Assessment of Heavy Metal and Mineral Levels in Hair Samples from Dogs with Mammary Neoplasms

Emanuela BADEA¹, Gheorghe Valentin GORAN¹, Cristina ȚOCA², Victor CRIVINEANU¹

¹ Faculty of Veterinary Medicine, UASVM of Bucharest, 105 Splaiul Independenței, 050097, 5th district, Romania, EU
² IDAH of Bucharest, 63 Doctor Staicovici, 050557, 5th district, Romania, EU
* corresponding author: emanuela.badea@gmail.com

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ABSTRACT
Neoplasms involve abnormal tissue growths developing in an uncoordinated, persistent manner, faster than adjacent normal tissues. Several researchers have studied the possible implications of heavy metals and mineral levels on human mammary neoplasms using hair analysis. The study’s objective was to assess the levels of heavy metals and other mineral elements in dogs suffering from mammary neoplasms. Hair samples were analyzed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). All analyzed elements registered higher levels in clinically healthy dogs. The interaction between health status and keeping conditions significantly influenced the levels of Al, Ca, Fe, Mg, Ni, Co, Pb, and V. Concurrently, dogs with mammary neoplasms living outdoors registered the highest levels for most minerals, compared to dogs with mammary neoplasms living indoors, suggesting a possible implication of pollution.

Keywords: heavy metals, minerals, mammary neoplasms, dogs, hair.

INTRODUCTION
Neoplasms are a disease of the genome, involving a pathological activity concerning proliferation, differentiation and death of particular cells, abnormal tissue growths developing in an uncoordinated, persistent manner, faster than adjacent normal tissues. Neoplasms can be either benign or malignant, with different causes determining the onset of different types of cancer. (Dobson, and Lascelles, 2011; Meuten, 2017; Morris, and Dobson, 2001) Mammary gland tumors are the most commonly diagnosed neoplasms in female dogs (Pérez et al., 1998; Philibert et al., 2003; Sarli et al., 2002; Yamagami et al., 1996), some researchers stating that they account for 70% of all cancer cases. (Merlo et al., 2008). In tact status is the most common cause of mammary cancer (Dorn et al., 1968; Schneider et al., 1969; Sorenmo et al., 2000), along with increased age and progestagen treatment (Perez et al., 2000). Several researchers have studied the possible implications of heavy metals and mineral levels on human mammary neoplasms by hair analysis. (Joo et al., 2009; Pasha et al., 2010; Wang et al., 2006; Yasuda et al., 2009)

The study’s objective was to assess the levels of heavy metals and other mineral elements in dogs suffering from mammary neoplasms.

MATERIALS AND METHODS
The study was conducted on 30 female dogs, divided into two groups: the study group (SG) consisting of dogs suffering from mammary neoplasms (n = 15), and the control group (CG) consisting of clinically healthy dogs (n = 15). All dogs suffering from mammary neoplasms were post-op diagnosed with adenocarcinoma, using histopathological examination. In the SG, all dogs were above 7 years of age, of which 7 dogs lived...
indoors, and 8 lived outdoors. All dogs in the CG were above 5 years of age, of which 5 dogs lived indoors, and 10 lived outdoors. Based on keeping conditions, the groups were further divided into SGI (study group dogs living indoors), SGO (study group dogs living outdoors), and CGI (control group dogs living indoors), CGO (control group dogs living outdoors), respectively.

All hair samples were collected from the flank region, placed in disposable paper envelopes, labeled, and transported to the laboratory. The samples were degreased, washed, and rinsed. Each sample weighed roughly 0.5g, and was digested using 5 ml HNO₃ 65% Suprapur PA and 1 ml HCl fuming 37% Selectipur PA, then diluted to 10 ml with ultrapure water and analysed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Samples were analysed using Perkin-Elmer - Elan DRC II ICP-MS spectrometer (RF1100 W; reading time 30 s, washing time 30 s, nebuliser gas flow 0.5 L min⁻¹; auxiliary gas flow 0.5 L min⁻¹; sample injection pump flow 50 rpm). Results for each sample are the average of three successive measurements. (Tudoreanu, 2005) Calibration curves were developed using standard solutions of 0.001 ppm, 0.01 ppm, 0.1 ppm, 1 ppm, 5 ppm, 10 ppm, 50 ppm obtained by dilution from a multi-element ICP MERCK standard containing 1000 ml L⁻¹ of heavy metals (Al, As, Cd, Cr, Ni, Pb, Sr) and other mineral elements (Ca, Co, Cu, Fe, K, Mg, Mn, Se, V, Zn).

Statistical analysis was performed using VassarStats: Website for Statistical Computation (http://vassarstats.net/). One-Way ANOVA was performed for all samples’ heavy metal and mineral concentrations.

RESULTS AND DISCUSSION
Mean heavy metal and mineral levels in dogs suffering from mammary adenocarcinoma and clinically healthy dogs, along with p-value resulting from One-Way ANOVA, are presented in Tab. 1.

Table 1. Study group and Control group heavy metal and other mineral elements mean concentrations (mg•kg⁻¹)

<table>
<thead>
<tr>
<th>Element</th>
<th>Study group</th>
<th>Control group</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al</td>
<td>66.00</td>
<td>215.14</td>
<td>0.29</td>
</tr>
<tr>
<td>As</td>
<td>BDL***</td>
<td>0.84</td>
<td>-</td>
</tr>
<tr>
<td>Ca</td>
<td>1045.81</td>
<td>2300.22</td>
<td>0.18</td>
</tr>
<tr>
<td>Cd</td>
<td>0.02</td>
<td>0.03</td>
<td>0.72</td>
</tr>
<tr>
<td>Co</td>
<td>0.17</td>
<td>0.30</td>
<td>0.50</td>
</tr>
<tr>
<td>Cr</td>
<td>2.66</td>
<td>3.79</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Cu</td>
<td>11.74</td>
<td>12.30</td>
<td>0.83</td>
</tr>
<tr>
<td>Fe</td>
<td>106.16</td>
<td>302.43</td>
<td>0.29</td>
</tr>
<tr>
<td>K</td>
<td>631.25</td>
<td>1199.77</td>
<td>0.29</td>
</tr>
<tr>
<td>Mg</td>
<td>133.23</td>
<td>288.78</td>
<td>0.13</td>
</tr>
<tr>
<td>Mn</td>
<td>4.17</td>
<td>13.32</td>
<td>0.53</td>
</tr>
<tr>
<td>Ni</td>
<td>0.76</td>
<td>1.01</td>
<td>0.44</td>
</tr>
<tr>
<td>Pb</td>
<td>0.74</td>
<td>1.25</td>
<td>0.28</td>
</tr>
<tr>
<td>Se</td>
<td>0.20</td>
<td>0.65</td>
<td>0.60</td>
</tr>
<tr>
<td>Sr</td>
<td>1.67</td>
<td>4.49</td>
<td>0.09</td>
</tr>
<tr>
<td>V</td>
<td>1.28</td>
<td>2.95</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Zn</td>
<td>101.74</td>
<td>117.44</td>
<td>0.49</td>
</tr>
</tbody>
</table>

* p-value resulting from One-Way ANOVA
** BDL – below method’s detection limit
All analyzed elements were insignificantly higher in clinically healthy dogs compared to the study group, with only chromium \((p < 0.05)\) and vanadium \((p < 0.01)\) being significantly higher.

For comparison with other findings in scientific literature related to heavy metal and other mineral elements levels in hair samples taken from patients with breast cancer, Tab. 2 reveals what methods of element quantification other researchers used, along with their results.

Al (Romanowicz-Makowska et al., 2011), As (Benderli Cihan et al., 2011; Zhang et al., 2007), Ca (Benderli Cihan et al., 2011), Cd (Ionescu et al., 2006; Benderli Cihan et al., 2011; Romanowicz-Makowska et al., 2011), Co (Benderli Cihan et al., 2011), Cr (Ionescu et al., 2006), Cu (Geraki et al., 2004; Geraki et al., 2002; Margaliot et al., 1983; Mulay et al., 1971), Fe (Geraki et al., 2004; Geraki et al., 2002; Ionescu et al., 2006), K (Geraki et al., 2004), Mg (Santoliquido et al., 1976; Mulay et al., 1971), Mn (Mulay et al., 1971; Benderli Cihan et al., 2011), Ni (Ionescu et al., 2006; Benderli Cihan et al., 2011; Rizk and Sky-Peck, 1984), Pb (Ionescu et al., 2006; Benderli Cihan et al., 2011), Se (Benderli Cihan et al., 2011; Rizk and Sky-Peck, 1984), Sr (Rizk and Sky-Peck, 1984), Zn (Kelleher et al., 2009; Geraki et al., 2004; Geraki et al., 2002; Margaliot et al., 1983; Santoliquido et al., 1976; Mulay et al., 1971; Ionescu et al., 2006; Benderli Cihan et al., 2011) registered higher levels in human cancerous breast tissue compared to noncancerous breast tissue. The present study found that all these elements were decreased in hair samples taken from dogs suffering from mammary cancer, compared to hair samples taken from clinically healthy dog. These findings suggest that the analyzed elements could not reach the hair in order to accumulate, as they might be retained in the cancerous mammary tissue.

Additionally, Cd (Johnson et al., 2003; Silva et al., 2012; Byrne et al., 2013) and Cr (Martin et al., 2003) form a high-affinity complex with the hormone binding domain of the estrogen receptors in the mammary gland. These findings suggest that the analyzed elements could not reach the hair in order to accumulate, as they might be retained in the cancerous mammary tissue.

<table>
<thead>
<tr>
<th>Type of hair sample</th>
<th>Method</th>
<th>Higher in cancer patients</th>
<th>Higher in control samples</th>
<th>The same in both groups</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>Flame atomic absorption spectroscopy</td>
<td>Fe, Zn</td>
<td>Ca, Mg</td>
<td></td>
<td>Skrajnowska et al. (2014)</td>
</tr>
<tr>
<td>Human</td>
<td>Flame atomic absorption spectroscopy</td>
<td>Ca, Cd, Co, Cr, Cu, K, Mg, Mn, Ni, Pb, Sr, Zn</td>
<td>Al, Fe</td>
<td></td>
<td>Pasha et al. (2010)</td>
</tr>
<tr>
<td>Human</td>
<td>Mass spectrometry</td>
<td>Al, As, K</td>
<td>Ca, Cr, Cu, Fe, Mg, Mn, Pb, Se, Zn</td>
<td>Cd</td>
<td></td>
</tr>
<tr>
<td>Human</td>
<td>Inductively coupled plasma mass spectrometry</td>
<td>Al, Ca, Cd, Co, Cr, Cu, Fe, Pb, Zn</td>
<td>K, Mg, Mn, Ni, Se, V</td>
<td></td>
<td>Yasuda et al. (2009)</td>
</tr>
<tr>
<td>Human</td>
<td>Atomic absorption spectrometer with deuterium background correction</td>
<td></td>
<td>Zn</td>
<td></td>
<td>Memon et al. (2007)</td>
</tr>
<tr>
<td>Human</td>
<td>Flame atomic absorption spectroscopy</td>
<td>Cu, Fe</td>
<td>Ca, Mg, Zn</td>
<td></td>
<td>Wang et al. (2006)</td>
</tr>
<tr>
<td>Human</td>
<td>Graphite furnace atomic absorption spectroscopy</td>
<td>Cr</td>
<td>Mn</td>
<td></td>
<td>Kilic et al. (2004)</td>
</tr>
</tbody>
</table>
further suggest that Cd and Cr cannot accumulate in hair because they are retained in the mammary tissue.

Ca also has a reduced intestinal absorption and elevated endogenous fecal concentration in human patients suffering from breast cancer (Coombes et al., 1977), providing additional evidence that Ca cannot accumulate in cancer patient's hair.

Vanadium has the potential of being an antitumor agent (Manna et al., 2011; Ray et al., 2007; Bishayee et al., 2000). This suggests that dogs with cancer lacked V’s protection, thus leading it to also not be found in hair, compared

**Figure 1.** Mean concentrations of Ca and K based on health status and keeping conditions (SGI = study group living indoors; SGO = study group living outdoors; CGI = control group living indoors; CGO = control group living outdoors)

**Figure 2.** Mean concentrations of Al, Fe, Mg, and Zn based on health status and keeping conditions (SGI = study group living indoors; SGO = study group living outdoors; CGI = control group living indoors; CGO = control group living outdoors)
to the significantly higher levels of V (p < 0.01) registered in the hair of clinically healthy dogs, as the present study revealed.

Mean concentrations of heavy metals and other mineral elements based on health status and keeping conditions are shown in Fig. 1-4. Between SGI, SGO, CGI, and CGO, CGI overall registered the highest levels for most heavy metals and other mineral elements: Al, As, Ca, Co, Cr, Fe, K, Mg, Mn, Ni, Pb, Sr, and V. The remaining elements (Cd, Cu, Se, Zn) were higher in CGO.

Between SGI and SGO, SGI registered the highest levels of K, all other elements being higher in the SGO.

**Figure 3.** Mean concentrations of Cu, Cr, Mn, Ni, Sr, and V based on health status and keeping conditions (SGI = study group living indoors; SGO = study group living outdoors; CGI = control group living indoors; CGO = control group living outdoors)

**Figure 4.** Mean concentrations of As, Cd, Co, Pb, and Se based on health status and keeping conditions (SGI = study group living indoors; SGO = study group living outdoors; CGI = control group living indoors; CGO = control group living outdoors)
Other researchers have concluded that heavy metals and other mineral elements register higher levels in the hair of individuals who have been in contact with polluted environments (Barana et al., 2013; Skibniewski et al., 2013; Tomza-Marciniak et al., 2012; Skibniewska et al., 2011; Miranda et al., 2004).

The results obtained in this study partially confirm other findings from the scientific literature regarding higher levels of heavy metals and other mineral elements being found in individuals from polluted environments.

CONCLUSIONS
All analyzed elements were elevated in clinically healthy dogs’ hair compared to dogs with mammary adenocarcinoma, as they might be retained in the cancerous mammary tissue, leading them to not accumulate in the hair of dogs with cancer.

The absence of vanadium’s protecting effect against mammary cancer may explain the significantly lower levels found in the hair of dogs with mammary adenocarcinoma. Between dogs with cancer living indoors and those living outdoors, with the exception of K, all analyzed elements were higher in the outdoor dogs, suggesting a possible implication of environmental pollution.

REFERENCES


