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Chromium, cadmium and lead in feline mammary neoplasms

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ABSTRACT

This study aimed to evaluate concentrations of heavy metals (chromium (Cr), cadmium (Cd), and lead (Pb)) in feline mammary tissues, and to correlate their concentration with clinical and pathological findings with reported impact in prognosis. In this study, a total of 62 tissue samples from 15 female cats with malignant epithelial tumors (MET; n=40 masses from 10 cats) and non-neoplastic benign lesions (H&D; n=22 masses from 5 cats) were evaluated. Tissue Cr, Cd, and Pb levels in Group MET were not significantly different from those in Group H&D (P>0.05). In Group MET, Cr in Grade 1 (n=9) tended to be higher than in Grade 2 (n=9) and Grade 3 (n=22), but it could not reach the significance (P=0.054). Also, tissue Cr levels were not significantly related to the tumor sizes (P>0.05). The highest tissue Cd level was observed in T1 (0-2 cm), which was significantly higher than in T2 (2-3 cm) (P<0.01). Also, Cd levels in T2 were lower than in T3 (> 3cm) (P<0.05). Additionally, tissue Pb levels in T3 were significantly higher than those in T2 and T1 (P<0.05). Tissue Cr levels were significantly higher in the presence of ulceration on the tumor (P<0.05), while tissue Cd and Pb levels were not significantly different (P>0.05). To our knowledge, this is the first report evaluating Cr, Cd, and Pb concentrations in feline mammary tissues with respect to clinicopathological parameters. These insights emphasize the complex roles of heavy metals in cancer, which could vary greatly depending on the biological environment and the specific properties of each metal.

Keywords: cadmium, chromium, lead, feline mammary tumor

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Introduction

Mammary tumors are the third most common type of neoplasm in queens (Zappulli et al., 2015). They usually present (90%) malignant features that can be defined as progressive and rapidly growing, highly invasive neoplasms primarily associated with large necrotic areas, superficial ulceration, and metastases (Martín De Las Mulas et al., 2000). Metastases are most frequently seen in regional lymph nodes, lungs, liver, and pleura. Although survival time can vary with the clinical staging of the tumor, it averages 12 months in untreated cats (Morris, 2013). The incidence of mammary tumors in cats also increases with age, and they occur mainly in middle-aged to older queens. Feline mammary tumors are considered a suitable model for human breast cancer studies due to their similarities in terms of late age of onset, incidence, histopathological features, biological behaviour, and metastasis pattern (Vilhena et al., 2020). Exposure to many environmental chemicals increases the risk for cancer by inducing epithelial-mesenchymal transition (Pesonen and Vähäkangas, 2022). Metalloestrogens are metals that activate the estrogen receptor in the absence of estradiol. These bivalent cations include cadmium (Cd), calcium, cobalt, copper, nickel, chromium (Cr), lead (Pb), mercury, and tin (Byrne et al., 2013). Metal estrogens that are Cd, Pb, and Cr are non-essential metals and, although they do not contribute to the body's metabolic processes, their presence negatively impacts health (Rezaei et al., 2019). Additionally, Cd and Cr are known to have a high affinity for binding to estrogen receptors in human mammary glands (Byrne et al., 2013; Martin et al., 2003). A study investigated the heavy metals in hair samples from dogs with mammary adenocarcinoma and suggested that Cd and Cr do not accumulate in hair but remain in the mammary tissue (Badea et al., 2018). Animal studies have indicated that a low concentration of Pb in drinking water may promote the development and accelerate the growth of mammary murine tumors (Schrauzer, 2008). This study aimed to evaluate concentrations of heavy metals (Cd, Cr, Pb) in feline mammary tissues, and to correlate their concentration with clinical and pathological findings with reported impact in prognosis.

Materials and Methods

All animal procedures were carried out with the approval of the Unit Ethical Committee at the Istanbul University-Cerrahpaşa, Faculty of Veterinary Medicine (Approval number: 2021/07).

Animals and study design

A total of 62 mammary tissue samples from 15 female cats diagnosed with malignant epithelial tumors and/or non-neoplastic benign lesions were included in the study. Based on histopathological evaluations, the study groups were divided into two groups: malignant epithelial tumor (MET; n=40 masses from 10 cats) and hyperplasia and dysplasia (H&D; n=22 masses from 5 cats). The ages and weights of the cats ranged from 9 to 16 years and 3 to 4.5 kg, respectively. They were all kept indoors and fed commercially available dry food. The cats resided in Istanbul, Türkiye (41°N, 28°E). During clinical examinations, the mammary glands were macroscopically investigated for the presence of possible tumor masses and signs of tumor invasion. The presence of inflammation and/or ulceration on the tumoral masses was also recorded. Macroscopic examination of regional lymph nodes was conducted through palpation to identify potential tumor metastases. Pulmonary metastases were assessed by using three view thoracic radiography. Routine complete blood counts and biochemical tests were performed prior to surgical intervention to mitigate anesthesia risks. Bilateral mastectomy was the chosen treatment protocol for all cats.

Histopathology

All tissue samples collected from mammary glands were fixed in 10% neutral buffered formalin solution for 48 hours and subsequently processed (i.e., tissue embedding, sectioning, and H&E staining). The pathology assessment plan was conducted in a blinded manner by two pathologists, and final diagnoses were made by mutual agreement. For the pathology assessment, a histopathological examination of mammary tissue samples was performed based on the evaluation system by Mills et al. (2015) to determine tumor type and grade, as previously reported in Uçmak et al. (2023). Low-grade carcinomas were defined as Grade 1, intermediate-grade carcinomas were defined as Grade 2, and high-grade carcinomas were defined as Grade 3 (Mills et al., 2015). The histopathological examination also encompassed intra-mammary and regional lymph nodes (axillary or popliteal) to determine the possible metastasis in these regions. The classification of tumor sizes for feline mammary tumors was performed according to the TNM system as T1 (< 2 cm maximum diameter), T2 (2-3 cm maximum diameter), and T3 (>3 cm maximum diameter). Tumor inflammation was evaluated based on the criteria established by Karayannopoulou et al. (2010). The assessment relied on the presence and density of inflammatory cell infiltration within the tumor stroma and/or the surrounding tissue. Infiltration was classified as positive (inflammation: yes) when moderate to severe accumulation of mononuclear and/or polymorphonuclear inflammatory cells was observed, either diffusely or focally, within or adjacent to the tumor. Conversely, cases with no infiltration or only minimal inflammatory cell presence were considered negative (inflammation: no).

Analysis of trace and toxic elements

The analysis of trace and toxic elements in all samples was conducted using an Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES; Thermo iCAP 6000 series) at the Element Analysis Laboratory within Cerrahpaşa Faculty of Medicine, İstanbul University-Cerrahpaşa. Specifically, chromium (Cr), lead (Pb), and cadmium (Cd) elements were assessed utilizing an ICP-OES device. Tissue samples were stored in sterile eppendorf tubes at a temperature of -80ºC without contact with any chemicals (such as formaldehyde) until analysis. Before the measurement of toxic and trace elements, standard solutions were prepared, calibration curves were generated, and quality control procedures were performed.

The quality assurance process for the ICP-OES analysis included utilizing appropriate test solutions sourced from Chem-Lab NV, each containing 2000 ppm (mg/L) of the tested elements. Standard solutions for all elements were prepared by diluting standards obtained from Chem-Lab NV, each containing 1000 ppm (mg/L) of the tested elements, in deionized water. A 3-point calibration was conducted using these standard and blank solutions as reference materials. This calibration process yielded reproducible and linear calibration curves for analysis, with correlation coefficients determined for each measured element. The calibration graph was generated from the ICP-OES device utilizing blank and standard solutions, and subsequent analyses of the prepared samples were carried out at the elemental level. Measurements were performed three times for each sample, and then the average of these measurements was calculated for analysis. In this study, the appropriate wavelengths of Cr (267.716 nm), Pb (220.353 nm), and Cd (228.802 nm) were selected for analysis using the ICP-OES device. Information regarding the instrumental settings used during ICP-OES measurements can be found in Table 1.

Table 1. ICP-OES device parameters for determination of trace and toxic elements in samples.

Parameters	Assigned value
Plasma gas flow rate	15 L/min
Argon carrier flow rate	0.5 L/min
Sample flow rate	1.51 L/min
The speed of peristaltic pump	100 rpm
RF Power	1150 W

Fresh tissue samples were brought to room temperature for measurement and prepared for element measurement using the wet digestion method (Uçmak et al., 2023). The levels of trace and toxic elements are reported as µg/gsample wet weight of the samples. The quality control analysis showed a recovery rate ranging between 96% and 104%. The results of the ICP-OES method validation are presented in Table 2.

Statistical analysis

The minimum sample size required was determined using G*Power 3.1.9.7 software (Heinrich-Heine-

Table 2. The results of ICP-OES method validation for Cr, Pb, and Cd elements.

Elements	Quality Control (QC)	LOD (ppm)	LOQ (ppm)	Expected Concentration (ppm)	Measured Concentration (n=3) (ppm)	Precision (RSD%)	Recovery (%)
Cr	QC-1	0.001	0.002	0.050	0.048	1.701	96
	QC-2			0.100	0.104	1.629	104
Pb	QC-1	0.001	0.003	0.100	0.100	0.816	100
	QC-2			0.500	0.508	0.334	101.6
	QC-3			1.000	0.991	0.125	99.1
Cd	QC-1	0.000	0.001	0.100	0.102	1.226	102
	QC-2			0.500	0.513	0.243	102.6
	QC-3			1.000	0.983	0.172	98.3

QC: Quality control; LOD: limit of detection; LOQ: limit of quantitation; RSD: relative standard deviation.

Universität Düsseldorf, Düsseldorf, Germany). Calculations revealed that a total sample size of n = 62 would be sufficient to provide 85% power with 0.05 α-error probability, for determining the correlation between the evaluated parameters. Additionally, a sample size of 21 for each group (Group MET and H&D) would be sufficient to provide 80% power with an α-error probability of 0.05 in detecting differences between two independent means. Statistical analyses were performed using SPSS version 23.0. The normal distribution of the data was verified with the Shapiro Wilk test and after which the appropriate method for comparing the groups was selected. The comparison of the trace and toxic elements relative to tumor size and histological grades were conducted using one-way ANOVA. Differences in tissue trace and toxic elements related to the tumor type, metastasis, tumor invasion to peripheral muscles, ulceration, and inflammation were assessed using Student's t-test. Relationships among the parameters were examined using the Pearson correlation test. Values were presented as mean ± standard error of the mean (SEM). The significance level was accepted as $P < 0.05$.

Results

Total mastectomy specimens obtained from 15 female cats, each containing 8 mammary lobes, were selected. A total of 120 mammary lobes were examined microscopically for the presence of lesions. Subsequently, 62 mammary lobes harboring lesions diagnosed as malignant epithelial tumors or hyperplastic and dysplastic changes were included in the study. A total of 58 tumors, which consisted of benign tumors, inflammatory lesions, and mesenchymal-origin tumors such as chondrosarcoma and carcinosarcoma, were excluded from the study. The cats had an average age of 12.55 ± 0.45 years and weighed 3.65 ± 0.42 kg. The mean age of five cats with H&D was 9.75±0.59 years and the mean age of ten cats with MET was 13.94±0.62 years. The samples included from five Persian and 10 mixed-breed cats. Out of these, 10 cats were intact while the remaining five had been spayed. Among the studied cases, intra-mammary lymph node metastases, axillary lymph node metastases, and pulmonary metastases were observed in nine cats. Specifically, the two cats with Grade 2 adenocarcinomas presented with only intra-mammary lymph node metastases. Seven cats had Grade 3 tumors characterized as comedocarcinoma, adenocarcinoma, and squamous cell carcinoma, showed axillar lymph node metastases, intra-mammary lymph node metastases, and pulmonary metastases. The affected cats had multiple tumoral masses along the mammary chains with varied histopathological subtypes. Thirty tumors in group MET were localized on the abdominal and inguinal mammary lobes.

Histopathological examination findings

Histopathological analysis revealed 22 cases of benign hyperplastic and dysplastic lesions. Hyperplastic mammary glands were characterized by the elevated numbers of lining cuboidal epithelial cells. As for dysplastic changes, the glandular epithelial cells having polygonal to cuboidal structures were elevated in number and showed a higher mitotic rate. Hyperplastic lobular pattern associated with secretion and ectasia in ducts and mammary glands were viewed in these altered tissues (Fig 1A). Besides that, there were cases associated with the fibroadenomatous alterations (Figs 1B & 1C). MET group included carcinoma in situ (n=3), carcinoma simple-tubular or tubulopapillary pattern (n=23), comedocarcinoma (n=10), and squamous cell carcinoma (n=4). In-situ carcinomas appeared as non-uniform tubule structures without exceeding the basement level (Fig 1D).

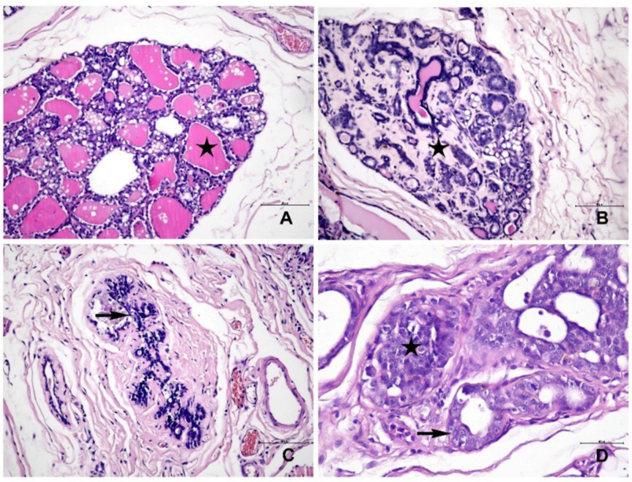

Figure 1.A. Hyperplastic mammary glands with secretory activity (star); **1.B.** Fibrous hypertrophy, benign fibroadenomatous change (star); **1.C.** Fibrous hypertrophy surrounding a duct (arrow); **1.D.** A group of neoplastic cells (star) and tubular structures (arrow), carcinoma in situ case, H&E, A, B&C: Bar=90 µm. D: Bar=40 µm

Carcinoma-simple tubular or tubulopapillary tumors exhibited well-defined tubular and papillary structures lined by neoplastic epithelial cells with varying degrees of differentiation. Most of the areas showed uniform and well-differentiated cells, with others displaying more pleomorphism (Fig 2A). In some cases, these findings were accompanied by tightly packed solid areas (Fig 2B), characterized by prominent nuclear pleomorphism with hyperchromasia and abnormal nuclear morphology. The nucleoli of these neoplastic cells were either single, prominent, and vesicular, or small and multiple. Several cases revealed invasion in lymphatic vessels and metastatic foci in related lymph nodes (Figs 2C and 2D).

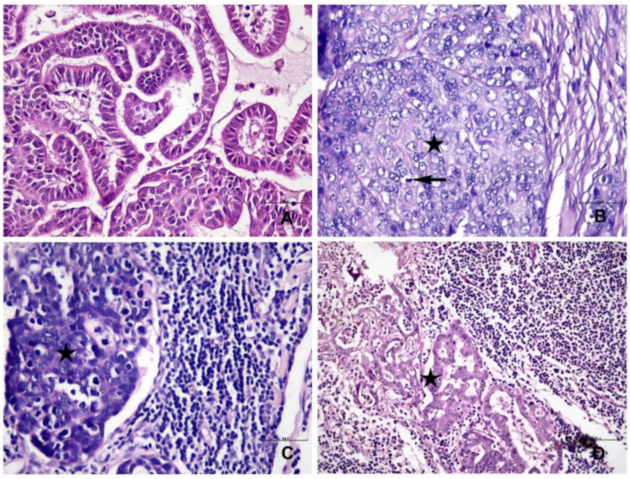

Figure 2.A. Carcinoma simple-tubulopapillary type, sections of papillary structures; **2.B.** Densley packed neoplastic cells in solid areas (star), mitotic figure (arrow); **2.C** and **2.D.** Foci of micrometastasis (stars) in adjacent lymphoid tissues of tubular carcinoma cases, H&E, A, B&C: Bar=40 µm. D: Bar=90 µm

Tumors diagnosed as comedocarcinoma were solid tumors with large necrotic areas in the centrum (Figs 3A and 3B). Surrounding these necrotic areas, there were malignant epithelial cells showing variation in size and shape, indicating distinctive pleomorphism with high nuclear-to-cytoplasmic ratio, and prominent nucleoli. Squamous cell carcinomas were diagnosed by the presence of the squamous epithelial cells arranged in nests, sheets, and cords. The neoplastic cells typically had abundant eosinophilic cytoplasm and centrally located prominent hyperchromatic nuclei. The most distinctive finding of these carcinomas was keratinization, characterized by the existence of eosinophilic keratin pearls (Figs 3C and 3D).

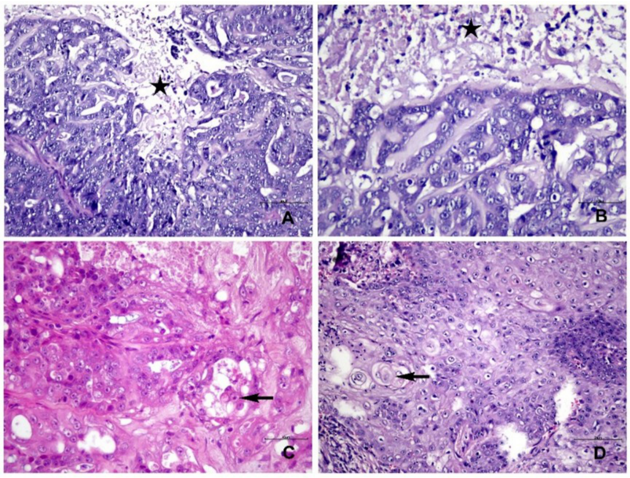

Fig. 3.A and **3.B.** Comedocarcinoma, a few nodular areas with centrally located necrosis (stars); **3.C.** A tubular carcinoma showing squamous cell differentiation (arrow); **3.D.** Squamous cell carcinoma, composing neoplastic squamous cells showing keratinization (arrow), A&D: Bar=90 µm, B&C: Bar=40 µm.

In some inflammatory cases, edema was observed in the epidermal and dermal layers, along with varying degrees of haemorrhage and necrosis, including necrotic tumor tissues. In these areas, inflammatory cells such as macrophages, polymorphonuclear leukocytes, and mononuclear cells were identified. Lymphocytes, plasma cells, and macrophages were scattered among the tumor foci. Neoplastic tubules or small clusters of epithelial cells were seen aggressively invading the surrounding muscle tissue in two cases. In a few cases, metastases to the intramammary lymph node were noted, particularly in the paracortical sinuses or cortical areas.

Elemental analysis results

Elemental analysis results related to the clinicopathological parameters were detailed in Table 3. The differences of tissue Cr, Cd, and Pb levels between the groups were not significant (P>0.05). The differences of the element concentration in Group MET were evaluated in terms of inflammation, histological grading, tumours' size, ulceration and invasion to peripheral muscles (Table 3). Within the MET group, elements related to metastases were not statistically significant (P>0.05). Tissue Cr levels in Grade 1 tumors of Group MET tended to be higher compared to Grades 2 and 3, but the difference was not significant (P=0.054). Tissue Cr level tended to be higher in the presence of invasion to the peripheral

Table 3. Elemental analysis results related to the clinicopathological parameters.

Clinicopathological parameters		Cr (ppm)	Cd (ppm)	Pb (ppm)
Tumor types	MET (n=40)	0.27±0.04	0.03±0.004	0.13±0.01
	H&D(n=22)	0.24±0.03	0.02±0.006	0.13±0.02
Inflammation	(+) (n=15)	0.28±0.05	0.03±0.006	0.13±0.03
	(-) (n=25)	0.19±0.02	0.02±0.004	0.13±0.02
Grade (G)	G1 (n=9)	0.37±0.12	0.03±0.005	0.16±0.06
	G2 (n=9)	0.22±0.02	0.04±0.015	0.10±0.02
	G3 (n=22)	0.19±0.02	0.02±0.004	0.12±0.02
Tumor size (T)	T1 (n=20)	0.30±0.05	$0.04±0.006^a$	$0.11±0.03^a$
	T2 (n=14)	0.17±0.02	$0.01±0.003^b$	$0.10±0.01^a$
	T3 (n=6)	0.16±0.04	$0.03±0.009^a$	$0.26±0.04^b$
Ulceration	(+) (n=14)	$0.16±0.01^a$	0.02±0.005	0.14±0.02
	(-) (n=26)	$0.29±0.05^b$	0.03±0.005	0.12±0.02
Invasion to peripheral muscles	(+) (n=10)	0.17±0.02	0.02±0.005	0.18±0.03
	(-) (n=30)	0.26±0.04	0.03±0.005	0.11±0.02

muscles while tissue Pb level tended to be higher in the absence of invasion to the peripheral muscles but they both failed to be significant (P=0.09). According to Pearson correlation test, Cr was considerably associated with both Cd (R=0.600, P<0.01) and Pb (R=0.564, P<0.001) while Cd showed no significant association with Pb (R=0.247, P>0.05).

Discussion

Hyperplastic and neoplastic formations seen in the mammary glands of carnivores may result from excessive or repeated stimulation by steroid hormones. Given the impact of sexual steroid hormones on the development of mammary tumors, unneutered or late-neutered cats are particularly susceptible to this disease (Beauvais et al., 2012). Accordingly, 10 out of 15 cats with mammary tumors in this study were intact.

Duran et al. investigated the trace element concentrations in commercially available cat and dog foods (dry and wet) in Türkiye (Duran et al., 2010). They found that the trace metal concentrations (Cr, Cd, Pb) in the pet food samples met the nutritional standards set by the Association of American Feed Control Officials. Furthermore, Altınok-Yipel et al. (2022) determined the levels of heavy metal contamination in canned and pouched cat foods with 15 different brands collected from local Istanbul markets. Their findings indicated that the levels were below the legal or recommended limits and consuming these foods at the recommended rate did not pose a health risk to adult cats and kittens. Thus, the commercially sold pet foods in Türkiye were used to feed the cats in this study, which complied with acceptable quality and element concentration standards, and likely did not contribute to high levels of heavy metal ingestion.

Chromium (Cr)

Heavy metals tend to accumulate predominantly in the parenchymal component of tumoral tissue, including breast cancer tissue. This accumulation occurs because cancer cells, through the dysregulation of endo-exocytosis, attract heavy metals as they proliferate within the blood vessels, leading to increased heavy metal content in the human body (Romaniuk et al., 2017). Carcinogenic metals like Cr, arsenic, and Cd disrupt DNA synthesis and repair processes, causing toxic effects across various tissues and organs in humans (Koedrith et al., 2013). Heavy metals adversely impact on cell growth, proliferation, differentiation, damage repair mechanisms and apoptosis (Balali-Mood et al., 2021). The interaction of reactive oxygen and nitrogen species with cellular macromolecules is a common mechanism of toxicity and carcinogenesis due to Cr exposure (Aggarwal et al., 2019). Romaniuk et al. (2017) observed an increase in heavy metal concentrations in neoplastic tissues with the progression of carcinogenesis, suggesting that heavy metals may promote breast cancer progression through various pathogenetic mechanisms. Unlike the findings of Romaniuk et al. (2017), in this study, the concentrations of heavy metals in feline mammary tissue did not vary significantly regarding histopathological classification. To our knowledge, this is the first report investigating Cr in feline mammary tissues. Although Cr levels were higher in malignant epithelial tumors compared to the hyperplasia and dysplasia group, the difference was not statistically significant (P>0.05). This discrepancy may be due to the absence of healthy mammary tissue samples and the inclusion of neoplastic tissues/lesions in both study groups. These results suggest that Cr maintains its toxic effects in both malignant tumors and hyperplastic and dysplastic conditions. Choi et al. (2019) found no significant clinicopathological correlations between Cr levels and lymph node metastases. Similarly, Cr levels were not significantly different in the presence of metastases in this study. Chromium is known to exert carcinogenic effects on humans and other animals (O'Brien et al., 2003). In this study, Cr levels were significantly higher in malignant mammary tumors with skin ulceration compared to non-ulcerative malignant mammary tumors (P<0.05), suggesting that elevated chromium levels may exacerbate the deterioration of skin and mucous membranes. Also, Cr levels tended to be higher in the presence of invasion (P=0.09). This result is thought to be achieved because of Cr on DNA damage responses and processes like cell cycle arrest or apoptosis, as reported by O'Brien et al. (2003).

Cadmium (Cd)

The concentration of Cd may be influenced by various cofounders such as hormones, hormone receptor positivity, or the presence of other elements (Huff et al., 2007; Antila et al., 1996). Romanowicz-Makowska et al. (2011) reported that the concentration of Cd in normal breast tissue was significantly lower than in breast cancer tissue (P<0.05). Conversely, Antila et al. (1996) did not observe a significant difference in tissue Cd concentration between the breast cancer patients and healthy controls (P>0.05). Wu et al. (2006) reported that serum Cd levels were higher in patients with malignant breast diseases compared to those with benign breast conditions (P<0.01). In this study, although the mean tissue Cd concentration in Group MET was higher than in Group H&D, the difference was not statistically significant (P>0.05). Nevertheless, the presence of the highest Cd concentration in Group MET supports the toxic effect of Cd on mammary tissue in cats. Future studies should investigate tissue Cd concentration in subtypes of malignant mammary tumors in cats. He et al. (2017) found that blood Cd level was positively associated with distant metastasis in patients with invasive breast cancer. Ponce et al. (2015) reported that Cd disrupts cell to cell interactions by disrupting E-cadherin, thereby enhancing the cancer cells' ability to spread from the primary tumor in humans. In this study, no difference was found between tissue Cd levels in metastatic and non-metastatic malignant epithelial tumors; in fact they were very similar, and contradictory results could be due to differences in the analysed materials. Future studies should evaluate blood Cd concentrations in cats with metastatic and non-metastatic mammary tumors. Tumor grading quantifies the clinical aggressiveness of a neoplasm based on specific histological features (Avallone et al., 2021). Romanowicz-Makowska et al. (2011) observed no significant difference between Cd concentration and histological grades of breast cancer. Similarly, in this study, tissue Cd concentration in Group MET did not significantly vary with histological grades (P>0.05). The researchers (Avallone et al., 2021) noted that the insignificance of results might be due to the relatively small size of groups across grades I, II, and III. Similarly, the size of subgroups of the histological grades is thought to be small to provide an accurate estimate of the effect.

Jablonska et al. (2017) observed the highest Cd content in smaller breast tumors (T1) and suggested that while Cd might promote carcinogenesis, it could also delay tumor onset by inhibiting angiogenesis. Similarly, in this study, the highest Cd concentration was found in tumors measuring less than 2 cm in diameter (P<0.05). Therefore, the increased presence of this toxic metal in smaller tumors might be due to Cd's inhibitory effects on breast cancer cell growth or differentiation as the researchers suggested (Jablonska et al., 2017).

Lead (Pb)

The assessment of heavy metals and other mineral elements in biological samples has been extensively studied in relation to cancer. Badea et al. examined hair samples from dogs with mammary neoplasms and found no significant difference in lead (Pb) concentrations compared to a control group of clinically healthy dogs (Badea et al., 2018). Anđelković et al. observed the Pb levels in breast cancer tissue did not differ from the benign tissue Pb levels of the control group (Anđelković et al., 2022). Distribution of

Pb in tumor and normal tumor free breast tissue from the women with malignant and benign breast lesions were evaluated and no statistical significance was observed (Siddiqui et al., 2006). These findings are consistent with those of the current study, where tissue Pb concentrations in Group MET did not significantly differ from those in Group H&D. Mehari et al. suggested that the insignificance in Pb results could stem from its low cytotoxic effect on breast cancer cells (Mehari et al., 2017). In harmony with Mehari et al. (2017), it was suggested that insignificant results could be obtained between groups due to the low cytotoxic effect of Pb. In an in vitro study with a breast cancer cell line, Martin et al. demonstrated that Pb significantly stimulates the growth of cancer cells (Martin et al., 2003). Alatise and Schrauzer (2010) stated that Pb levels in hair samples of breast cancer patients were directly related to tumor volumes, in accordance with the tumor growth-promoting effects of Pb. In this study, the highest tissue Pb concentration was determined in tumors more than 3 cm in diameter (T3). This result has supported the stimulatory effect of Pb on tumor growth. Histological grading helps to quantify the putative clinical aggressiveness of a neoplasm (Avallone et al., 2021). Güzel et al. (2012) observed the highest concentrations of Pb in high-grade prostate carcinoma tissue samples, although no similar studies have been conducted on breast cancer (Güzel et al., 2012). The current study observed the highest Pb concentration in grade 1 tumors, although this was not considerably significant ($P>0.05$). This might be explained by the ability of Pb to induce oxidative stress and DNA damage regardless of tumor grade, as noted by the researchers (Valko et al., 2005).

Moreover, heavy metals such as Cr, Cd, and Pb are recognized not only for their toxicity but also for their carcinogenic potential. Waalkes et al. reported that Cd, Pb, mercury, and tin were toxic because they can mimic or block the functions of other essential metals, contributing to their carcinogenicity (Waalkes et al., 2000). Furthermore, Cd, Cr, and Pb have been demonstrated to be carcinogenic and induce breast cancer (Men et al., 2020). In line with the previous reports, positive correlations were found between Cr and Pb ($P<0.001$; $R=0.564$) and Cr and Cd ($P<0.01$; $R=0.600$) in feline mammary tissues in this study. Similar results are thought to be achieved due to their synergistic effects in the carcinogenic process of mammary tumors.

Conclusions

Indoor cats share their environment with humans, exposing them to the same indoor contaminants and associated health risks, including cancer. This shared exposure makes pet animals, particularly cats and dogs, relevant natural models for human cancer research due to epidemiological and clinicopathological similarities between spontaneous tumors in pets and their human counterparts. To our knowledge, this is the first report evaluating Cr, Cd, and Pb concentrations in feline mammary tissues with respect to clinicopathological parameters. These insights emphasize the complex roles of heavy metals in cancer, which could vary greatly depending on the biological environment and the specific properties of each metal.

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Any additional data supporting this study are available from the authors upon reasonable request.

Conflict of interest

The authors declare no conflict of interest.

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