razdvojenih elektroforezom u agaroznom gelu, kao i određivanje koncentracija proteina akutne faze kod kuja sa neoplazijom mlečne žlezde. Evaluacija je sprovedena na dvanaest ženki pasa sa palpabilnim pojedinačnim ili višestrukim nodulima u parenhimu mlečne žlezde i na deset klinički zdravih kuja bez tumora da bi se uporedile moguće razlike u dobijenim rezultatima. Krvni serum je korišćen za gel elektroforezu na agarozi glavnih frakcija proteina u serumu krvi i za analizu koncentracija ukupnih proteina u serumu i proteina akutne faze pasa: serumski amiloid A, haptoglobin, C-reaktivni protein i α_1-kiseli glikoprotein. Koncentracije ukupnih serumskih proteina bile su nešto veće kod kuja sa tumorima mlečne žlezde. Elektroforeza serumskih proteina je pokazala niže srednje koncentracije albumina i α_1-globulina kod obolelih pasa, dok su koncentracije α_2- i β_1-globulina bile značajno veće (P=0,0032 i P=0,0021) u poređenju sa psima bez tumora mlečne žlezde. U koncentracijama proteina akutne faze dobijene su značajno veće srednje koncentracije C-reaktivnog proteina i haptoglobina kod pasa sa tumorima mlečne žlezde (P=0,0025 i P=0,0002). Vrednosti α_1-kiselog glikoproteina nisu značajno varirale između kuja sa i bez tumora mlečne žlezde. Prikazani podaci sugerišu da neoplastični procesi u mlečnoj žlezdi takođe mogu da promene elektroforetski obrazac proteina krvnog seruma i indukuju promene u proizvodnji nekih inflamatornih proteina.