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Evaluation of local expressions of acute phase proteins in white muscle disease in lambs by the immunohistochemical method

Evaluación inmunohistoquímica de expresiones locales de proteínas de fase aguda en la enfermedad del músculo blanco en corderos

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ABSTRACT

White muscle disease (WMD) and/or nutritional muscular dystrophy is defined as a disease with a generally acute course that results in degeneration and necrosis of the heart muscle. In this study, it was aimed to reveal local tissue expressions of acute phase proteins such as C-reactive protein (CRP), Serum amyloid-A (SAA) and Haptoglobin (Hp) in lambs with WMD. The study material consisted of 27, one-six months old lamb heart tissues, 6 of which were healthy and 21 with WMD. The lambs were necropsied, and the relevant heart samples were taken into neutral formaldehyde. Afterwards, paraffin blocks were obtained by going through routine tissue follow-up processes. Sections were taken from paraffin blocks and stained with Hematoxylin-Eosin (H-E) and Immunohistochemical methods. Histopathologically, control groups exhibited normal histology. Hyaline degeneration, Zenker necrosis, calcification, inflammatory cell infiltration and an increase in connective tissue were detected in the heart tissues of lambs with WMD. Lambs with WMD had significantly increased CRP and SAA proteins compared to control (P<0.01). However, there was no difference between the groups in Hp (P>0.05). In conclusion, local tissue expressions of CRP, SAA and Hp in lambs with WMD were identified immunohistochemically for the first time. It is possible to say that CRP and SAA may play important roles in the pathophysiology of WMD and that CRP and SAA may provide more sensitive results in the diagnosis and prognosis of the disease.

Key words: White muscle disease; immunohistochemistry; C-reactive protein; serum amyloid-A; haptoglobin

RESUMEN

La enfermedad del músculo blanco (EMB) y/o distrofia muscular nutricional se define como una enfermedad con un curso generalmente agudo que resulta en la degeneración y necrosis del músculo cardíaco. En este estudio, el objetivo fue revelar la expresion tisular local de proteínas de fase aguda, como la proteína C-reactiva (PCR), el amiloide A sérico (SAA) y la haptoglobina (Hp) en corderos con EMB. El material de estudio estuvo compuesto por 27 tejidos de corazón de cordero de uno a seis meses de edad, 6 sanos y 21 con EMB. Se realizó la necropsia de los corderos y las muestras de corazón correspondientes se pusieron en formaldehído neutro. Posteriormente, se obtuvieron bloques de parafina mediante los procesos de seguimiento tisular rutinarios. Se tomaron secciones de bloques de parafina y se tiñeron con los métodos de Hematoxilina-Eosina (H-E) e Inmunohistoquímico. Histopatológicamente, los grupos de control presentaron una histología normal. En los tejidos cardíacos de corderos con EMB se detectó degeneración hialina, necrosis de Zenker, calcificación, infiltración de células inflamatorias y aumento del tejido conectivo. En comparación con el control, los corderos con EMB mostraron un aumento significativo de las proteínas PCR y SAA (P<0,01). Sin embargo, no hubo diferencia entre los grupos en la tinción de HE (P>0,05). En conclusión, las expresiones tisulares locales de PCR, SAA y Hp en corderos con EMB se identificaron inmunohistoquímicamente por primera vez. Es posible afirmar que PCR y SAA pueden desempeñar papeles importantes en la fisiopatología de la EMB, y que PCR y SAA pueden proporcionar resultados más sensibles en el diagnóstico y pronóstico de la enfermedad.

Palabras clave: Enfermedad del músculo blanco; inmunohistoquímica; proteína C-reactiva; amiloide-A sérico; haptoglobina

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INTRODUCTION

White muscle disease (WMD) and/or nutritional muscular dystrophy is defined as a disease with a generally acute course that results in degeneration and necrosis of the heart muscle. The etiology of the disease is vitamin E (VitE) and/or Selenium (Se) deficiency [1, 2]. WMD causes heart failure–related death in young animals that tend to grow rapidly. Pathomorfologically, it manifests with degeneration, paleness, fibrosis, necrosis and calcification in the heart muscle [2, 3, 4]. WMD is usually seen in kids (*Capra aegagrus hircus*), lambs (*Ovis aries*), calves (*Bos taurus*) and camels (*Camelus*) [5, 6, 7]. WMD shows a global incidence of around 1%. Its incidence is around 20–30% in Türkiye and New Zeland [8].

Selenium (Se) is normally included in the structure of the glutathione peroxidase (GSH-Px) enzyme [9]. GSH-Px neutralizes the effects of hydrogen peroxide and lipid hydroperoxide, which cause cell protein destruction and necrosis. Vitamins E (Vit E) plays a major role in inhibiting excessive peroxide formation and prevents hyaline degeneration [9, 10]. The metabolic function of Se is closely related to vitamin E. Both agents act to protect biological membranes from oxidative damage [11]. While Se deficiency is seen in animals grazing on pastures, Vit E deficiency is more common in animals fed with forage. Deficiencies of these two agents cause lipoperoxidation, muscle degeneration and calcification in various tissues [3, 12, 13].

Some free radicals formed after the decrease of antioxidant defense as a result of Vit E and Se deficiency cause oxidative stress. As free oxygen radicals play a major role in the pathogenesis of the disease by causing very important pathological changes such as degeneration of proteins in tissues, lipid peroxidation and necrosis of heart muscle [4, 12, 14]. In Se and vit E deficiencies, lipid peroxidation and hydrogen peroxide cannot be cleared from the muscles due to the decrease in GSH-Px activity. In addition, increased reactive oxygen species (ROS) levels serve as markers for oxidative stress, and lipid peroxidation and instability of the redox system are associated [13]. In many studies, it has been reported that oxidative stress plays a very important role in the pathogenesis of WMD [4, 13, 14, 15]. In this context, oxidative stress is seen as an important cause of degenerative and necrotic changes in related tissues.

Inaccurate results can be obtained due to the fact that the GSH-Px enzyme is affected by some factors and the detection of Se and Vit E in body fluids is difficult and does not always give accurate results [16, 17, 18]. In addition, it is difficult to reach a clear WMD diagnosis based solely on clinical symptoms because it can be confused with many infections and mineral balance disorders such as cerebrocortical necrosis (CCN), listeriosis, borna disease, enzootic ataxia, polyarthritis [19, 20, 21]. Therefore, diagnosis based on autopsy findings is frequently preferred today [3, 4].

Acute phase response (APR) is known as a series of inflammatory responses of the host in conditions such as infection, trauma and tissue damage. Pyrogen cytokines are mediators of APR. APR is induced by a series of pro-inflammatory cytokines (IL-1, IL-6, TNF-α) released by inflammatory cells to produce Acute Phase Protein (APP) in response to tissue damage [22, 23, 24]. APR is crucial in ensuring that homeostatic mechanisms quickly regain normal physiological function by isolating and neutralizing pathogens, minimizing tissue damage and initiating repair [25]. Proteins whose blood levels fluctuate markedly at the onset of inflammation are called Acute Phase proteins (APP). APP are blood proteins that assess the response of the immune system in cases of trauma, infection or inflammation. The main production site of APP is the liver [22, 23]. In conditions such as infection, trauma, and tissue damage, some blood proteins decrease over time and are called negative APPs, while those with increased blood protein amounts are called positive APPs. C-reactive protein (CRP), Serum amyloid-A (SAA) and Haptoglobin (Hp) are in the group of positive APPs [22, 26, 27].

CRP is a highly important phylogenetically conserved plasma protein that participates in the systemic response to inflammation. CRP constitutes an important part of APR, and its values in the blood increase in many infectious processes [28, 29]. It plays a role in many processes, such as chemotaxis, inhibition of cytokine production, and modulation of monocytes and macrophages. In cases such as infection and tissue damage, its value in the blood can increase up to 50,000 times [30, 31].

SAA consists of 2 different groups of apolipoproteins: acute phase SAA (A-SAA) and structural SAAs (C-SAA) [32]. It has effects such as SAA, inducing chemotaxis and anti-inflammatory effects. In addition, it has functions such as fat metabolism and transport and stimulation of enzymes that break down the extracellular matrix [31, 33]. SAA secretion is observed in the acute phase of inflammation, and therefore it is often preferred to differentiate between acute and chronic inflammation. SAA levels can increase 1000 times during APR [32, 33, 34].

Hp is known as a very important APP in ruminants. Hp plays an important role in many biological processes, such as bacteriostatic effect, stimulation of angiogenesis and binding to haemoglobin [22, 31]. Normally, its amount in the blood is quite low, whereas when the immune system is stimulated, it may increase 100 times [26, 31]. It has been stated in many studies that it is useful to evaluate the severity of the inflammatory response in natural or experimental infections such as endometritis, enteritis, pneumonia, mastitis, endocarditis and abscess [23, 31, 35].

In recent years, APPs have been frequently preferred in both human and veterinary fields as markers of inflammation, infection and trauma. Not enough studies are available in the literature for the detection and evaluation of APPs in WMD. This study aimed to evaluate local tissue expressions of acute phase proteins such as Hp, CRP and SAA in heart tissue in WMD, a metabolic disease, by immunohistochemistry.

MATERIAL AND METHODS

Animal materials

The material of the study consisted of heart samples from 21 WMD positive male lambs (*Ovis aries*, merino, 1–6 months) and 6 healthy male lambs (*Ovis aries*, merino, 1–6 months), a total of 27 heart samples. Heart tissues were obtained from different farms suffering from WMD disease in Sivas and Yozgat provinces. Additionally, Musculus gracilis and intercostal muscles were examined in all VMD positive animals. Heart tissues of the necropsied lambs were taken into neutral formalin for histopathological and immunohistochemical examination. The study was approved by the Sivas Cumhuriyet University HADYEK ethics committee (31.07.2023, Decision no; 616).

Histopathological and Immunohistochemical examination

After 24–48 h neutral formalin fixation of heart tissues, routine tissue follow-up procedures were performed. Afterwards, they were embedded in paraffin and paraffin blocks were obtained. 4–5 µm

sections were taken from paraffin blocks onto ground slides with a microtome (Leica RM 2255, Germany), stained with Hematoxylin–Eosin and examined under light microscopy (Olympus BX51, Tokyo, Japan) [36, 37].

For the immunohistochemical examination, 4–5 µm thickness sections were taken on adhesive slides. Immunohistochemical staining was performed following the procedure with a commercial kit (UltraVision Detection System Anti-Polyvalent, HRP (Horseradish Peroksidaz) (Ready-To-Use, TP-060-HL, Lab Vision, USA) [38].

The antigen retrieval process was performed by (microwave oven-UTD-1420, Utest, Turkey) in Tris-Ethylenediaminetetraacetic acid (EDTA) (pH 9, 10x) solution at 750 watts for 20 min. Rabbit Anti-Hp (Polyclonal antibody, Proteintech, USA, Cat No:16665-1-AP, 1/200 dilution), Rabbit Anti-SAA (Polyclonal antibody, Proteintech, USA, Cat No: 20398-1-AP, 1/200 dilution) and Rabbit Anti-CRP (Polyclonal antibody, Proteintech, USA, Cat No: 24175-1-AP, 1/200 dilution) primers were used. Primers were incubated for 90 min. As chromogen, 3.3 diaminobenzidine (DAB) was used and counterstained with Mayer's-Hematoxylin. The sections were then examined under a light microscope at 20X magnification by a blinded pathologist (Olympus BX51, Tokyo, Japan). Immunohistochemical scoring none;0, little;1, moderate;2, severe;3 was evaluated [38].

Statistical analysis

SPSS (Inc., Chicago, USA 25.0) statistical program was used to evaluate the data between groups. Immunohistochemical scores were evaluated using the t-test. The group mean was given as Mean ± SE. The accepted significance limit was $P<0.05$.

RESULTS AND DISCUSSION

Macroscopic Results

The cardiac muscle tissues of the animals in the control group had a normal macroscopic appearance.

Hyaline degeneration, Zenker necrosis and areas of calcification were seen in the heart tissue of WMD animals (FIG 1). Areas with hyaline degeneration and Zenker necrosis were usually pale and similar to fish and/or chicken meat. In addition, calcification areas were found in the cardiac tissue in 12 cases.

In addition, pallor was detected macroscopically in the *Musculus gracilis* in 5 cases and in the intercostal muscles in 3 cases in cadavers. No calcification was observed in non-cardiac muscles.

Microscopic Results

Histopathologically, control animals showed normal histology (FIG 2, A). In the WMD group, degenerative and necrotic muscle fibers were found, citration was lost, and these fibers had a pinkish and swollen appearance (FIG 2, B-C). In this group, calcification areas were found disseminated (FIG 2, E). In addition, mononuclear cell filtration and increased connective tissue were observed in places (FIG 2, D-E).

In the immunohistochemical examination, immunohistochemical scores in the Control and WMD groups are given in TABLE I. Mild or no immunoreactivity was found with the primers Hp, CRP, and SAA in the control group. Moderate and severe staining was found in CRP and SAA in the WMD group compared to the control group ($P<0.01$). In particular, immunopositivity was observed in degenerative and necrotic muscle fibres and inflammatory cell infiltrations (FIG 3). Hp in the WMD group presented similar immunopositivity compared to the control group and was not significant ($P>0.05$).

WMD shows two different clinical forms, acute (cardiac) and subacute. The acute form is characterized by cardiac muscle degeneration and sudden death, especially in young animals, while the subacute form usually presents with skeletal muscle degeneration

TABLE I
Immunohistochemical scores of CRP, SAA, Hp in healthy and WMD animals

Groups	CRP	SAA	Hp
Control	0.67 ± 0.21^{b}	0.83 ± 0.16^{b}	0.50 ± 0.21^{a}
WMD	2.23 ± 0.16^{a}	2.07 ± 0.21^{a}	0.69 ± 0.13^{a}
	$P<0.01$	$P<0.01$	$P>0.05$

Group averages are given as Mean + SE. [a,b]: Indicates statistical significance between values in the same column (C: Control, WMD: White muscle disease, CRP: C-reactive protein, SAA: Serum amyloid-A, Hp: Haptoglobin)

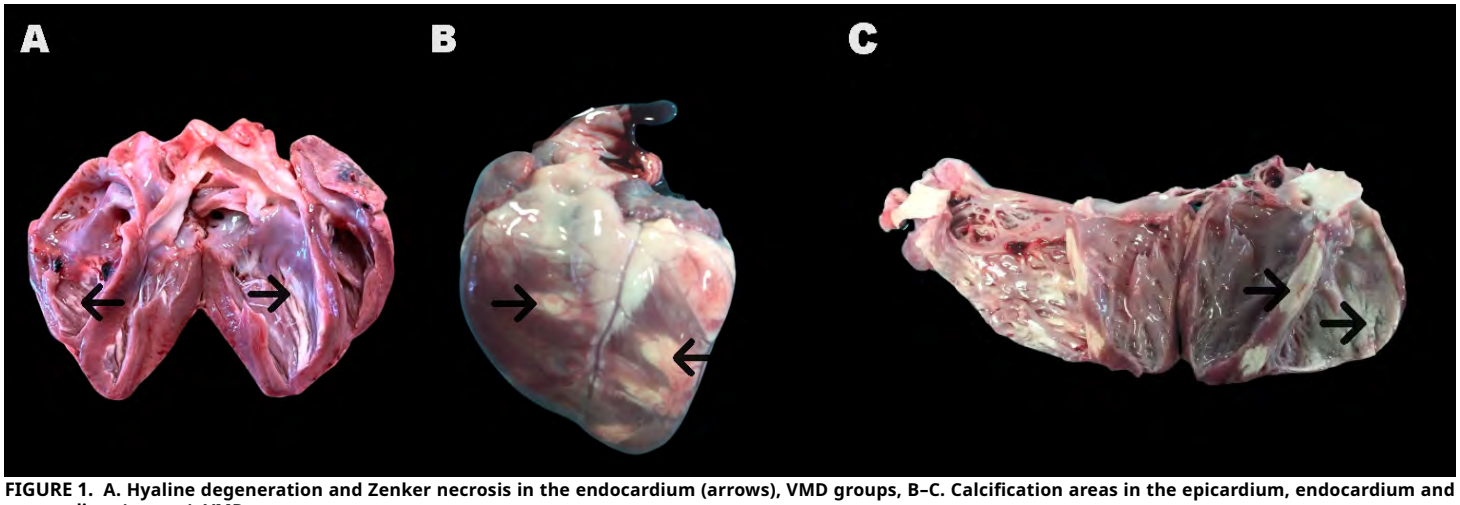

FIGURE 1. A. Hyaline degeneration and Zenker necrosis in the endocardium (arrows), VMD groups, B-C. Calcification areas in the epicardium, endocardium and myocardium (arrows), VMD groups

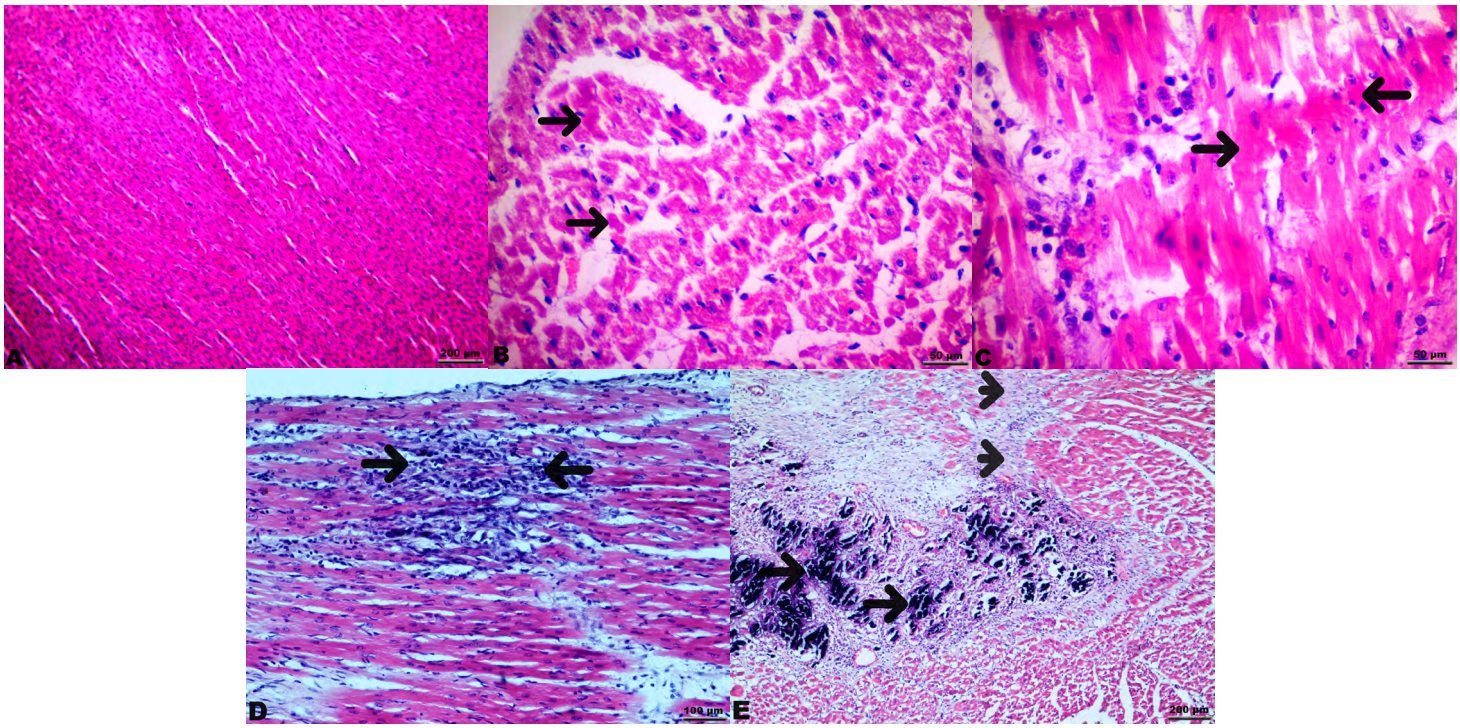

FIGURE 2. Histopathological examination of the Control (A) and WMD (B–E) groups, Hematoxylin–Eosin, A. Normal histological appearance of the control group, 100X. B–C. Hyaline degeneration and zenker necrosis (arrows), WMD groups, 400X. D. Inflammatory cell infiltration (arrows), VMD groups, 200X. E.Calcification areas (arrows) and fibrosis (arrowheads), WMD groups, 100X

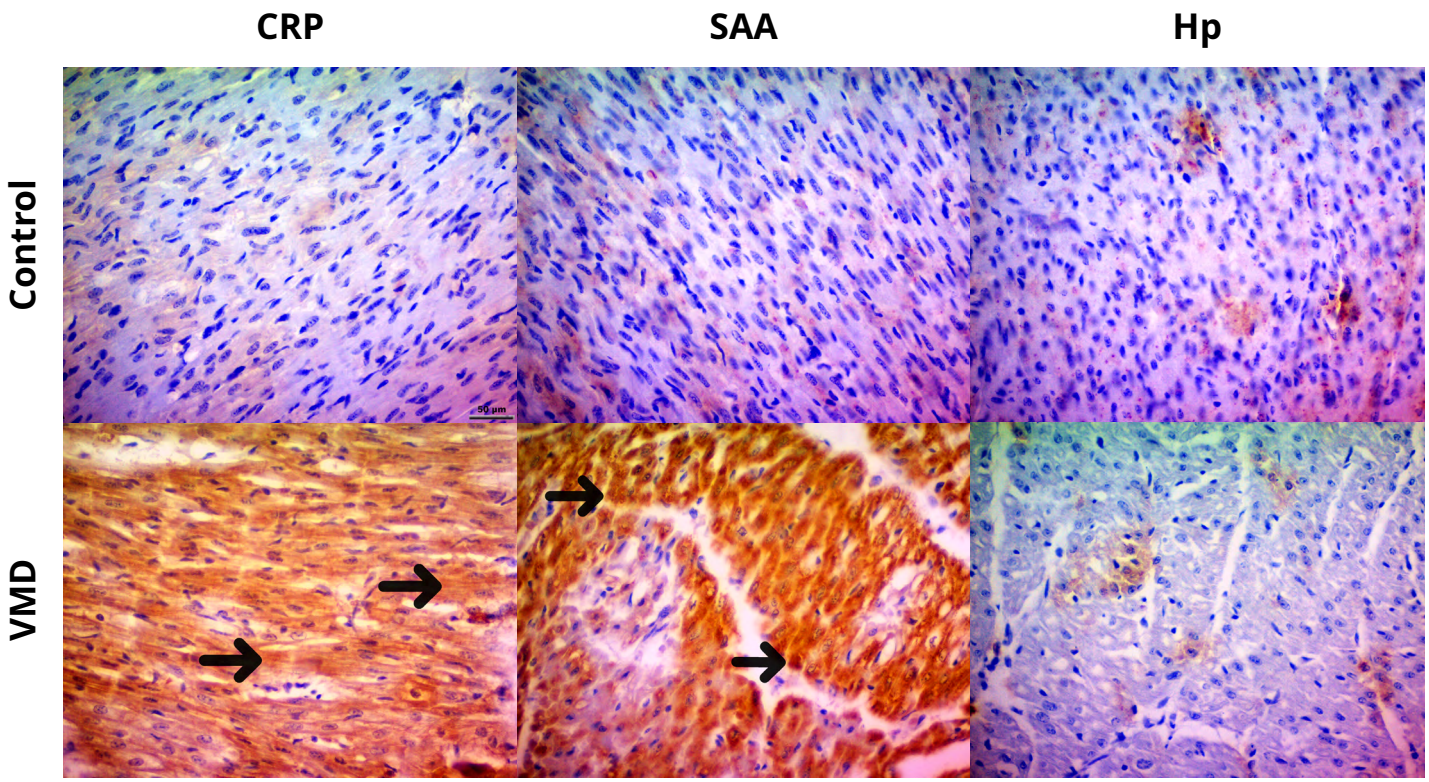

FIGURE 3. Microscopic view of immunohistochemical staining between groups (WMD; White muscle disease, Relevant expressions in degenerative and necrotic cells (arrows), CRP; C–reactive protein, SAA; Serum amyloid–A, Hp; Haptoglobin)

[4, 20]. The main clinical forms of the disease are inability to stand up, loss of appetite, weakness, difficulty in standing, short and steep steps, shortness of breath, and curvature of the back [3, 4, 20]. Most of the clinical findings mentioned in this study were recorded in lambs.

In the study, lesions were generally located in heart tissue. In the cadavers, paleness was detected macroscopically in the Musculus gracilis in 5 cases and in the intercostal muscles in 3 cases. It was stated that treatment procedures were applied to the lambs in our study by the patient owners, but no positive results were obtained. This situation can be explained by the fact that it is not possible to treat patients with acute heart failure.

Previous studies have reported that the disease mostly affects the heart, legs and other muscles [3, 21]. Macroscopically, hyaline degeneration, Zenker necrosis and areas of calcification have been reported [3, 4].Histopathologically, degenerative and necrotic changes in the heart muscle have been reported. In particular, these muscle fibres are stated to be irregular, swollen and homogeneously pink. In addition, dystrophic calcification areas, connective tissue increases and inflammatory cell infiltrations in the interstitium are reported in these necrotic parts [3, 14, 21]. In this study, macroscopically, similar to previous study findings, hyaline degeneration, Zenker necrosis and calcification areas were detected in the heart tissue of lambs with WMD (FIG 1). In particular, areas with hyaline degeneration and Zenker necrosis were generally pale in color and had a fish and/or chicken meat–like appearance. Although this appearance is seen macroscopically in WMD, it is necessary to support it with histopathological examination. Because it is reported that the skeletal muscles of lambs normally appear whitish, gross findings alone are not sufficient for diagnosis [3, 10]. Microscopically, degenerative and necrotic muscle fibers (FIG 2 B–C) were found in lambs with WMD, citration was lost, and these fibers had a pinkish and swollen appearance. Additionally, an increase in mononuclear cell filtration and connective tissue was observed in some places (FİG 2 D–E), and our findings were consistent with the literature [3, 4].

In recent years, CRP protein is frequently evaluated in the veterinary field for diagnosis and prognosis in many infectious and inflammatory conditions [26, 35]. No studies were found in the literature to detect CRP in WMD. In the related study, a significant level of CRP immunoreactivaty was found in the WMD group compared to the control group (P<0.01, FIG 3). This study on WMD revealed that CRP can accumulate locally, although we have no evidence of blood concentrations. It also seems to play an important role in the pathophysiology of WMD.

The high activity of CRP protein has been reported in studies on many cardiovascular diseases in human medicine. In addition, some studies have reported a positive correlation between oxidative stress and CRP levels [39, 40, 41, 42]. It has been reported in a study that oxidative stress may be a determinant of CRP levels [40]. Therefore, it can be concluded that high immunoreactivity in lambs with WMD may be related to possible oxidative stress. Immunoreactivity, especially in degenerative and necrotic muscle fibres strengthens this idea.

SAA, another acute phase protein, has been evaluated in the veterinary field in the diagnosis and prognosis of many infections or inflammatory conditions in recent years [26, 35]. No study was found in the literature to detect any SAA in WMD. In this study, there was a significant increase in SAA protein in the WMD group compared to the control group (P<0.01, FIG 3). Local production of biologically SAA in different tissue damage has been reported in different studies and has been attributed to the role of host immunity [43, 44]. In particular, in a study conducted in dairy calves with bovine respiratory disease, it was predicted that the increase in SAA concentrations was associated with host immunity [44]. The current study revealed that SAA accumulated locally, although it did not have any findings about blood concentrations associated with systemic APR. Although we cannot say for sure because the study material consisted of lambs (1–6 monthly), the increased local expression of SAA in WMD may result from the host's immunity and/or accumulate as a result of systemic APR. In this context, more comprehensive studies are needed in the future. Our findings suggest that SAA protein plays an important role in the pathophysiological process of WMD.

In this study, Hp protein was similar in the WMD group compared to the control group (P>0.05). These findings suggested that Hp does not play an active role in the pathophysiological process of WMD in lambs.

When the findings of this study are evaluated, the accumulation of locally relevant APPs in WMD, which is a metabolic disease, shows that the acute phase reaction is not only a systemic reaction but an important part of the possible local immune response. In particular, CRP and SAA show that they may play an important role in the pathophysiology of WMD. The most important limitation of the present study is the lack of relevant blood concentrations. It was suggest that more detailed information about APR can be obtained in future studies by evaluating blood concentrations of relevant APPs in WMD.

CONCLUSION

Our results show that the cardiac (acute) form is more effective in the Sivas and Yozgat regions of Turkey. In addition, local tissue expressions of CRP, SAA and Hp in lambs with WMD were determined immunohistochemically for the first time. It is possible to say that CRP and SAA may play an important role in the pathophysiology of WMD, and that CRP and SAA may give more sensitive results in the diagnosis and prognosis of the disease.

Conflict of interest

There is no conflict of interest between the authors.

BIBLIOGRAPHIC REFERENCES

[1] Dierenfeld ES. Vitamin E deficiency in zoo reptiles, birds, and ungulates. J. Zoo. Wildl. Med. [Internet]. 1989 [cited 20 July 2023]; 20(1):3–11. Available in: https://bit.ly/4akKt6j.

[2] Abutarbush SM, Radostits OM. Congenital nutritional muscular dystrophy in a beef calf. [Internet]. Can. Vet. J. 2003 [cited 18 July 2023]; 44(9):738–739. Available in: https://bit.ly/3GMGbXI. Cited in: PubMed; PMID:14524629.

[3] Yavuz O. The pathological investigations on nutritional myopathy causing lamb deaths in neonatal period. J. Bahri Dagdas Anim. Res. [Internet]. 2017 [cited 18 July 2023]; 6(2):1–8. Available in: https://bit.ly/3Tn2U4g.

[4] Karakurt E, Karataş Ö, Dağ S, Beytut E, Mendil AS, Nuhoğlu H, Yıldız A. Evaluation of 4–Hydroxy–2–Nonenal, Dityrosine and 8–Hydroxy–2–Deoxyguanosine Expressions in Lambs with White Muscle Disease. Firat Univ. Saglik Bilim. Vet. Derg. [Internet] 2021 [cited 23 May 2023]; 35(2):109–113. Available in: https://bit.ly/3TvU1p1.

[5] Kandeel M, Al-Taher A, Venugopala KN, Marzok M, Morsy M, Nagaraja S. Camel Proteins and Enzymes: A Growing Resource for Functional Evolution and Environmental Adaptation. Front. Vet. Sci. [Internet]. 2022; 9:911511. doi: https://doi.org/gstj65

[6] White C, Rewell L. Vitamin E and selenium status of sheep during autumn in Western Australia and its relationship to the incidence of apparent white muscle disease. Aust. J. Exp. Agric. [Internet]. 2007; 47(5):535-543. doi: https://doi.org/d58mqj

[7] Ortiz-Morales O, Ramírez-Bribiesca JE, Hernández-Bautista J, Hernandez-Sanchez D, Bárcena-Gama JR, Hernández-Trujillo E, Díaz-Sánchez VM, Garrido-Fariña G, López-Ojeda JC, Hernández-Rodriguez M. Effect of Supranutritional Dosage Selenium in Neonatal Goat Kids on Productive Performance, Physicochemical Profiles in Meat, Selenium Levels in Tissues, and Histopathological Findings. Biol. Trace. Elem. Res. [Internet]. 2023: 201:4374-4388. doi: https://doi.org/k8z6

[8] McDowell LR, Valle G, Cristaldi L, Davis PA, Rosendo O, Wilkinson NS. Selenium availability and methods of selenium supplementation for grazing ruminants. Proceedings 13th. Annual Florida Ruminant Nutrition Symposium.Gainesville, Florida, USA: IFAS, University of Florida. 2002. p. 86-102.

[9] Tórtora-Pérez J. The importance of selenium and the effects of its deficiency in animal health. Small Rumin. Res. [Internet]. 2010; 89(2-3):185-192. doi: https://doi.org/frz8tv

[10] Cooper B, Valentine B. Chapter 3. Muscle and tendon. In: Maxie MG, editor. Jubb, Kennedy and Palmer's Pathology of Domestic Animals: Volume 1. 6th ed. Philadelphia, USA: Saunders LTD. 2015: p 214-218.

[11] McDowell L, Williams S, Hidiroglou N, Njeru C, Hill G, Ochoa L, Wilkinson NS. Vitamin E supplementation for the ruminant. Anim. Feed Sci. Technol. [Internet]. 1996; 60(3-4):273-296. doi: https://doi.org/cczm44

[12] Ataollahi F, Mohri M, Seifi HA, Pingguan-Murphy B, Wan Abas WAB, Osman NAA. Evaluation of copper concentration in subclinical cases of white muscle disease and its relationship with cardiac troponin I. PLoS One. [Internet]. 2013; 8(2):e56163. doi: https://doi.org/f4k66r

[13] Kozat S, Gunduz H, Deger Y, Mert N, Yoruk IH, Sel T. Studies on serum α-tocopherol, selenium levels and catalase activities in lambs with white muscle disease. Bull. Vet. Inst. Pulawy. 2007; 51(2):281-284.

[14] Yumusak N, Yigin A, Polat PF, Hitit M, Yilmaz R. Expression of ADAMTS-7 in myocardial dystrophy associated with white muscle disease in lambs. Pol. J. Vet. Sci. [Internet]. 2018; 21(1):119-126. doi: https://doi.org/k8z7

[15] Kozat S, Altug N, Yuksek N, Ozkan C. Evaluation of the levels of homocysteine, troponin I, and nitric oxide in lambs with subclinical white muscle disease. Kafkas Univ. Vet. Fak. Derg. [Internet]. 2011; 17(3):441-444. doi: https://doi.org/k8z8

[16] Nizamlıoğlu M, Tiftik AM, Turgut K, ve Traş B. [Investigation of Vitamin E, Glutamic oxalacetic transaminase (GOT), creatine kinase (CK) and lactate dehydrogenase (LDH) activities in white muscle disease of lambs]. Doğa Türk Vet. Hay. Derg. 1991; 15:59-64.Turkish.

[17] Keleş İ, Dede S, Keleş H, Değer Y, Altuğ N. Studies on some antioxidant vitamin concentrations in lambs with stiff-lamb disease. Yüzüncü Yıl Univ. Vet. Fak. Derg. 2000; 11(1):79-82.

[18] Beytut E, Erişir M, Aksakal M. [Reduced glutathione and malondialdehyde levels with catalase enzyme activity in the heart, skeletal muscle and liver of lambs with white muscle disease]. Kafkas Univ. Vet. Fak. Derg. 2001; 7(1):1-5. Turkish.

[19] Arshad MA, Ebeid HM, Hassan F-u. Revisiting the effects of different dietary sources of selenium on the health and performance of dairy animals: a review. Biol. Trace Elem. Res. [Internet]. 2021; 199:3319-3337. doi: https://doi.org/gpbgsw

[20] Paynter DI. Diagnosis of mineral deficiencies. In: Master DG, White CL, editors. Detection and Treatment of Mineral Nutrition Problem in Grazing Sheep. Canberra, Australia: Australian Centre for International Agricultural Research. 1996. p. 45-56. ACIAR Monograph N° 37.

[21] Gunes V, Ozcan K, Citil M, Onmaz AC, Erdogan HM. Detection of myocardial degeneration with point-of-care cardiac troponin assays and histopathology in lambs with white muscle disease. Vet. J. [Internet]. 2010; 184(3):376-378. doi: https://doi.org/bqjz3h

[22] Murata H, Shimada N, Yoshioka M. Current research on acute phase proteins in veterinary diagnosis: an overview. Vet. J. [Internet]. 2004; 168(1):28-40. doi: https://doi.org/c8tdm3

[23] Petersen HH, Nielsen JP, Heegaard PMH. Application of acute phase protein measurements in veterinary clinical chemistry. Vet. Res. [Internet]. 2004; 35(2):163-187. doi: https://doi.org/bjtm9p

[24] Cerón JJ, Eckersall PD, Martínez-Subiela S. Acute phase proteins in dogs and cats: current knowledge and future perspectives. Vet. Clin. Pathol. [Internet]. 2005; 34(2):85-99. doi: https://doi.org/cm573x

[25] Cray C, Zaias J, Altman NH. Acute phase response in animals: a review. Comp. Med. 2009; 59(6):517-526. Cited in: PubMed; PMID: 20034426.

[26] Eckersall P, Bell R. Acute phase proteins: Biomarkers of infection and inflammation in veterinary medicine. The Vet. J. [Internet]. 2010; 185(1):23-27. doi: https://doi.org/fb64df

[27] Coşkun A, İsmail Ş. [Clinical Use of Acute Phase Proteins in Cattle]. Saglik Bilim. Derg. [Internet] 2011; [cited 18 May 2023]; 20(3):240-246. Turkish. Available in: https://bit.ly/4anQaQU.

[28] Pathak A, Agrawal A. Evolution of C-reactive protein. Frontiers immunol. 2019; 10:943. doi: https://doi.org/k82x

[29] Niu W, Wan Y, Li M, Wu Z, Zhang L, Wang J. The diagnostic value of serum procalcitonin, IL-10 and C-reactive protein in community acquired pneumonia and tuberculosis. Eur. Rev. Med. Pharmacol. Sci. 2013; 17(24):3329-3333. Cited in: PubMed; PMID: 24379064.

[30] Horadagoda NU, Knox KMG, Gibbs HA, Reid SWJ, Horadagoda A, Edwards SER, Eckersall PD. Acute phase proteins in cattle: discrimination between acute and chronic inflammation. Vet. Rec. [Internet]. 1999; 144(16):437-441. doi: https://doi.org/bt6hb6

[31] Jain S, Gautam V, Naseem S. Acute-phase proteins: As diagnostic tool. Pharm Bioallied Sci. [Internet]. 2011; 3(1):118-127. doi: https://doi.org/dhncrg

[32] Zhang Y, Zhang J, Sheng H, Li H, Wang R. Chapter Two–Acute phase reactant serum amyloid A in inflammation and other diseases. In: Makowski GS, editor. Advances in Clinical Chemistry. Vol 90. [Internet]. Cambridge (MA), USA: Academic Press. 2019. p. 25–80. doi: https://doi.org/k82z

[33] De Buck M, Gouwy M, Wang JM, Van Snick J, Opdenakker G, Struyf S, Van Damme J. Structure and expression of different serum amyloid A (SAA) variants and their concentration–dependent functions during host insults. Curr Med Chem. [Internet]. 2016; 23(17):1725–1755. doi: https://doi.org/gk5wqh

[34] Urieli–Shoval S, Linke RP, Matzner Y. Expression and function of serum amyloid A, a major acute–phase protein, in normal and disease states. Curr. Opin. Hematol. [Internet]. 2000; 7(1):64–69. doi: https://doi.org/cfqsv2

[35] El–Deeb W, Fayez M, Elsohaby I, Salem M, Alhaider A, Kandeel M. Investigation of acute–phase proteins and cytokines response in goats with contagious caprine pleuropneumonia with special reference to their diagnostic accuracy. Peer J. [Internet]. 2020; 8:e10394. doi: https://doi.org/gpgxkc

[36] Kazak F, Deveci MZY, Akcakavak G. Eucalyptol alleviates cisplatin–induced kidney damage in rats. Drug. Chem. Toxicol. [Internet]. 2022; 1–8. doi: https://doi.org/k823

[37] American Registry of Pathology; Luna LG, editor. Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology. 3rd ed. New York: Mc Graw–Hill. 1968. 258 p.

[38] Akcakavak G, Kazak F, Deveci MZY. Eucalyptol Protects against Cisplatin–Induced Liver Injury in Rats. Biol. Bull. [Internet]. 2023; 50:987–994. doi: https://doi.org/k824

[39] Dabak M, Karataş F, Gül Y, Kizil Ö. Investigation of selenium and vitamin e deficiency in beef cattle. Turkish J. Vet. Anim. Sci. [Internet] 2002 [cited 24 April 2023]; 26(4):741–746. Available in: https://bit.ly/47ZTzUw.

[40] Abramson JL, Hooper WC, Jones DP, Ashfaq S, Rhodes SD, Weintraub WS, Harrison DG, Quyyumi AA, Vaccarino V. Association between novel oxidative stress markers and C–reactive protein among adults without clinical coronary heart disease. Atherosclerosis. [Internet]. 2005; 178(1):115–121. doi: https://doi.org/cmk2xg

[41] Cottone S, Mulè G, Nardi E, Vadalà A, Guarneri M, Briolotta C, Arsena R, Palermo A, Riccobene R, Cerasola G. Relation of C–reactive protein to oxidative stress and to endothelial activation in essential hypertension. Am. J. Hypertens. [Internet]. 2006; 19(3):313–318. doi: https://doi.org/bdgb69

[42] Noren–Hooten N, Ejiogu N, Zonderman AB, Evans MK. Association of oxidative DNA damage and C–reactive protein in women at risk for cardiovascular disease. Arterioscler. Thromb. Vasc. Biol. [Internet]. 2012; 32(11):2776–2784. doi: https://doi.org/k825

[43] Eklund KK, Niemi K, Kovanen P. Immune functions of serum amyloid A. Crit. Rev. Immunol. [Internet]. 2012; 32(4):335–348. doi: https://doi.org/gk54gq

[44] Joshi V, Gupta VK, Bhanuprakash AG, Mandal RSK, Dimri U, Ajith Y. Haptoglobin and serum amyloid A as putative biomarker candidates of naturally occurring bovine respiratory disease in dairy calves. Microb. Pathog. [Internet]. 2018; 116:33–37. doi: https://doi.org/gdd72z