Serum concentration of magnesium in dogs suffering from tumors of the perianal glands

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Abstract. The aim of this study was to determine serum magnesium concentrations in dogs with benign and malignant tumors of the perianal glands. The magnesium concentration was determined in samples taken from 45 tumor-affected and 17 healthy males. Twenty one dogs suffered from malignant tumors (Malignant group), while benign changes were diagnosed in 24 cases (Non-malignant group). All dogs with neoplastic disease were treated with antiandrogens and antiestrogens. Determination of magnesium concentrations in serum obtained from dogs before the start of treatment (baseline), and one and six months later was performed using atomic absorption spectrophotometry. One-way ANOVA and the post-hoc Tukey’s HSD test were used to compare differences between the groups investigated and time-related changes. Significantly higher serum magnesium concentrations were found in the group with malignant tumors when compared to the control and non-malignant groups (P<0.001). Serum magnesium concentrations in dogs with non-malignant neoplastic changes had increased gradually by 17% and 41% at one and six months when compared to the baseline values (P<0.05). In conclusion, the malignant neoplastic process in dogs was associated with a higher baseline serum magnesium concentration. Thus, determination of the serum magnesium concentration might be helpful for diagnostic differentiation between malignant and benign perianal tumors in dogs.

Key words: magnesium, dogs, perianal tumors, serum

Magnesium is the fourth most abundant cation in the body, behind sodium, potassium and calcium, and the second most prevalent intracellular cation. The major pool of magnesium is distributed in intracellular form. Over 50% of the total amount of magnesium in the body is deposited in the skeletal system, while 20% of this element is stored in the skeletal muscles. The remaining 20% of the magnesium pool is deposited in intracellular structures. Approximately 1% of the magnesium stores resides within the extracellular fluid compartment [1, 2]. Around 20-30% of serum magnesium is bound to proteins, while the rest in this compartment is ionized and biologically active [3].

The skin is an organ rich in glands. In dogs, the skin of the perianal region consists of structures and glands exclusive to this region. The most frequent oncological diseases in dogs are skin neoplasms accounting for up to 30% of all cases [4]. In dogs, neoplasms originating from the glands of the perianal region account for
about 15-17% of skin neoplastic cases. Their occurrence in old males is very high in comparison to females or castrated males, indicating androgens as important factors that increase the risk of neoplastic disease [5]. Among the various tumor types found in this region in dogs, adenomas represent over 80%. Most perianal region adenomas are benign; however, malignant adenocarcinomas can occur in this region and may progress to systemic symptoms such as apathy, muscle weakness, polyuria and polydipsia associated with mineral metabolism disturbances, hypercalcemia and hypophosphatemia [6]. Furthermore, metastases from perianal carcinomas may occur in the lungs, liver, spleen and lumbar lymph nodes [7, 8].

Considering the fact that tumor growth may influence mineral metabolism, the aim of the study was to determine and compare serum magnesium concentrations in dogs suffering from benign and malignant neoplastic changes originating from the perianal glands. Furthermore, changes in serum magnesium concentrations after one month and six months of treatment in these dogs were evaluated in this study.

### Methods and materials

Experimental procedures used throughout this study were approved by The II Local Ethics Committee on Animal Experimentation of the University of Life Sciences in Lublin, Poland.

#### Experimental design and sampling procedure

Magnesium concentrations in serum were determined in samples obtained from 45 tumor-affected and 17 healthy male dogs of different breeds. All of the dogs were treated pharmacologically in the Clinic of Animal Surgery, Faculty of Veterinary Medicine, University of Life Sciences in Lublin between 2008 and 2011. The treatment was performed as a function of hormonal evaluation of the serum samples, since perianal tumors are considered to be hormone-dependent. In dogs with significantly elevated serum levels of testosterone, an antiandrogen (Androcur, Schering A.G., Germany) was administered per os (5 mg/kg b.wt./day) over four weeks if effective (90% cases), or for an additional three weeks until the neoplastic changes had disappeared (10% cases). In dogs with serum estrogen levels exceeding 7 pg/mL, an antiestrogen (Tamoxifen, Polfa Kutno S.A., Poland) was administered for four weeks at a daily dose of 1 mg/kg b.wt. In a few cases, the antiestrogenic treatment was prolonged by three weeks until the perianal tumors had disappeared completely. The ages of the dogs varied between nine and 14 years. All dogs were fed a standard diet without additional mineral supplementation. To differentiate the tumor type, neoplastic tissue samples were biopsied under local anesthesia with 2% lignocainum gel (Lignocainum, Jelfa S.A., Jelenia Góra, Poland). Before the tissue sampling procedure, the animals were sedated with an i.m. injection of xylazine (Sedazin®, Biowet Puławy, Puławy, Poland) at a dose of 2 mg/kg b.wt. The diameter of the neoplastic tissue samples was 0.5 mm and these were fixed in 10% buffered formaldehyde solution and analyzed histopathologically at the Department of Pathological Anatomy, Faculty of Veterinary Medicine, University of Life Sciences in Lublin. The histological examinations showed that of the 45 patients, 21 dogs suffered from malignant tumors (Malignant group), while 24 dogs were diagnosed with benign perianal tumors (Non-malignant group; table 1). Age- and sex-matched dogs in good health comprised the control group (n = 17), although they qualified for surgery on the basis of a diagnosis other than neoplastic disease. The control dogs were subjected to blood collection in the same way as the other groups.

#### Evaluation of magnesium concentration

Determination of magnesium concentrations in the serum obtained from the dogs was performed using an atomic absorption spectrophotometric method and PYE UNICAM apparatus (Pye Unicam Ltd., Cambridge, UK). For this, 1 mL samples were diluted with distilled water at a 1:1 ratio and

### Table 1. Histopathological differentiation of the neoplastic changes in dogs suffering from perianal tumors.

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Malignant (n = 21)</th>
<th>Benign (n = 24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epithelioma</td>
<td>Carcinoma (n = 9)</td>
<td>Adenoma (n = 24)</td>
</tr>
<tr>
<td>(n = 12)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
underwent a drying process, which was performed in quartz dishes at 80°C for 72 hours. After drying, the samples were subjected to mineralization by calcination at 450°C. The ash obtained after mineralization was dissolved in spectrally clean hydrochloric acid (Merck, Darmstadt, Germany), which had previously been mixed with deionized water at a 1:1 ratio. The magnesium concentration was determined directly from the water phase. The wavelength used for the determination of magnesium was set at 285.2 nm. A calibration curve was determined before the measurements.

Statistical analysis
All values are presented as means ± SEM. Statistical analysis was performed using Statistica software (version 6.0) and ANOVA. The post-hoc Tukey’s HSD test was used to compare differences between the groups investigated and time-related changes. The differences between mean values were considered as statistically significant at P ≤ 0.05.

Results
Magnesium concentrations in serum from control dogs and those suffering from non-malignant and malignant neoplastic changes are shown in table 2. Significantly higher values for serum magnesium concentrations were found in the group with malignant tumors when compared to the control and non-malignant groups (both P < 0.001). The differences between the groups reached 173% and 126%, respectively. The time-related changes in serum magnesium concentrations in dogs suffering from benign and malignant tumors are shown in table 2. Serum magnesium concentrations in dogs with non-malignant neoplastic changes were significantly higher, (12%), when compared to the values obtained five months earlier (P = 0.05). However, in the group with malignant tumors, no statistically significant differences in serum magnesium concentrations obtained one and six months after the start of treatment were found when compared to the values measured at baseline (P > 0.05).

Discussion
The results obtained in this study have shown changes in magnesemia in dogs suffering from malignant perianal tumors. It was shown that the malignant neoplastic process, involving the perianal glands in dogs, is associated with higher serum magnesium concentrations when compared to healthy dogs or those with non-malignant neoplastic changes. Considering the similar serum magnesium levels in groups of healthy dogs and those with non-malignant tumors, it may be postulated that determination of this parameter in blood samples may be useful for diagnostic identification of malignant tumors. Compared to histopathological examination, determination of magnesium concentration in serum is relatively fast and does not require animal sedation or anesthesia, either general or local. Furthermore, magnesium determination in serum is cost- and time-effective and could be part of a routine blood examination. The current study has also revealed time-related changes in serum magnesium concentrations in these patients. As the result of magnesium evaluation at one- and six-month intervals after the start of treatment of dogs with non-malignant neoplastic process, a stable, higher concentration of this element in serum was observed when compared to the baseline value. Contrary to these observations, the significantly higher serum magnesium concentrations found in the group of dogs with malignant perianal tumors remained unchanged throughout the six-month period following the start of treatment. The results obtained in this study are in accordance with a previous report on dogs, where the highest magnesium concentrations were found in malignant tumor tissue of skin when compared to a group of healthy controls and dogs suffering from non-malignant neoplastic skin changes [9]. However, the evaluation of serum magnesium concentrations in dogs in the previous
Table 2. Magnesium concentration (mg/L) in serum from control dogs and those suffering from non-malignant and malignant neoplastic changes before treatment (baseline) and after one month and six months of treatment.

<table>
<thead>
<tr>
<th>Time</th>
<th>Before treatment</th>
<th>After one month of treatment</th>
<th>After six months of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group (n = 17)</td>
<td>9.672 ± 0.602</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Non-malignant group (n = 21)</td>
<td>11.672 ± 0.304 a</td>
<td>13.675 ± 0.688 b</td>
<td>16.453 ± 0.453 c</td>
</tr>
<tr>
<td>Malignant group (n = 24)</td>
<td>26.410 ± 0.966 ab</td>
<td>25.057 ± 0.952 a</td>
<td>28.131 ± 0.779 b</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM.

* Statistically significant differences versus the control and non-malignant groups for P<0.001.

ab Different letters indicate statistically significant differences between mean values within row for P<0.05.

The experiment did not reveal significant differences between healthy controls and dogs suffering from skin tumors [9]. Other investigations on dogs with non-malignant and malignant tumors of the mammary glands have shown that tissue magnesium concentrations are elevated only in the group of animals with malignant neoplastic changes when compared to healthy controls. Similarly to the results obtained in the studies on dogs with neoplastic skin changes, serum magnesium concentrations in dogs with mammary gland tumors remained unchanged in comparison to the healthy controls [10]. Data obtained from blood analyses in both previous investigations conflict with the results reported in the current study, showing significant differences in serum magnesium concentrations between dogs with malignant perianal tumors and healthy dogs and those suffering from non-malignant changes. The differences observed between the results obtained in all of these studies on dogs demonstrate the variability in magnesium metabolism depending on the tumor type and the stage of malignancy. Thus, as opposed to the possible diagnostic usefulness of serum magnesium determination in dogs with perianal tumors, the value of serum magnesium evaluation in dogs with skin and mammary gland tumors is limited [9, 10].

The relationship between magnesium and cancer is very complex but can be anticipated from the well-established requirement of magnesium for neoplastic cell proliferation. Basic and pre-clinical studies have confirmed that magnesium deficiency may induce both anti- and pro-tumor effects [11]. Investigations in humans have also shown significant changes in magnesium metabolism in patients suffering from lung, breast, ovarian and pharyngeal cancers. While the magnesium concentration was found to be higher in erythrocytes in patients with neoplastic disease, its serum level was lower when compared to control subjects [12]. Results obtained in a trial involving 494 patients with different grade prostate cancer have shown that low blood magnesium levels and a high calcium/magnesium ratio were significantly associated with high-grade prostate cancer. These findings have shown that magnesium affects prostate cancer risk perhaps through interacting with calcium [13]. Epidemiological studies on the possible association between the risk of lung cancer in women (7,064 participants) and the levels of calcium and magnesium in drinking water have shown a significant trend toward a decreased risk of lung cancer in women with increasing magnesium levels in drinking water [14]. Observations in mice have shown significant retardation of primary tumor growth by up to 70% in a group receiving an Mg-deficient diet, while Mg supplementation caused a significant increase in primary tumor burden in mice [15]. In other studies on tumor-bearing mice, significantly higher magnesium concentrations were reported in erythrocytes, while its plasma and tissue content was not influenced [16]. Other results obtained in magnesium-deficient mice have revealed that low magnesium concentrations limit and foster tumorogenesis, since inhibition of tumor growth at its primary site is observed in the face of increased metastatic colonization. Furthermore, in vitro experiments have shown that cultured neoplastic cells tend to accumulate magnesium, confirming the significantly higher serum concentration of the cation observed in the current study [17]. The high metabolic demand of
malignant neoplastic changes for magnesium requires its increased supply by circulating blood from main stores within the organism. As we have previously reported in dogs, tumor tissue magnesium concentrations rise with the increased tumor malignancy [9, 10].

It was shown that high intracellular levels of magnesium provide a metabolic advantage to neoplastic cells, contributing to alterations of the genome, and promoting the acquisition of an immortal phenotype [17]. Magnesium influences the process of carcinogenesis both by affecting oxidative stress and consequent oxidative DNA modifications that might lead to mutagenesis, and by affecting DNA repair mechanisms that maintain genomic stability. It is also involved in a wide range of biochemical reactions crucial to cell proliferation, differentiation, angiogenesis, and apoptosis [18]. Studies on women with breast cancer revealed significantly higher levels of magnesium in cancerous tissues as compared to the unaffected control cells [19]. On the other hand, findings resulting from cohort studies on humans have shown that a higher intake of dietary magnesium is associated with a lower risk of colorectal tumors due to its inhibiting action on c-myc expression and ornithine decarboxylase activity in the mucosal epithelium of the intestine [20, 21].

In conclusion, this study has shown that the malignant neoplastic process in dogs was associated with high serum magnesium concentrations. This indicates disturbances of magnesium metabolism in dogs suffering from malignant perianal tumors. Considering these data, it may be postulated that determination of the magnesium concentration in serum may be useful for diagnostic differentiation of perianal tumors in dogs.

Disclosure

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References


