

## Communication

[Comunicação]

### Lipid peroxidation in female dogs bearing mammary gland carcinomas

[Peroxidação lipídica em cadelas portadoras de carcinomas mamários]

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Oxidative damage to cellular macromolecules, such as lipids, proteins and nucleic acids, by increased production of reactive oxygen species (ROS) is suggested to be involved in the pathogenesis of malignant tumors. ROS can be produced during carcinogenesis by infiltrating macrophages and neutrophils during immune activation and/or tumoral necrosis or due to tumor hypoxia and reperfusion (Halliwell, 2007). ROS primary targets are the polyunsaturated fatty acids in cell membranes, causing lipid peroxidation, whose products can induce nuclear damage and consequently mutagenesis and carcinogenesis. Thus, it is suggested that the generation of ROS may be considered as both a consequence and a cause of the carcinogenic process. An easily performed biomarker of lipid peroxidation includes the determination of thiobarbituric acid reactive substances (TBARS). Several human studies have demonstrated that plasma and/or tissue levels of TBARS are increased in association with harmful life-style conditions (Nielsen *et al.*, 1997) and several types of cancers including breast cancer (Preedy, 2014). In dogs, studies are scarce and controversial. While Kumaraguruparan *et al.* (2005) observed higher TBARS levels in bitches bearing mammary adenocarcinomas relative to healthy females, other studies involving dogs with lymphomas (Winter *et al.*, 2009) and mammary neoplasms (Szczubial *et al.*, 2004) did not show significant differences compared to control animals. One problematic feature in these oncological studies that try to link lipid peroxidation and carcinogenesis in dogs refers to the fact that they often include animals bearing heterogeneous tumors within the experimental

groups and these tumors are all grouped together even presenting different stages of progression.

In view of the still conflicting results regarding oxidative stress in dogs with malignant neoplasms, the present study aimed to compare the plasma levels of TBARS in clinically normal bitches and those with diagnosis of mammary carcinoma. The present investigation was approved by the Ethics Committee on Animal Use (CEUA) of the University Cruzeiro do Sul (São Paulo, SP, Brazil) under protocol number 031/2016 and the consent of the owners was obtained for all participating dogs.

Thirty-six female dogs of different breeds and ages ranging from 3 to 15 years were selected. Eighteen of them were grouped in group I after receiving histopathologic diagnosis of simple tubular mammary carcinoma staged as Stage 4 [T - any tumor size; N1 (positive); M0] according to the T (tumor size), N (lymph node status) and M (metastasis) system (Sorensen *et al.*, 2013), and 18 healthy female dogs were included in group II. Clinically normal females had to be more than 3 years old without intestinal or blood parasites and had to present a normal physical examination at least 3 months before blood collection. Ultrasonographic evaluation of lymph nodes and liver and radiological examination of the lungs for metastasis research of canine mammary tumor were performed. Three milliliters of blood were collected from the cephalic vein and divided into two parts. One milliliter was treated with the anticoagulant EDTA and used for complete blood cell count and determination of hemoparasites; the remainder was used to determine TBARS concentrations according to Fraga *et al.* (1988), with the results expressed in micromoles of TBARS per milliliter of plasma.

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The normality of the data was assessed using Kolmogorov-Smirnov test and analysis was made using the unpaired t-test. Data were reported as means  $\pm$  standard deviation (SD) and significance was set at  $P < 0.05$ .

Demographic characteristics of both groups (carcinoma-bearing and clinically normal female dogs) are shown in Table 1. Individual data for breed, age and plasma TBARS (in  $\mu\text{mol/mL}$ ) are shown in Table 2.

Table 1. Demographic characteristics of the female dogs bearing mammary gland carcinomas and clinically normal

Variables	Carcinoma-bearing female dogs (n=18)	Clinically normal female dogs (n=18)
Age, years; mean $\pm$ SD (range),	9.28 $\pm$ 2.95 (3-15)	7.78 $\pm$ 1.77 (4-11)
Breed, number (%)		
Mixed	10 (55.56%)	11 (61.11%)
Pure breed	8 (44.44%)	7 (38.89%)

Age analyzed by unpaired t-test; breed by Chi-square test.

Table 2. Breed, age and plasma TBARS in  $\mu\text{mol/mL}$  for female dogs bearing mammary gland carcinomas and those clinically normal

Mammary carcinoma-bearing dogs	Breed and age	TBARS ( $\mu\text{mol/mL}$ )	Clinically normal dogs	Breed and age	TBARS ( $\mu\text{mol/mL}$ )
1	Poodle, 11 yrs	7.41	1	Mixed, 10 yrs	6.54
2	Poodle, 11 yrs	6.89	2	Poodle, 8 yrs	6.12
3	Mixed, 15 yrs	8.06	3	Mixed, 10 yrs	5.93
4	Poodle, 6 yrs	6.42	4	Mixed, 8 yrs	6.49
5	Mixed, 12 yrs	8.10	5	Dobermann, 7 yrs	5.95
6	Poodle, 3 yrs	7.04	6	Cocker Spaniel, 6 yrs	5.92
7	Boxer, 8 yrs	8.25	7	Mixed, 4 yrs	6.51
8	Mixed, 11 yrs	7.01	8	Mixed, 9 yrs	6.61
9	Cocker Spaniel, 8 yrs	6.98	9	Collie, 8 yrs	6.14
10	Mixed, 11 yrs	6.95	10	Labrador Retriever, 10 yrs	6.35
11	Poodle, 10 yrs	7.23	11	Mixed, 7 yrs	6.64
12	Mixed, 13 yrs	10.92	12	Toy Fox Terrier, 7 yrs	6.12
13	Mixed, 8 yrs	8.87	13	Mixed, 6 yrs	6.32
14	Mixed, 9 yrs	8.84	14	Mixed, 8 yrs	6.69
15	Mixed, 6 yrs	11.26	15	Toy Fox Terrier, 8 yrs	6.34
16	Mixed, 6 yrs	6.20	16	Mixed, 6 yrs	4.67
17	Mixed, 8 yrs	9.49	17	Mixed, 11 yrs	5.03
18	Poodle, 11 yrs	7.66	18	Mixed, 7 yrs	6.11
Mean $\pm$ SD	9.28 $\pm$ 2.95 yrs	7.98 <sup>a</sup> $\pm$ 1.43 $\mu\text{mol/mL}$		7.78 $\pm$ 1.77 yrs	6.14 <sup>b</sup> $\pm$ 0.53 $\mu\text{mol/mL}$

TBARS and age analyzed by unpaired t-test. Distinct letters represent significant different results ( $P < 0.0001$ ).

Female dogs diagnosed with mammary carcinomas in group I had significantly higher plasma levels of TBARS (mean of  $7.98 \pm 1.43 \mu\text{mol/mL}$ ,  $P < 0.0001$ ) compared to females considered healthy from group II (mean of  $6.14 \pm 0.53 \mu\text{mol/mL}$ ). Average age in

carcinoma-bearing bitches and those clinically normal did not differ significantly (respectively, 9.28 and 7.78 years), indicating that such difference could not be partially attributed to age variation between both groups. Also, there was no significant difference in the proportion of

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pure breeds in the carcinoma-bearing females group (8/18) compared to that in the clinically normal females group (7/18).

These results are in agreement with those already obtained by Kumaraguruparan *et al.* (2005) in female dogs with mammary carcinomas. They also corroborate the findings of Macotpet *et al.* (2003), who found significantly higher serum levels of TBARS in dogs diagnosed with different types of tumors, such as mammary gland carcinomas, mast cell tumors, osteosarcomas, lymphomas, melanomas, fibrosarcomas, hemangiosarcomas, among others. However, our results are inconsistent with those observed by Szczubial *et al.* (2004) comparing normal dogs and females bearing mammary gland tumors.

As ROS are difficult to measure with standard biochemical techniques due to their high

reactivity and short half-life (Macotpet *et al.*, 2013), TBARS appear to be commonly used as biomarkers of oxidative stress in various pathological conditions, including tumors.

As this study is a cross-sectional investigation, we can not infer that oxidative stress causes cancer or vice-versa, but the finding of increased plasma levels of TBARS in female dogs carrying mammary carcinomas suggests an apparent association between lipid peroxidation, oxidative stress and the occurrence of mammary gland tumors. It also supports the possibility of considering antioxidant therapy while deliberating other therapeutic options for canine mammary tumors.

Keywords: dogs, cancer, mammary gland, oxidative stress, TBARS.

### RESUMO

*O estresse oxidativo causa peroxidação lipídica e formação de substâncias reativas ao ácido tiobarbitúrico (TBARS), processo que está comprovadamente associado à progressão de neoplasias malignas em seres humanos. Por sua vez, espécies reativas de oxigênio (EROs) são produzidas no processo carcinogênico, de forma que a geração de EROs parece ser, ao mesmo tempo, causa e consequência dele. Em cães, a associação da peroxidação lipídica com a carcinogênese permanece ainda obscura, com estudos escassos, de resultados conflitantes, que, muitas vezes, incluem, dentro de um mesmo grupo amostral, animais portadores de tumores heterogêneos dos pontos de vista morfológico e comportamental, além de estes se apresentarem em estágios bastante distintos. Nesse contexto, buscou-se, na presente investigação, avaliar a concentração plasmática de TBARS em fêmeas híginas e portadoras de carcinomas mamários com diagnóstico histopatológico de carcinoma mamário tubular simples estágio 4, com comprometimento de linfonodos, porém sem metástases detectadas. Foi observado que as cadelas diagnosticadas com carcinoma mamário tiveram níveis plasmáticos de TBARS significativamente maiores (média de  $7,98 \pm 1,43 \mu\text{mol/mL}$ ,  $p < 0,0001$ ) em relação às fêmeas consideradas híginas (média de  $6,14 \pm 0,53 \mu\text{mol/mL}$ ), o que sugere associação entre câncer e maior ocorrência de estresse oxidativo.*

*Palavras-chave: cães, câncer, estresse oxidativo, mama, TBARS*

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