Arginine, Symmetric and Asymmetric Dimethylarginine Levels in Canine Leishmaniasis

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Abstract

The study aimed to investigate the levels of arginine, symmetric dimethylarginine (SDMA), and asymmetric dimethylarginine (ADMA) in dogs with canine leishmaniasis (CanL) and their relationship with some renal and cardiovascular parameters. A total of 60 dogs were enrolled, including 40 with CanL and 20 healthy controls. The CanL group was divided into four stages based on clinical and laboratory findings. The levels of plasma arginine, SDMA, and ADMA were determined by high performance liquid chromatography (HPLC). The data from the healthy group were compared with those from the CanL group, and according to the stages. In dogs with CanL, systolic and diastolic blood pressure, plasma creatinine, cystatin-C, phosphorus, potassium, and low-density lipoprotein concentrations, the urine protein/creatinine ratio, the amount of nitric oxide, and creatine kinase-MB activity were higher, while the high-density lipoprotein concentration was lower compared to healthy controls. The concentration of arginine was low (p < 0.05) and the levels of ADMA (p < 0.001) and SDMA (p < 0.05) were high in dogs with CanL. There were no statistically significant differences in arginine concentration among the different stages of CanL. However, the concentration of plasma ADMA was higher in all stages of CanL compared to the healthy group, and the concentration of plasma SDMA was higher in Stage IV compared to the healthy group and Stage III. The present study demonstrates for the first time a decrease in arginine concentration and an increase in ADMA concentration in dogs with CanL. The increase in SDMA concentration in CanL dogs is consistent with previous studies. These findings may serve as a source of further diagnostic and therapeutic research on the renal and cardiovascular pathophysiology of CanL. It is suggested that more clinical studies, including patient follow-up and treatment, would be beneficial in further elucidating the changes observed in CanL.

Introduction

Canine leishmaniasis (CanL) is a zoonotic, systemic disease caused by *Leishmania infantum* and transmitted by sand flies. It has a chronic course and may take years for clinical signs to appear. During this period, the disease can cause irreversible damage to various systems due to both the agent itself and the immune complexes that form in the body (Solano-Gallego et al. 2009). Common clinical findings in CanL include lymphadenopathy, weight loss, fever, epistaxis, exfoliative dermatitis, hyperkeratosis, onychogriposis, uveitis, and conjunctivitis. Laboratory findings may include mild anemia, thrombocytopenia, hypoalbuminemia, hypergammaglobulinemia, and azotemia. CanL has been the subject of numerous studies due to the pathologies it causes in the renal and cardiovascular systems, the lack of a definitive treatment for the agent, the prevalence of the disease, its chronic course, and the risk of zoonosis (Solano-Gallego et al. 2011; Paltrinieri et al. 2016; Roura et al. 2021).

Asymmetric dimethylarginine (ADMA) and symmetrical dimethylarginine (SDMA) are molecules that are produced during the proteolysis stage in the body through the process of arginine methylation. They may have toxic properties and are also used as biomarkers due to their specific metabolism (Tain and Hsu 2017). SDMA levels are often used as a biomarker for the diagnosis and prognosis of renal dysfunction, while ADMA is associated with cardiovascular diseases (Yerramilli et al. 2014; Valente et al. 2021). There
have been numerous studies in recent years examining the role of arginine and its metabolites in various diseases and conditions in humans and other species (Tain and Hsu 2017). However, studies on dogs have been limited, with most focusing on conditions such as atrial fibrillation, mitral insufficiency and heart failure (Ohnishi et al. 2002; Pedersen et al. 2006; Moesgaard et al. 2007; Valente et al. 2021).

The aim of this study was to determine arginine, ADMA, and SDMA levels in dogs with CanL and to reveal their relationship with other renal and cardiovascular parameters. We hypothesized that elevated ADMA and SDMA levels may play a role in the pathophysiological mechanisms of CanL. By evaluating ADMA and SDMA as markers for CanL in dogs at different stages of the disease, we hope to contribute to the literature and provide insights for diagnosis and treatment by revealing the arginine metabolism.

Material And Methods

This was a prospective, observational study of dogs naturally infected with CanL that presented at the Aydin Adnan Menderes University Veterinary Faculty Animal Hospital in the south-west region of Turkey over a period of 11 months from April 2018 to March 2019. Ethical approval for the study was obtained from the Aydin Adnan Menderes University Animal Experiments Local Ethics Committee on 17 April 2017, with approval number 64583101/2017/031. All dogs included in the study were recruited after informed consent was obtained from their owners.

Study Population

Dogs with CanL were selected from cases presenting with one or more clinical findings consistent with the disease. The initial diagnosis of CanL was based on a positive result from a rapid patient-side immunoassay test kit (Snap Leishmania, Idexx, USA) on a blood/plasma sample. Infection was confirmed by serological evaluation using an indirect fluorescent antibody test (IFAT) on serum samples at the Aydin Adnan Menderes University Faculty of Medicine/Veterinary Medicine Department of Parasitology. Dogs with CanL were divided into subgroups based on the severity of the disease according to clinical, serological, and laboratory findings and the recommendations of the Leishvet group (Solano-Gallego et al. 2011).

Exclusion criteria included treatment with any medication known to interfere with renal and cardiovascular function or against CanL either at presentation or within 8 weeks prior to presentation. Dogs had to be free of any other vector-borne diseases that could affect the variables. A rapid test kit (SNAP 4Dx plus, Idexx, USA) was used to exclude ehrlichiosis, anaplasmosis, borreliosis, and dirofilariasis. A control group of healthy dogs presented to the clinic for vaccination or health check-ups was also included. The control group consisted of dogs of both sexes, aged between 2 and 8 years, of medium size, with a normal body condition score, and without any abnormalities in clinical and laboratory evaluations (hematological, biochemical, serological, Snap 4Dx plus).
Detailed anamnesis data was collected for each of the dogs. As part of the systemic clinical examination, the main findings associated with CanL were evaluated and clinical scores were calculated (with scores ranging from 0–3 based on severity, and a maximum total score of 87) based on previously published literature (Proverbio et al. 2014). Body weight and condition were also assessed. Clinical examination and sampling were performed on a single occasion.

**Laboratory Analysis**

Blood samples were collected from the cephalic vein of the forelimb using tubes with and without anticoagulant, after at least 8 hours of fasting to minimize the impact of food intake on the results. Urine samples were collected either voluntarily or through catheterization. The samples were centrifuged to obtain serum and plasma, which were then stored at -20°C until analysis.

As part of the laboratory analysis, a complete blood count was performed using an automatic device (Abacus Junior Vet 5, Diatron, Hungary), including measurements of leukocytes (WBC), erythrocytes (RBC), hematocrit (HCT), platelets (PLT), mean erythrocyte volume (MCV), and mean erythrocyte hemoglobin concentration (MCHC). Routine serum biochemical tests, including urea, creatinine, total protein (TP), albumin (ALB), phosphorus, potassium, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and cholesterol, were performed using the colorimetric method on an autoanalyzer (RX Monaco, Randox, United States) with commercial test kits. The protein/creatinine ratio in the urine was calculated by measuring the protein and creatinine concentrations in the urine samples.

Plasma arginine, SDMA, and ADMA levels were analyzed using the High-Performance Liquid Chromatography (HPLC) method. The measurements were made using an HPLC device (Shimadzu Prominence, Japan) and commercial test kits that use a fluorimetric method (ADMA, SDMA, Arginine test, Eureka, Italy). While a similar method has been used in previous studies on dogs (Moesgaard et al. 2007), method validation (intra-assay/inter-assay coefficient of variability) was performed by repeating the measurements.

Plasma nitric oxide (NO) amount was measured using the colorimetric method, which determines the levels of nitrate and nitrite based on the Griess reaction, with a commercial test kit (Nitrate/Nitrite Colorimetric Assay Kit, Cayman Chemicals, United States). Serum cystatin C (Abbkine KTE20086 Canine Cystatin C, China) and plasma endothelial nitric oxide synthase (eNOS) (BT-LAB, E0341Ca Canine eNOS kit, China) concentrations were measured using dog-specific ELISA assays according to the manufacturer's instructions. Plasma Troponin I, Myoglobin, and CK-MB concentrations were measured using a Fluorescent Immununassay rapid test device (Finecare, Wondfo Biotech Co. Ltd, Finecare, China) and its commercial test kits.

**Statistical Analysis**
The distribution of numerical data was evaluated using the Kolmogorov-Smirnov or Shapiro-Wilk tests. For parameters with a non-normal distribution, transformation was applied. Nonparametric tests were used to analyze parameters with an abnormal distribution. The Mann-Whitney U test was used to compare the healthy and CanL dog groups, and the Kruskal-Wallis test and paired comparison method were used to determine differences between more than two groups of dogs with CanL by stage. The Pearson test was used to assess the correlation between plasma concentrations of arginine, ADMA, and SDMA and all other parameters measured in the study. Statistical analysis was performed using a statistical software package (SPSS 22.0, SPSS Inc., Chicago, USA). Probability values of p < 0.05 were considered statistically significant in all analyses. The median and interquartile range of values for the clinical, hematological and biochemical parameters, according to stages of CanL and healthy dogs, are presented in tables.

Results

The 40 dogs with CanL included in the study were divided into four groups based on serological, clinical, and laboratory findings: Stage I (n = 12), Stage II (n = 14), Stage III (n = 7), and Stage IV (n = 7). The 40 dogs with CanL and groups according to the stages of the disease were then compared with a healthy control group (n = 20).

No significant differences were observed in age [years: median (range); 6 (4,25–7,75) vs 5 (4–7)], sex (female/male; 9/11 vs 22/18) or body weight [kg: median (range); 20 (18,25–23,75) vs 21 (17,25–26,75)] between healthy and CanL dogs. The healthy group included 11 crossbreds, 3 Golden retrievers, 3 English pointers, 2 Labrador retrievers, and 1 boxer, while the CanL group included 26 crossbreds, 4 Golden retrievers, 3 Labrador retrievers, 2 Rottweilers, 2 Terriers, 2 Dobermans, and 1 Cocker spaniel.

IFAT values for dogs with CanL ranged from 1/64 to 1/2048. Amastigotes were detected in the lymph aspirates of 22 out of 40 dogs that were positive with rapid test kits. The IFAT values for dogs in the Stage I group ranged from 1/64 to 1/128, while those in the Stage II and III group ranged from 1/64 to 1/512. The IFAT values for dogs in the Stage IV group had titration steps ranging from 1/512 to 1/2048. When dogs were grouped according to the different stages of CanL, the IFAT values were found to be higher in the Stage IV group compared to the Stage I and Stage II groups (p < 0.001). Lymphadenomegaly, conjunctivitis, uveitis, epistaxis, anorexia, weight loss, dry exfoliative dermatitis, onychogriposis, alopecia, auricular dermatosis, and nasal hyperkeratosis were the most common findings in dogs with CanL. The clinical score was found to be higher in dogs in the Stage III and Stage IV groups compared to those in the Stage I group (p < 0.001) when the dogs were grouped according to the different stages of CanL.

Systolic (p < 0.001) and diastolic (p < 0.01) blood pressures were found to be higher in dogs with CanL compared to healthy dogs. Systolic blood pressure was found to be higher in dogs in the Stage I (p < 0.05), Stage II (p < 0.01), Stage III, and Stage IV (p < 0.001) groups compared to healthy dogs, and diastolic blood pressure was found to be higher in dogs in the Stage III and Stage IV (p < 0.01) groups compared to healthy dogs. While there was no difference in WBC counts in the hematological evaluation, RBC (p < 0.05) and PLT (p < 0.01) counts and HCT, MCV, and MCHC values (p < 0.001) were found to be
lower in dogs with CanL compared to healthy. According to the stages, HCT value was lower in Stage II, III, and IV group dogs and the MCV value was lower in Stage I, II, and III group dogs compared to the healthy group. The MCHC value was lower in the Stage I and II groups, and the PLT count was lower in the Stage II group compared to the healthy group. Table 1 presents clinical, parasitological, and hematological findings classified by stages in CanL and healthy dogs.
Table 1
Clinical, parasitological, and hematological findings classified by stages in CanL and healthy dogs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Healthy (n = 20)</th>
<th>Stage I (n = 12)</th>
<th>Stage II (n = 14)</th>
<th>Stage III (n = 7)</th>
<th>Stage IV (n = 7)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFAT</td>
<td>-a</td>
<td>64b</td>
<td>128bc</td>
<td>256c</td>
<td>512c</td>
<td>0,000</td>
</tr>
<tr>
<td></td>
<td>(64–80)</td>
<td>(128–204)</td>
<td>(128–512)</td>
<td>(512–1024)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinical score</td>
<td>0a</td>
<td>5b</td>
<td>11bc</td>
<td>22c</td>
<td>24cd</td>
<td>0,000</td>
</tr>
<tr>
<td></td>
<td>(3–7)</td>
<td>(8–14)</td>
<td>(18–24)</td>
<td>(22–34)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>110a</td>
<td>130b</td>
<td>136b</td>
<td>148b</td>
<td>165b</td>
<td>0,000</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>65a</td>
<td>70a</td>
<td>70a</td>
<td>80b</td>
<td>80b</td>
<td>0,000</td>
</tr>
<tr>
<td></td>
<td>(65–70)</td>
<td>(60–80)</td>
<td>(70–80)</td>
<td>(80–110)</td>
<td>(80–110)</td>
<td></td>
</tr>
<tr>
<td>WBC (x10^9/L)</td>
<td>10,12</td>
<td>11,99</td>
<td>10,17</td>
<td>12,5</td>
<td>11,01</td>
<td>0,357</td>
</tr>
<tr>
<td></td>
<td>(7,22–12,06)</td>
<td>(6,8–13,57)</td>
<td>(9,35–14,75)</td>
<td>(10,28–16,43)</td>
<td>(9,65–14,27)</td>
<td></td>
</tr>
<tr>
<td>RBC (x10^{12}/L)</td>
<td>6,23</td>
<td>5,23</td>
<td>5,55</td>
<td>5,7</td>
<td>4,87</td>
<td>0,091</td>
</tr>
<tr>
<td></td>
<td>(5,86–6,76)</td>
<td>(3,82–6,76)</td>
<td>(4,12–6,22)</td>
<td>(3,7–6,97)</td>
<td>(3,24–6,09)</td>
<td></td>
</tr>
<tr>
<td>HCT (%)</td>
<td>45,08a</td>
<td>36,85ab</td>
<td>34,85b</td>
<td>33,49b</td>
<td>31,91b</td>
<td>0,000</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>76a</td>
<td>68b</td>
<td>62b</td>
<td>62b</td>
<td>66ab</td>
<td>0,000</td>
</tr>
<tr>
<td></td>
<td>(74–78)</td>
<td>(60–71)</td>
<td>(57–69)</td>
<td>(52–64)</td>
<td>(63–71)</td>
<td></td>
</tr>
<tr>
<td>MCHC (g/dL)</td>
<td>38,4a</td>
<td>33,7b</td>
<td>31,4b</td>
<td>37,2ab</td>
<td>33,2ab</td>
<td>0,000</td>
</tr>
<tr>
<td></td>
<td>(36,1–41,0)</td>
<td>(31,1–37,7)</td>
<td>(31,2–32,5)</td>
<td>(31,9–38,4)</td>
<td>(30,1–36,3)</td>
<td></td>
</tr>
<tr>
<td>PLT (x10^9/L)</td>
<td>294a</td>
<td>216ab</td>
<td>114b</td>
<td>144ab</td>
<td>283ab</td>
<td>0,022</td>
</tr>
</tbody>
</table>

Medians with different superscript letters indicate significant differences (P < 0.05) among each group
Plasma creatinine, cystatin C, phosphorus, and potassium concentrations and the urinary protein/creatinine ratio were found to be higher in dogs with CanL (p < 0.001).

Table 2 lists some biochemical and renal biomarker findings classified by stages in CanL and healthy dogs. Urea, creatinine, cystatin C, and phosphorus concentrations were found to be higher in the Stage III and IV groups compared to healthy dogs and Stage I dogs. The urine protein/creatinine ratio was found to increase in all groups compared to the healthy group, and it was observed that the Stage IV group had a higher ratio than the Stage I group. Potassium concentration was found to be higher in the Stage II, III, and IV groups compared to the healthy group.
Table 2

Some biochemical and renal biomarker findings classified by stages in CanL and healthy dogs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Healthy</th>
<th>Stage I</th>
<th>Stage II</th>
<th>Stage III</th>
<th>Stage IV</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 20)</td>
<td>(n = 12)</td>
<td>(n = 14)</td>
<td>(n = 7)</td>
<td>(n = 7)</td>
<td></td>
</tr>
<tr>
<td>Urea (mg/dL)</td>
<td>21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>30&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>60&lt;sup&gt;b&lt;/sup&gt;</td>
<td>151&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0,000</td>
</tr>
<tr>
<td></td>
<td>(15–31)</td>
<td>(11 – 2)</td>
<td>(12–57)</td>
<td>(40–68)</td>
<td>(86–196)</td>
<td></td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0,91&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0,65&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1,05&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1,7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4,72&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0,000</td>
</tr>
<tr>
<td></td>
<td>(0,68 - 0,97)</td>
<td>(0,37 - 1,2)</td>
<td>(0,89 - 1,47)</td>
<td>(1,53 - 1,83)</td>
<td>(2,45 - 7,12)</td>
<td></td>
</tr>
<tr>
<td>Cystatin-C (µg/L)</td>
<td>457&lt;sup&gt;a&lt;/sup&gt;</td>
<td>325&lt;sup&gt;a&lt;/sup&gt;</td>
<td>525&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>850&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1655&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0,000</td>
</tr>
<tr>
<td>Urine Protein/Creatinine</td>
<td>0,1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0,2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0,75&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0,000</td>
</tr>
<tr>
<td></td>
<td>(0,1 - 0,1)</td>
<td>(0,19 - 0,32)</td>
<td>(0,6 - 0,97)</td>
<td>(1–4)</td>
<td>(3–10)</td>
<td></td>
</tr>
<tr>
<td>Phosphorus (mg/dL)</td>
<td>4,26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4,59&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4,72&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>5,50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8,85&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0,000</td>
</tr>
<tr>
<td></td>
<td>(3,82 - 4,45)</td>
<td>(3,96 - 4,77)</td>
<td>(4,51 - 5,50)</td>
<td>(5,1–5,9)</td>
<td>(7,42 - 12,25)</td>
<td></td>
</tr>
<tr>
<td>Potassium (mg/dL)</td>
<td>4,04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4,12&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>4,28&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4,29&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0,000</td>
</tr>
<tr>
<td></td>
<td>(3,91 - 4,12)</td>
<td>(4,04– 4,24)</td>
<td>(4,11 - 4,36)</td>
<td>(4,26 – 4,5)</td>
<td>(5,05–7,45)</td>
<td></td>
</tr>
<tr>
<td>TP (mg/dL)</td>
<td>6,44</td>
<td>6,8</td>
<td>6,7</td>
<td>7,55</td>
<td>5,8</td>
<td>0,281</td>
</tr>
<tr>
<td></td>
<td>(5,86 - 7,45)</td>
<td>(6,2–7,15)</td>
<td>(5,7–10,7)</td>
<td>(5,55 – 8,2)</td>
<td>(5,48 – 5,9)</td>
<td></td>
</tr>
<tr>
<td>ALB (mg/dL)</td>
<td>2,69&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3,25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2,7&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2,3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2,1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0,000</td>
</tr>
<tr>
<td></td>
<td>(2,42 - 2,88)</td>
<td>(2,77 – 3,43)</td>
<td>(2,48 – 2,9)</td>
<td>(1,94 – 2,55)</td>
<td>(1,83 – 2,6)</td>
<td></td>
</tr>
</tbody>
</table>

Medians with different superscript letters indicate significant differences (P < 0.05) among each group

There were no statistical differences in cholesterol and myoglobin concentrations and troponin and eNOS activities between the CanL and healthy. In dogs with CanL, low HDL concentration (p < 0.001), high LDL concentration (p < 0.001), high NO amount (p < 0.01), and high CK-MB activity (p < 0.05) were observed.

Table 3 presents the cardiovascular biomarker findings classified by stages in CanL and healthy dogs in the study. The differences in HDL, LDL, myoglobin, and NO concentrations and troponin and CK-MB
activities were significant. Compared to the healthy group, HDL concentration was lower and LDL concentration was higher in all stages of the disease. NO concentration was higher in the Stage II, III, and IV groups compared to the healthy and Stage I groups. Troponin activity was higher in the Stage IV group than other groups. CK-MB activity was higher in the Stage III group than in healthy, Stage I, and II groups, and myoglobin concentration was higher in the Stage IV group than in healthy, Stage I, and II groups. NO concentration was higher in the Stage II, III, and IV groups compared to healthy and Stage I groups.

Table 3
Cardiovascular biomarker findings classified by stages in CanL and healthy dogs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Healthy (n = 20)</th>
<th>Stage I (n = 12)</th>
<th>Stage II (n = 14)</th>
<th>Stage III (n = 7)</th>
<th>Stage IV (n = 7)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Troponin (ng/mL)</td>
<td>0,1&lt;sup&gt;a&lt;/sup&gt; (0,1 – 0,1)</td>
<td>0,1&lt;sup&gt;a&lt;/sup&gt; (0,1 – 0,1)</td>
<td>0,1&lt;sup&gt;a&lt;/sup&gt; (0,1 – 0,1)</td>
<td>0,1 ± 0&lt;sup&gt;ab&lt;/sup&gt; (0,1 – 0,1)</td>
<td>0,1&lt;sup&gt;b&lt;/sup&gt; (0,17 – 0,37)</td>
<td>0,000</td>
</tr>
<tr>
<td>Myoglobin (ng/mL)</td>
<td>5,15&lt;sup&gt;a&lt;/sup&gt; (4,47 – 7)</td>
<td>6,3&lt;sup&gt;a&lt;/sup&gt; (4,67 – 7,79)</td>
<td>4,23&lt;sup&gt;ab&lt;/sup&gt; (3,04 – 6,56)</td>
<td>6&lt;sup&gt;abc&lt;/sup&gt; (4,51 – 8)</td>
<td>9,04&lt;sup&gt;c&lt;/sup&gt; (6,56 – 12,41)</td>
<td>0,042</td>
</tr>
<tr>
<td>CK-Mb (ng/mL)</td>
<td>0,3&lt;sup&gt;a&lt;/sup&gt; (0,3 – 0,3)</td>
<td>0,3&lt;sup&gt;a&lt;/sup&gt; (0,3 – 0,3)</td>
<td>0,3&lt;sup&gt;a&lt;/sup&gt; (0,3 – 0,3)</td>
<td>3,5&lt;sup&gt;b&lt;/sup&gt; (0,3 – 5)</td>
<td>0,3&lt;sup&gt;ab&lt;/sup&gt; (0,3 – 2,2)</td>
<td>0,000</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>141&lt;sup&gt;a&lt;/sup&gt; (138 – 152)</td>
<td>58&lt;sup&gt;b&lt;/sup&gt; (52 – 68)</td>
<td>67&lt;sup&gt;b&lt;/sup&gt; (56 – 86)</td>
<td>77&lt;sup&gt;b&lt;/sup&gt; (44 – 87)</td>
<td>75&lt;sup&gt;b&lt;/sup&gt; (60 – 82)</td>
<td>0,000</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>23,1&lt;sup&gt;a&lt;/sup&gt; (21,6 – 25,4)</td>
<td>67,9&lt;sup&gt;b&lt;/sup&gt; (53,2 – 82,8)</td>
<td>55,3&lt;sup&gt;b&lt;/sup&gt; (32,1 – 66,9)</td>
<td>37,1&lt;sup&gt;b&lt;/sup&gt; (30,9 – 108,9)</td>
<td>58,8&lt;sup&gt;b&lt;/sup&gt; (45,9 – 104,1)</td>
<td>0,000</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>212 (183 – 215)</td>
<td>205 (178 – 214)</td>
<td>215 (184 – 262)</td>
<td>241 (158 – 308)</td>
<td>246 (216 – 324)</td>
<td>0,134</td>
</tr>
<tr>
<td>NO (µM)</td>
<td>4,69&lt;sup&gt;a&lt;/sup&gt; (4,09 – 5,45)</td>
<td>3,7&lt;sup&gt;ab&lt;/sup&gt; (3,13 – 8,19)</td>
<td>7,41&lt;sup&gt;b&lt;/sup&gt; (5,42 – 8,78)</td>
<td>7,1&lt;sup&gt;b&lt;/sup&gt; (5,63 – 9,46)</td>
<td>8,39&lt;sup&gt;b&lt;/sup&gt; (8,23 – 9,5)</td>
<td>0,000</td>
</tr>
<tr>
<td>eNOS (pg/mL)</td>
<td>1633 (1398 – 1855)</td>
<td>1746 (1436 – 1995)</td>
<td>1755 (1441 – 2002)</td>
<td>1977 (1222 – 2375)</td>
<td>1850 (1540 – 1892)</td>
<td>0,942</td>
</tr>
</tbody>
</table>

Medians with different superscript letters indicate significant differences (P < 0.05) among each group.
In dogs with CanL, arginine concentrations were low (p < 0.05), while ADMA (p < 0.001) and SDMA (p < 0.05) levels were high as presented in Fig. 1.

Table 4 displays the levels of arginine, ADMA and SDMA classified by stages in CanL and healthy dogs. It was observed that the differences in arginine concentration in dogs with CanL grouped by stage and healthy groups were not statistically significant. However, the mean plasma arginine concentration was lower in dogs in the Stage II, III, and IV groups compared to the healthy and Stage I groups. Plasma ADMA concentration was higher in dogs of all stages compared to the healthy group (p < 0.001). Plasma SDMA concentration was higher in Stage IV group dogs compared to the healthy and Stage III groups (p < 0.05).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Healthy</th>
<th>Stage I</th>
<th>Stage II</th>
<th>Stage III</th>
<th>Stage IV</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine (µmol/L)</td>
<td>168 (130–181)</td>
<td>154 (128–170)</td>
<td>138 (117–167)</td>
<td>136 (127–1138)</td>
<td>118 (107–162)</td>
<td>0,149</td>
</tr>
<tr>
<td>ADMA (µmol/L)</td>
<td>0,42a (0,12 – 0,73)</td>
<td>2,47b (0,35 – 4,98)</td>
<td>2,19b (0,7 – 3,5)</td>
<td>2,01b (1,63 – 3,5)</td>
<td>4,62b (1,82 – 6,56)</td>
<td>0,000</td>
</tr>
<tr>
<td>SDMA (µg/dL)</td>
<td>6,06a (4,72 – 7,14)</td>
<td>10,00ab (4,59 – 16,53)</td>
<td>7,40a (3,85 – 10,75)</td>
<td>4,80ab (3,80 – 10,00)</td>
<td>12,80b (9,16 – 13,80)</td>
<td>0,044</td>
</tr>
</tbody>
</table>

Medians with different superscript letters indicate significant differences (P < 0.05) among each group.

A negative correlation was found between arginine concentration and platelet count and potassium concentration. A positive correlation was found between ADMA concentration and urea, creatinine, phosphorus, potassium, cystatin C concentrations, troponin activity, and urine protein/creatinine ratio. A weak negative correlation was found between SDMA and cholesterol concentrations.

**Discussion**

In this study, it was found that dogs with CanL had low arginine concentrations and high ADMA and SDMA concentrations. These findings suggest a relationship between arginine metabolism impairment and CanL, leading to increased dimethylarginine levels and indicating the potential use of arginine metabolism as a diagnostic and therapeutic target in CanL. Additionally, dogs with CanL had an atherogenic lipid profile characterized by low HDL and high LDL concentrations, which may contribute to the cardiovascular pathophysiology of CanL. Nitric oxide concentration was also found to be higher in dogs with CanL, possibly due to the oxidative and inflammatory processes associated with the disease.
According to the staging criteria of CanL (Solano-Galego et al. 2011) and International Renal Interest Society (IRIS, 2023), the renal profile was normal in stages I and II, with first or second degree chronic renal failure found in stage III, and third or fourth degree chronic renal failure found in Stage IV. The hematological profile showed that RBC (p < 0.05) and PLT (p < 0.01) counts and HCT, MCV, and MCHC values (p < 0.001) were all significantly lower in dogs with CanL. Among the routine biochemical parameters, plasma creatinine, phosphorus, and potassium concentrations and urinary protein/creatinine ratio were all significantly higher in dogs with CanL (p < 0.001). These clinical, hematological, and biochemical findings were found to be consistent with the literature (Solano-Gallego et al. 2009; Paltrinieri et al. 2016; Roura et al. 2021).

SDMA levels have been evaluated in the early diagnosis of renal diseases in cats and dogs through various studies, and its benefits have been demonstrated in relation to serum urea and creatinine concentrations (Braff et al. 2014; Hall et al. 2014a; Hall et al. 2014b; Yerramilli et al. 2014; Hall et al. 2015; Nabity et al. 2015). However, there is ongoing debate about the use of SDMA in routine clinical practice for the diagnosis and monitoring of treatment (Santoro 2018). Some current studies have found that SDMA does not change during treatment monitoring in dogs with CanL (Pardo-Marin et al. 2017; Torrent et al. 2018). Another study found that SDMA was not effective in distinguishing between acute kidney injury and chronic kidney failure in dogs (Dahlem et al. 2017). In the present study, we found differences in urea, creatinine, cystatin C, phosphorus, and potassium concentrations and urinary protein/creatinine ratio in healthy dogs and dogs at different stages of CanL, which were consistent with the literature (Pasa et al. 2009; Paltrinieri et al. 2016). Plasma SDMA concentration was found to be significantly higher in the Stage IV group compared to the healthy and Stage III groups (p < 0.05). However, only three dogs with CanL had SDMA concentrations above > 14 µg/dl, which is the first stage of renal failure according to IRIS. It is possible that this is due to the different methods used for SDMA analysis. In addition, there was no significant increase in the SDMA levels of dogs in the Stage I and II groups, which are thought to be in the early stages of renal damage, compared to healthy dogs. Pardo-Marin et al. (2017) found that changes in serum SDMA levels before and after treatment were not significant in dogs with and without proteinuria. Torrent et al. (2018) reported that increases in urine protein/creatinine ratio (> 0.5), SDMA (> 19 µg/dL), and creatinine concentrations (≥ 1.4 mg/dL) were detected in 47%, 15%, and 9% of dogs with CanL, respectively, and there were no significant changes in SDMA levels after 6 months of follow-up. The lack of an increase in SDMA levels in the early stages in this study is consistent with other studies conducted in dogs with CanL (Pardo-Marin et al. 2017; Torrent et al. 2018). In addition, limited value of SDMA levels distinguishing between LeishVet clinical stages was consistent with another study (Giapitzoglou et al. 2020). When evaluating the results of our study, it appears that the potential benefits of using SDMA levels for the early diagnosis of renal dysfunction should continue to be investigated, particularly in comparison to more classical and economical methods such as creatinine concentration or urine protein/creatinine ratio.

There have been case reports (Mendes et al. 2014) and studies (Silvestrini et al. 2012; Xenoulis et al. 2014; Ural et al. 2017) that have investigated troponin concentrations in CanL. A study that evaluated Troponin-I levels as a marker of myocardial damage in CanL found that it was increased in 40% of dogs
Another study found that Troponin-I activities were not above the reference range (> 0.5 ng/mL) before treatment or at the 2nd and 4th weeks after treatment in dogs diagnosed with CanL and treated with a combination of allopurinol and meglumine antimonate or allopurinol alone (Xenoulis et al. 2014). In the present study, Troponin-I values were above the reference range in three cases in the Stage IV group and one in the Stage III group. Serum Troponin-I activity was found to be < 0.1 ng/mL in all cases in the Stage I and II groups. The increases in Ck-MB activity and myoglobin concentration that accompanied the increase in Troponin-I activity in the Stage III and Stage IV groups are significant in terms of indicating myocardial damage in cases in these stages. However, in addition to the cases in the Stage I and II groups, some cases in the Stage III and IV groups had relevant cardiac biomarkers within normal limits. Since our study did not involve repeated measurements, it was not possible to determine whether there was a persistent increase in Troponin-I, CK-MB, and myoglobin levels. The continuous increase in these values is important in demonstrating irreversible and ongoing cardiomyocyte damage (Wells and Sleeper 2008). In this context, it can be suggested that myocardial damage may not have progressed or may not have developed yet in some cases included in the study.

In many infections, changes in lipid and lipoprotein concentrations can occur as a result of either the direct effects of the agent or the indirect response of the organism to infection or inflammation (Liberopoulos et al. 2014). In this study, HDL concentration was found to be significantly lower in all groups with CanL compared to healthy dogs (p < 0.001). These changes in HDL levels are consistent with findings in dogs with babesiosis (Cunha et al. 2000; Mrljak et al. 2014) and CanL (Durgut et al. 2012). It is suggested that low levels of HDL may be associated with liver function disorders or decreased lipoprotein lipase activity caused by the effects of vector-borne diseases (Mrljak et al. 2014). Another reason for this may be the low activity of the antioxidant system, which protects HDL against oxidative damage, in dogs with CanL (Gultekin et al. 2017). In this study, LDL concentrations were found to be higher in dogs with CanL than in healthy dogs, as previously shown (Nieto et al. 1992; Durgut et al. 2012; Ruiz-Tapia et al. 2014; Gultekin et al. 2017). Increases in LDL concentration, known as "bad lipoprotein" increases or atherogeny, are a predisposing factor for atherosclerosis and cardiovascular diseases in humans (Litvinov et al. 2012). The decrease in HDL level and increase in LDL level observed in this study can also be considered an atherogenic lipid profile (Siewert et al. 2015).

In this study, it was found that NO concentration was higher in the Stage II, III, and IV groups compared to the healthy and Stage I groups. The increase in nitric oxide levels in dogs with CanL has also been reported in previous studies (Baldissera et al. 2015; Askar et al. 2019). It is suggested that this may be a part of the oxidative and inflammatory process related to the disease, and one of the main factors contributing to the increase is the release of nitric oxide during the phagocytosis of L. infantum amastigotes by macrophages (Panaro et al. 2008). The increase in nitric oxide concentrations found in this study is consistent with literature data and confirms its close relationship with the pathophysiology of CanL.

Nitric oxide synthesis capacity can be revealed by evaluating nitric oxide, arginine, ADMA, and eNOS parameters (Mels et al. 2016). In this study, plasma ADMA (p < 0.001) and SDMA (p < 0.05)
concentrations were higher and arginine concentrations were lower (p < 0.05) in dogs with CanL compared to the healthy group. At high concentrations, ADMA acts as an inhibitor of eNOS and reduces the synthesis of usable nitric oxide. The decrease in arginine levels during inflammatory processes may limit the beneficial effects of nitric oxide, such as the activation of macrophages and T-lymphocytes and vasodilation (Schwedhelm et al. 2008; Wijnands et al. 2015). In addition to these findings, the revelation of an atherogenic profile characterized by a decrease in HDL concentration and an increase in LDL concentration in all stages of plasma lipid analysis is important in understanding the cardiovascular pathophysiology in dogs with CanL. In fact, systolic and diastolic blood pressures in dogs with CanL were found to be higher than in healthy dogs, which supports these data in the context of clinical findings (p < 0.001). Therefore, considering the cardiovascular pathophysiology in the treatment of dogs with CanL may be important in terms of preventing or correcting clinical findings.

There are also some limiting factors that make it difficult to directly explain the changes in all parameters in this study. While data providing a foundation for future research are obtained with our study results, it may be more useful to evaluate a larger number of cases by repeating relevant measurements in experimental or natural infection models in dogs with CanL and by investigating their responses to treatment interventions.

As a result, a decrease in arginine concentration and an increase in ADMA concentration were demonstrated for the first time in dogs with CanL as a contribution to the literature. The increase in SDMA concentration in dogs with CanL was consistent with previous studies. It is thought that the findings obtained could be a source for future diagnostic and therapeutic studies to explain the renal and cardiovascular pathophysiology of CanL. It is concluded that more clinical studies, including patient follow-up and treatment, to detail the changes identified in CanL, would be beneficial.

Declarations

Funding

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval

The study was approved by the Animal Experiments Local Ethics Committee of Aydin Adnan Menderes University with the date 17.04.2017 and number 64583101/2017/031.

Consent to participate

Not applicable.

Consent to publish

Not applicable.

References


Figures
Figure 1 Concentrations of arginine (a), ADMA (b), and SDMA (c) in dogs with CanL and healthy. Boxes show the 25th and 75th percentile, whiskers show the 10th and 90th percentile. The line inside each box indicates the median. Dots represent results outside the slice.

Figure 1
See image above for figure legend