THE SYSTEMIC-IMMUNE INFLAMMATION INDEX IN NATURALLY OBESE DOGS

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ABSTRACT

Obesity is one of the most prevalent health problems in the canine population. This study aimed to evaluate the clinical usability of the systemic immune inflammation index (SII) in obese dogs. A total of 25 obese dogs compared with 10 normal-weight dogs. Complete blood counts, the neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) were measured in all dogs. The SII was calculated with the neutrophil count \times platelet count/lymphocyte count formula. C-reactive protein (CRP) concentrations were determined with dog-specific commercial ELISA test kits. The obese group had statistically higher WBC, neut, PLT, and SII than the control group. However, no significant difference between the groups in NLR, PLR, and CRP concentration. Additionally, a positive correlation between SII and serum CRP was determined. To the best of our knowledge, this is the first study to demonstrate the SII levels in obese dogs. Nevertheless, considering no statistical difference between inflammatory parameters such as NLR, PLR, and CRP between the two groups, large-scale studies are needed to reveal the reason for this increase in SII in obese dogs and understand its clinical utility.

Keywords: C-reactive protein, inflammation, obesity, neutrophil-to-lymphocyte ratio, systemic immune-inflammation index

INTRODUCTION

Obesity is a pathological condition characterized by excessive fat storage that leads to changes in various body functions, and it usually occurs when the ideal body weight increases by 15% (Laflamme, 2001; Piantedosi et al., 2016). It is the most common nutritional disease in dogs. Recently, the prevalence of overweight and obesity has been estimated to have increased by approximately 37% in dogs, affecting almost 59.3% of the dog population (Vedrine et al., 2021; Marchi et al., 2022). Risk factors for obesity in dogs are multifactorial. It includes dog-related characteristics and owner-specific factors (Preet et al., 2021). The primary factor in dog-related characteristics is breed. Beagles, Cocker Spaniels, Dachshunds, Labrador Retrievers and Golden Retrievers breeds are more likely to be obese. Other risk factors for canine obesity include age, gender, neutering, types of food, activity, and feeding frequency (Preet et al., 2021; Chiang et al., 2022).

The life span and various body functions are affected by obesity in humans and animals.
Like humans, canine obesity can precipitate or exacerbate various clinical conditions such as osteoarthritis, metabolic diseases, certain types of cancer, and respiratory disease (Piantedosi et al., 2016; Chiang et al., 2022). It may also contribute to a shortened lifespan, increased risk of surgery, and exercise intolerance (German et al., 2009; Piantedosi et al., 2016; Shepherd, 2021). In humans, obesity is considered a chronic inflammatory condition that causes increased production and release of proinflammatory mediators accompanied by increases in white blood cells (WBC), lymphocytes (Lymph), and C-reactive protein (CRP) (Radakovich et al., 2017; Barić Rafaj et al., 2017). However, the relationship between inflammation and obesity in veterinary medicine is controversial (Veiga et al., 2008; German et al., 2009; Tvarijonaviciute et al., 2012; Barić Rafaj et al., 2017).

Recently, the components of complete blood count (CBC) and the parameters derived from them have been widely used in disease diagnosis, treatment follow-up, prognosis, and determination of systemic inflammation due to their cheap and easy accessibility (Pierini et al., 2019; Park & Lee, 2022). Neutrophil-lymphocyte ratio (NLR) and platelet-lymphocyte ratio (PLR) are two of these parameters, and their clinical and diagnostic significance has recently been demonstrated in dogs with different diseases (Pierini et al., 2019; Becher et al., 2021; Neumann, 2021; Pierini et al., 2021; Dinler Ay, 2022). The systemic immune-inflammation index (SII) is derived from CBC like NLR and PLR and reflects local immune response and systemic inflammation (Yazlık et al., 2022). As a biomarker of the inflammatory process in human medicine, it has found its place as a prognostic factor in different malignancies (Sun et al., 2020). The SII has been evaluated in dogs with few studies on different diseases such as oral melanoma, chronic enteropathy, pyometra and diabetes mellitus (Cristóbal et al., 2022; Garcia et al., 2022; Yazlık et al., 2022).

With the prevalence of obesity in dogs increasing worldwide, there is a need to determine how routine veterinary testing may be affected by obesity. The inflammatory state is controversial in dogs. Some researchers suggest that obesity may be associated with a chronic, low level of inflammation (German et al., 2009; Barić Rafaj et al., 2017). However, there is limited research regarding this condition in dogs (Veiga et al., 2008; German et al., 2009; Tvarijonaviciute et al., 2012; Barić Rafaj et al., 2017). Also, no studies evaluate the SII in obese dogs. Therefore, this study aimed to assess the clinical usability of SII in obese dogs, which has recently been used as an inflammatory biomarker in humans.

MATERIALS AND METHODS

Aydın Adnan Menderes University Animal Research Ethics Committee has reviewed and approved all study procedures with protocol number 64583101/2022/117.

Dogs

The present study was conducted on 35 dogs of different ages, sexes, and breeds brought to the Aydın Adnan Menderes University Veterinary Faculty Research and Practice Hospital. The dogs' body condition scores (BCS) were evaluated on a 9-point scale (Laflamme, 2001). The dogs of the control group (n=10) were clinically healthy based on physical examination and laboratory findings. Moreover, these dogs were brought for annual routine control, vaccination and selective operation (Castration or ovariohysterectomy). The BCS of these dogs ranged from 4 to 5 points. Dogs with BCS > 7 were included in the obese group (n=25). All dogs show the exclusion criteria (diagnosed comorbidities such as inflammatory disease, neoplasm and endocrinopathies and ongoing treatment etc.) were removed from the study.

The control group included 4 Crossbreeds, 2 Golden retrievers, 2 Terriers, and 2 Cavalier King Charles breeds dogs. In the obese group,
there were 11 Crossbreds, 4 Golden retrievers, 3 Terriers, 2 Cockers, 2 Labrador retrievers, 2 cavalier King Charles and 1 French bulldog. The characteristics of dogs in the control and obese groups are shown in Table 1.

### Table 1: Characterization of the study population.

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Obese group</th>
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</thead>
<tbody>
<tr>
<td><strong>Number of dogs</strong></td>
<td>10</td>
<td>25</td>
</tr>
<tr>
<td><strong>Number of male/female (%) dogs</strong></td>
<td>3 (30%) / 7 (70%)</td>
<td>4 (16%) / 21 (84%)</td>
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<tr>
<td><strong>Neutered/spayed (%)</strong></td>
<td>5 (50%)</td>
<td>20 (80%)</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>5.9±2.07</td>
<td>6.68±1.33</td>
</tr>
<tr>
<td><strong>Body weight (kg) (mean±SD) (Min-Max)</strong></td>
<td>14.96±10.44 (3-29)</td>
<td>23.47±12.32 (6-43)</td>
</tr>
<tr>
<td><strong>Body condition score</strong></td>
<td>4-5</td>
<td>&gt; 7</td>
</tr>
</tbody>
</table>

Abbreviation: SD, standard derivation; Min, minimum; Max, maximum

**Laboratory Analysis**

Blood samples from dogs were collected via cephalic vein puncture into serum separator tubes and anticoagulant tubes containing K3-ethylenediaminetetraacetic acid (EDTA). CBC of dogs was performed with an automated blood cell counter (Abacus Vet5, Diatron, Budapest, Hungary) using samples with EDTA within 15 min after blood collection. WBC, neutrophil (Neut), Lymph, and platelet (PLT) counts were recorded directly from CBC. NLR and PLR were calculated as the ratio of absolute neut, and PLT counts to the absolute Lymph count, respectively. SII was calculated with the formula of neutrophil count x platelet count/lymphocyte count. Blood samples within the serum separator tubes were centrifuged after clot retraction at 2000 g for 10 min to obtain sera. Serum samples were stored at −20°C until the CRP analysis (within 1-2 months). Serum CRP concentrations were determined using a dog-specific ELISA commercial assay (Tridelta, Ireland).

**Data analysis**

Statistical analyses were conducted using Statistical Package for the Social Sciences (SPSS) 19.0 (Armonk, USA). WBC, neut, and PLT were normally distributed according to the Shapiro-Wilk test, while Lymph, NLR, PLR, SII, and CRP did not show normal distribution. However, considering the sample size of the groups, the nonparametric Mann-Whitney U test was used. Spearman correlation coefficient (rho) was determined to evaluate the correlation between SII and CRP.

**RESULTS**

The mean WBC, Neut, Lymph and PLT values and statistical comparisons of the obese and control groups are shown in Figure 1. In both groups, these four parameters were within the normal reference ranges reported for dogs. Nevertheless, the mean WBC (p = 0.026), Neut (p = 0.022), and PLT (p = 0.024) values of the obese group were statistically significantly higher than the control group. There was no statistically significant (p > 0.05) difference between these two groups regarding the mean lymphocyte counts (Figure 1).

The NLR, PLR, SII and serum CRP concentrations and statistical comparisons of the two groups are shown in Figure 2. The obese and control groups had no statistical differences in serum CRP concentration, NLR, and PLR values (p > 0.05 for each parameter). Nevertheless, SII was significantly higher in the obese group than in the control group (p = 0.028). Also, the Spearman correlation coefficient results showed a significant positive association between CRP and SII (rho = 0.681; p = 0.000) (Figure 3).
Figure 1: Box plot graphs showing the WBC (A), Neut (B), Lymph (C), and PLT (D) comparison between control and obese groups. "*" is statistically significant (p < 0.05). Abbreviations: LYMPH, lymphocyte; NEUT, neutrophil; PLT, platelet; WBC, white blood cell.

Figure 2: Box plot graphs showing the CRP (A), NLR (B), PLR (C) and SII (D) comparison between control and obese groups. "*" is statistically significant (p < 0.05). Abbreviations: CRP, C reactive protein; NLR, neutrophil to lymphocyte ratio; PLR, platelet to lymphocyte ratio; SII, systemic immune inflammation index.
DISCUSSION

Recently, the incidence of obesity has been increasing in pets and humans. This situation is a major concern in clinical practice as it can cause serious health problems (Barić Rafaj et al., 2017). The inflammatory state of obesity in dogs has still not been fully elucidated, and there are different opinions on this issue (Tvarijonaviciute et al., 2012; Barić Rafaj et al., 2017).

There are several studies on CBC components in obese dogs (German et al., 2009; Barić Rafaj et al., 2017; Martins et al., 2019). These studies demonstrated that clinically healthy overweight/obese dogs had a variety of haematological parameters that differed significantly from healthy control dogs (Radakovich et al., 2107). Leukocytosis can be expected in obese dogs because of granulopoiesis and lymphopoiesis induced by interleukins and interferons. However, most studies show that the mean WBC, neut and lymph counts were within reference limits in obese dogs, although higher than in dogs with normal body weight (German et al., 2009; Radakovich et al., 2017; Barić Rafaj et al., 2017; Martins et al., 2019). Similar to these studies, the total and differential leukocyte counts of the obese group were within the reference ranges in the current study. Also, WBC and neut counts were statistically significantly higher than the control group.

German et al. (2009) reported that obesity might predispose dogs to subclinical inflammatory conditions since the mean leukocyte counts of obese dogs are at the upper limit of the reference range and decrease significantly after weight loss. However, the exact role of proinflammation in canine overweight and obesity is currently unknown. On the other hand, studies in obese humans and dogs have shown that neutrophils are positively correlated with increased visceral fat (Ryder et al., 2014; Barić Rafaj et al., 2016), and an increase in total cholesterol causes increases in neutrophil counts. In the current study, WBC and neut counts of the obese group were statistically higher than the control group.

Considering that other inflammatory markers such as NLR, PLR and CRP were normal in the obese group, this elevation may be associated with increased visceral fat, as mentioned by the authors above. Also, a possible stress response may have caused a rise in these two parameters.

It has been reported that the platelet count increases in overweight dogs and that primary hemostasis is affected by this condition (Pasquini et al., 2013; Barić Rafaj et al., 2017). Barić Rafaj et al. (2017) report that the increase in PLT value in obese dogs may be associated with chronic low inflammation. Pasquini et al. (2013) also emphasize that the PLT value decreases after dieting in overweight dogs, and this is not related to the
inflammatory condition reported in humans since there is no change in CRP concentration. Similar to Pasquini et al. (2013), although the PLT values of the obese group were significantly higher than the control group in this study, there was no difference between the groups regarding CRP, the major acute phase protein (APP). Therefore, the increase in PLT may be associated with visceral adipose tissue becoming an additional source of thrombopoietin and increased thrombopoietin production (Purdy & Shatzel, 2021).

Acute phase proteins are proteins that concentrations rise quickly in the blood in texture demolition and inflammation. In recent years, they have been used in veterinary medicine for the differential diagnosis of many diseases and inflammatory conditions, evaluation of prognosis and determination of therapeutic efficacy (Eckersall & Bell, 2010). Some researchers suggest that obesity may be associated with chronic, low-grade inflammation. They also revealed that adipocytes have roles similar to T cells and macrophages in complement activation and proinflammatory cytokine production (German et al., 2009; Barić Rafaj et al., 2017). It has been reported that CRP concentration increases in obese people (Park et al., 2005). However, there are conflicting data on CRP concentration in dogs. German et al. (2009) and Barić Rafaj et al. (2017) report that APP levels are increased in obese dogs. However, while Tvarijonaviciute et al. (2011) emphasized no significant difference in APP levels in experimentally induced obesity in dogs, Veiga et al. (2008) reported that CRP concentration decreased in obese dogs. This study showed no statistical difference in CRP concentration between the obese and control groups. This result suggests that inflammation is not an important component in the canine obesity model, similar to the study of Tvarijonaviciute et al. (2011).

The SII is one of the new inflammatory markers obtained from CBC, such as NLR and PLR (Zhang et al., 2021; Ji & Wang, 2022). It has been demonstrated that high SII values in humans are associated with disease severity and poor prognosis in many diseases and malignancies (Zhang et al., 2021; Ji & Wang, 2022). There are a few studies on dogs with chronic enteropathy, melanoma and pyometra in veterinary medicine (Cristóbal et al., 2022; Garcia et al., 2022; Yazlık et al., 2022). It has been demonstrated that SII can be used as a diagnostic parameter in dogs with pyometra (Yazlık et al., 2022). In dogs with chronic enteropathy, SII reductions have also been reported with treatment (Cristóbal et al., 2022). There are no studies regarding SII in obese dogs. In humans, it has been shown that SII is significantly affected by body mass index, and SII is higher in obese adults/children than in individuals with normal weight (Furuncuoğlu et al., 2016). Similar to human research, the obese group had significantly higher SII levels than the control group in our study. No statistical difference between the obese and the control groups in inflammatory parameters such as CRP, NLR and PLR suggests that this increase may reflect the increase in platelet and neutrophil counts, not caused by inflammation. More extensive research is needed to understand the reason for this rise in SII.

There are several limitations to our study. First, the relatively low number of dogs included in the study. Many obese dogs were excluded from the study because of concurrent disease (e.g. another inflammatory or metabolic disease, cardiovascular problem) and lost data. Second, the number of pure breeds was low; It was not possible to statistically compare the data of obese and healthy dogs of the same breed. Finally, only CRP, the major APP in dogs, was evaluated to support the assessment of the inflammatory state in dogs due to the limited budget. Other APPs could not be evaluated.

**CONCLUSION**

In conclusion, the present study has shown that the SII is elevated in obese dogs, and
there was no difference in NLR, PLR and CRP concentrations. Furthermore, there was a positive correlation between SII and CRP. Large-scale studies are needed to reveal the reason for this increase in SII in obese dogs and to demonstrate its clinical utility.

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