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Prostatic acid phosphatase in serum and semen of dogs

Fosfatasa ácida prostática en suero y semen de perros

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RESUMEN

La incidencia de cáncer de próstata ha incrementado el uso de los marcadores celulares para detectar el cáncer en este tejido. Antígenos específicos del tejido o antígenos de diferenciación se encuentran en la superficie de las células normales. Clínicamente, estos antígenos son importantes para el diagnóstico de alteraciones en estos tejidos y para la inmunoterapia. Este estudio trata de evaluar la importancia de la fosfatasa ácida prostática en la próstata canina e investigar su concentración en el suero y en el plasma seminal de perros saludables de diferentes edades. La concentración de fosfatasa ácida prostática en el plasma seminal y en el suero fue evaluada por espectrofotometría, utilizando un kit comercial. Los niveles de la fosfatasa ácida prostática (PAP) no fueron diferentes de acuerdo con la edad y no presentaron correlación con la edad o con las dimensiones de las próstatas verificadas por ecografía. Los valores de concentración de PAP presentaron una gran variación en cada grupo. Sin embargo, son necesarios más estudios para evaluar el papel de la fosfatasa ácida prostática en la próstata canina y su importancia como una prueba de diagnóstico para los trastornos de la próstata.

Palabras clave: fosfatasa ácida prostática, próstata, plasma seminal, suero.

SUMMARY

The incidence of prostatic malignancy has increased the use of tissue markers to detect cancer. Tissue specific antigens or differentiation antigens are found on the surface of normal cells. Clinically, these antigens are important to diagnose alterations in the tissues and for immunotherapy. The objective of the present study was to evaluate the prostatic acid phosphatase concentration in blood and seminal plasma of intact and healthy dogs at different ages. The evaluation was carried out by spectrophotometer, using a commercial kit. The prostatic acid phosphatase (PAP) levels did not differ according to the age and did not correlate with age or prostatic dimensions verified by ultrasonography. The PAP concentration values varied greatly within each group. However, more studies are necessary to evaluate the role of prostatic acid phosphatase in the canine prostate and its importance as a diagnostic test for prostate disorders.

Key words: prostate, prostatic acid phosphatase, seminal plasma, serum.

INTRODUCTION

Although the prostate is found in all mammals, it has greater importance in men, dogs and chimpanzees (Steiner *et al* 1999) due to the frequency of disorders. The dog has been used as a model to study the development of prostate disease in human beings, because it is the one of the animal species that spontaneously develops prostatic hyperplasia and prostatic adenocarcinoma (Barsanti and Finco 1992).

Prostatic adenocarcinoma is the main prostatic neoplasia in humans and dogs, especially in middle aged and elderly individuals and medium to large sized dogs (Swinney 1998). Some authors have suggested that the

great majority of cases occur in dogs older than 8 years of age (average 8.9 years) (Leav and Ling 1968, Krawiec and Heflin 1992). The risk of developing cancer is 2.38 times higher in castrated than in intact dogs (Bell *et al* 1991, Johnston *et al* 2000).

The high prevalence of prostatic malignancy has increased the use of tissue markers to detect cancer (Alvizatos *et al* 1992). Tissue specific antigens are found on the surface of normal cells. Clinically, these antigens are important to diagnose alterations in the tissues and for immunotherapy (Souza and Toniollo 2001). Markers that reflect the biological activity of the tumor support the decision on how aggressive a treatment should be (Lewenhaupt *et al* 1990).

In humans, the detection of increased levels of prostatic glucoproteins in serum, mainly prostate specific antigen (PSA) and prostatic acid phosphatase (PAP), is important for early diagnosis. Prostatic acid phosphatase can be found in high concentrations in the serum and prostatic tissue of healthy men and in higher concentra-

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tions in the serum of patients with disseminated prostatic cancer. Therefore, it had been used for years as a diagnostic test for prostatic cancer in men until the discovery of PSA (Stamey *et al* 1994). However, for veterinary patients, tissue markers of cancer are not routinely used for screening purposes in Brazil. In Europe, some laboratories have developed a test to detect canine prostate specific esterase (CPSE) in canine serum to diagnose prostate diseases such as BPH or prostatitis.

Phosphatase is one of the most abundant prostatic proteins of the human seminal fluid, which is the best source for isolation and purification. However, prostatic acid phosphatase is also found in most human tissue, such as liver, kidneys, spleen, placenta, erythrocytes, brain, leucocytes, blood serum and others (Fink *et al* 1985, Ostrowski and Kuciel 1994). It could be a restrictive factor for using PAP as a diagnostic tool, because serum concentrations are high in benign prostatic hyperplasia (BPH) and in non-prostatic diseases (Alivizatos *et al* 1992). However, the highest expression of PAP occurs in the prostate (Solin *et al* 1990) and its increase in serum is probably due to a degeneration of the prostatic secretory epithelial cells induced by prostatic alterations. The use of PAP measurement in human serum is important for therapeutic monitoring and prognostic evaluation (Lewenhaupt *et al* 1990, Graddis *et al* 2011).

As in humans, the PAP secretion in dogs is hormone-dependent, but the quantitative alterations are less evident in the prostate of these animals because the concentration of this enzyme is lower than that from the human gland. Immunohistochemical methods and immunoelectronic microscopy have demonstrated that the enzyme is a high sensitive marker of the structural integrity of the prostate secretor epithelium in dogs (McEntee *et al* 1987, Souza and Toniollo 2001).

Some authors (Corazza *et al* 1994) have demonstrated promising results on the use of PAP as a biochemical marker in the differentiation between prostatic adenocarcinoma and BPH. The results obtained showed that, in normal males, the PAP concentration in serum increased significantly with age. Dogs with adenocarcinoma presented higher PAP levels than those with BPH, normal dogs and dogs with non-prostatic diseases. Another author (Souza 1998) found age differences for PAP values, suggesting that it would decrease according to the age.

Nevertheless, other authors (Bell *et al* 1995) found that PAP serum and seminal activity did not differ between normal dogs and dogs with prostatic disease, nor there was not any difference in PAP concentrations in serum and seminal fluid among dogs with varied prostatic disease.

The objective of this study was to quantify the PAP concentration in serum and seminal fluid of healthy dogs and verify correlation between PAP levels and age or prostatic dimensions.

MATERIAL AND METHODS

All the dogs used in this study had been referred to the sterilization program in the Department of Animal Obstetrics and Reproduction at the "Governador Laudo Natel" Veterinary Hospital, Jaboticabal Campus, São Paulo State University (UNESP). Thirty-six intact, mixed breed, male dogs, without clinical signs of reproductive or prostatic diseases, were divided into three groups (G) of twelve dogs, according to age: GI- 1 to 3 years; GII- 4 to 6 years; GIII- 7 years or older. The body weight of the animals varied from 6.4 to 31 kg.

All animals were examined clinically before any procedure. Anamnesis, physical exam and palpation of the prostate to assess its size, consistency, symmetry and location were carried out. The dogs were examined by ultrasonography¹, with sectorial 5.0 and 7.5 MHz probe on the pre-pubic region, to evaluate the echotexture and dimensions (craniocaudal and ventrodorsal) of the prostate.

After physical examination, a blood sample was obtained from the cephalic vein, labeled and sent to the laboratory to be centrifuged at 1500 g, during five minutes, to obtain the serum. The 0.5 mL samples were used to measure the PAP level. In order to store the samples for later analyses at -20°C, 10 mL sodium bisulphate (NaHSO₄) had to be added to each milliliter of the sample.

The semen was collected by manual stimulation, using a sterilized funnel and collector tube. The first and second fractions were disposed of and the third one was collected because it is richer in prostatic fluid and has fewer cells. The prostatic fluid was placed in a glass tube and taken to the laboratory and centrifuged at 1.500 g for five minutes to obtain the seminal plasma for PAP measurement. These samples were also stored at -20°C by adding 10 mL sodium bisulphate (NaHSO₄) for each milliliter of seminal fluid. The serum and seminal plasma were usually collected in the morning.

The PAP in serum and seminal plasma was measured at the Laboratory of Clinical Pathology, using the Acid Phosphatase kit². This determination uses monophosphate timolftaleine as substrate because it is highly specific for the prostatic isoenzyme. The concentrations were evaluated by spectrophotometer³.

STATISTICAL ANALYSIS

For statistical analysis the averages and standards deviations of PAP serum and seminal concentrations were obtained and also the prostate dimensions from each group. The Shapiro-Wilk normality test was applied to all the parameters evaluated, as recommended for studies

¹ Pie Medical Scanner 200

² Labtestb Diagnostica SA, Lagoa Santa, Minas Gerais

³ Labquest Diagnóstica, Lagoa Santa, Minas Gerais

with less than 50 observations. All of them met the normality requirements (kurtosis and skewness), except the semen PAP concentrations that were not skewness. The Bartlett test showed the homogeneity of the data. The variance analysis (ANOVA) was performed (table 1) and T Student test was applied to compare means of prostate dimensions and serum and semen PAP concentrations. The Pearson correlation test was performed to evaluate the relation among the parameters evaluated. The semen PAP concentration data was submitted to radical transformation.

RESULTS AND DISCUSSION

The prostate shape, size, contour and integrity were efficiently determined by ultrasonography. The canine prostate was predominantly round with a regular surface and presented homogenous parenchyma texture, characterized by hyperechoic areas within lower echogenicity areas (transonic regions) in all animals of GI and GII, but little anechoic sites were observed in only one dog of GIII, suggesting micro cysts related to BPH. The ultrasonographic exam was essential to characterize the morphology of the canine prostate and detect probable cavity lesions. The round form was reported previously (Cartee and Rowles 1983, Cooney *et al* 1992, Bussadori 1993). Prostate homogeneity texture was reported (Bus-

sadori 1993, Mattoon and Nyland 1995), but it is still a controversy. Muzzi (1999) verified an increased diffuse parenchyma echogenicity in altered prostate; but, in the present study, no alteration was observed in echogenic pattern even in cases of increased prostate or micro cysts. Prostate dimensions were close to previous reports (Ruel *et al* 1998, Di Santis *et al* 2001) in dogs at same ages.

Craniocaudal (CC) and ventrodorsal (VD) dimensions were recorded and indicated that GII and GIII had a similar prostate size, but it was statistically ($P < 0.005$) larger than GI (table 1). There was a positive and moderate significant ($P < 0.05$) correlation between prostate dimensions and animal age ($r = 0.4170$, $P = 0.0114$ for VD; $r = 0.5844$, $P = 0.0002$ for CC), suggesting that the prostate size increases according to the age, but no animal presented clinical signs of prostate disturbances.

Statistical analysis verified that there were no significant differences in serum or semen PAP concentrations among the groups (Table 2). However, the PAP concentrations varied within each group, presenting values from 0.022 to 0.78 U/L for serum and from 43 to 530 U/L for semen in GI; values from 0.048 to 0.460 U/L for serum and from 157 to 922 U/L for semen in GII; values from 0.137 to 0.670 U/L for serum and from 115 to 605 U/L for semen in GIII. There was no correlation between serum and semen PAP concentration, nor there was any correlation between levels of serum or semen PAP and age or prostatic dimensions.

No differences in the serum PAP concentrations were detected associated with age, which disagrees with other studies (Aumüller *et al* 1987, Corazza *et al* 1994,) that reported increasing PAP levels in human and dogs' serum, respectively, according to age. Another study (Souza 1998) noticed lower PAP levels in the serum when the age increased. However, it is similar to Reiman (2005) results that found no correlation between serum PAP concentrations and age or body weight. Amorim *et al* (2004) evaluated PAP concentrations in serum and urine of middle age (4-6 years old) and old dogs (7-11 years old) and observed difference only in urinary concentrations between the two groups. The values of this enzyme pre-

Table 1. Analysis of variance for ventral dorsal and craniocaudal prostate dimension, prostatic acid phosphatase (PAP) concentrations in serum and semen of dogs.

Análisis de la variancia para las dimensiones ventrodorsal y craniocaudal de la próstata, concentraciones de la fosfatasa ácida prostática (PAP) en el suero y semen de perros.

	VD	CC	Serum PAP	Semen PAP
F value	4.99	8.78	1.39	1.59
P value (significance)	0.0128	0.0009	0.2628	0.2185

Table 2. Means and standard deviations of ventrodorsal (VD) and craniocaudal (CC) dimensions of prostate (cm) and prostatic acid phosphatase (PAP) concentrations (U/L) in serum and seminal plasma of dogs from groups I (1-3 years old), II (4-6 years old) and III (7 years old or more).

Medias y desvíos padrones de las dimensiones ventrodorsal (VD) y craneocaudal (CC) de la próstata (cm) y las concentraciones de fosfatasa ácida prostática PAP (U/L) en el suero y en el plasma seminal de los perros de los grupos I (1-3 años de edad), II (4-6 años de edad) y III (7 años de edad o más).

Group	Prostate dimensions		PAP serum (U/L)	PAP semen (U/L)
	VD (cm)	CC (cm)		
I (n=12)	2.16 ± 0.88 ^b	2.25 ± 0.89 ^b	0.351 ± 0.22	245.77 ± 154.98
II (n=12)	3.00 ± 0.78 ^a	3.22 ± 1.02 ^a	0.288 ± 0.11	368.39 ± 217.13
III (n=12)	3.00 ± 0.55 ^a	3.84 ± 0.9 ^a	0.396 ± 0.14	306.88 ± 166.7

Averages followed by different letters (a, b), in columns, indicate statistical difference by T (Student) test ($P < 0.05$).

sented a high coefficient of variation in the present study, which indicated high heterogeneity of the individual values, maybe due to a sub clinical non-prostatic disease. It could suggest that the substrate used to measure the PAP concentration was not very specific.

The PAP values in the serum were lower than those obtained in some other studies (Corazza *et al* 1994, Souza 1998, Amorim *et al* 2004, Moura *et al* 2006). This could be explained by the difference in the reagent used by those authors, which contained tartarate, a PAP inhibitor, and the reagent used in this study, phosphate timoltaleine, a substrate for PAP.

The concentrations of this enzyme were higher in the seminal plasma than in the serum, confirming its prostatic origin, as described previously (Rosecrans *et al* 1987). The measurement of this enzyme in the prostatic fluid is important, because variations due to alterations in the gland can be easily detected, since this material has more PAP. Presence of PAP was observed in canine urine (Amorim *et al* 2004, Moura *et al* 2006), but the levels were lower than those observed for seminal plasma in that study. There was a variation in the serum and seminal PAP values within each studied in the present study, endorsing a previous report in which values of PAP found in the serum of 90 human patients with BPH ranging from 0.24 to 20.8 ng/mL, while in 39 patients with prostatic carcinoma these values ranged between 0.6 - 150 ng/mL (Alivizatos *et al* 1992). Hence, it may be suggested that maximum and minimum values for this enzyme range within large intervals.

Although the prostatic acid phosphatase concentrations did not vary in relation to age, we might not reject it as an important tissue marker. Thus, others studies are necessary to evaluate the role of prostatic acid phosphatase measurement as a diagnostic test for prostate disease.

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