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RESEARCH ARTICLE

Evaluation of the Levels of Homocysteine, Troponin I, and Nitric Oxide in Lambs with Subclinical White Muscle Disease

Süleyman KOZAT * Nuri ALTUĞ ** Nazmi YÜKSEK * Cumali ÖZKAN *

* Department of Internal Diseases, Faculty of Veterinary Medicine, University of Yüzüncü Yıl, TR-65080 Van - TURKEY
** Department of Internal Diseases, Faculty of Veterinary Medicine, University of Mustafa Kemal, TR-31040 Hatay - TURKEY

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Summary

White muscle disease (WMD) or Nutritional myodegeneration disease (NMD) is a degenerative disease of the cardiac and skeletal muscles. The level of serum activity of Plasma homocysteine (Hcy), cardiac troponin I (cTn I), Nitric oxide (NO) were investigated in lambs with subclinical NMD in this study. Ten healthy lambs and twenty lambs with subclinical NMD were used in this study. The blood samples were firstly taken at the onset of the disease and then 3 and 10 days after treatment in NMD, and taken once control group. The values of serum activity of creatin kinase (CK), aspartate aminotranferase (AST), alanine aminotranferase (ALT), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) were measured with autoanalyzer. Plasma concentration of Hcy, serum cTn I, and levels of NO were determined with ELISA method and value of activity erythrocyte glutathione peroxidase (GSH-Px) was measured with spectrophotometer. Before treatment, values of plasma Hcy, serum NO, cTn I, AST, ALT, ALP, CK, and LDH in lambs with NMD were higher than those of healthy ones ($P<0.001$), while GSH-Px activity of lambs with NMD are lower than those of healthy ($P<0.001$). After ten days of treatment, there were no significant differences between treated lambs with NMD and healthy lambs. Nutritional myodegeneration in lambs is associated with increased levels of Hcy, cTn I, and NO. Increased levels of Hcy, cTn I, and NO may be a result of selenium deficiency causing myocardial disorder in NMD lambs.

Keywords: *Nutritional Myodegeneration, Lamb, Homocysteine, Troponin I, Nitric oxide*

Subklinik Beyaz Kas Hastalıklı Kuzularda Homosistein, Troponin I ve Nitrik Oksit Düzeylerinin Değerlendirilmesi

Özet

Beyaz kas hastalığı veya besinsel kas dejenerasyonları, hayvanlarda iskelet ve kalp kaslarının dejeneratif değişiklikleri ile karakterize bir hastalığıdır. Bu çalışmada kuzulardaki subklinikal beyaz kas hastalığında plazma homosistein (Hcy), serum kardiak troponin I (cTnI) ve nitrik oksit (NO) düzeyleri araştırıldı. Çalışmada 10 sağlıklı ve 20 subklinikal beyaz kas hastalıklı kuzu kullanıldı. Kan örnekleri NMD kuzularda tedavi öncesi, tedavi sonrası 3. ve 10. günlerde ve kontrol grubunda bir kez alındı. Serum kreatinin kinaz (CK), aspartat aminotransferaz (AST), alanin aminotranferaz (ALT), alkalin fosfataz (ALP) ve laktat dehidrogenaz (LDH) değerleri otoanalizatör cihazıyla ölçüldü. Plazma homosistein konsantrasyonu, serum cTn I ve NO düzeyleri ELİSA cihazıyla ve eritrosit glutathione peroxidase (GSH-Px) aktivitesi ise spektrofotometre cihazıyla ölçüldü. Tedavi öncesi, kuzuların subklinik beyaz kas hastalığında GSH-Px aktiviteleri sağlıklı kuzulara göre daha düşük iken ($P<0.001$), plazma Hcy, serum NO, cTn I, AST, ALT, ALP, CK ve LDH değerleri sağlıklı kuzuların değerlerinden yüksek bulundu ($P<0.001$). Tedavi sonrası 10. günde elde edilen ortalama değerler, kontrol grubunun değerlerinden farklı değildi. Kuzuların subklinik beyaz kas hastalığında Hcy, cTn I ve NO değerlerindeki yükselmelerin, selenyum eksikliğinin sebep olduğu miyokardiyal hasarlarına bağlı gelişebileceği düşünüldü.

Anahtar sözcükler: *Beyaz Kas Hastalığı, Kuzu, Homosisteine, Troponin I, Nitrik oksit*

INTRODUCTION

White muscle disease is a nutritional and enzootic disease of lambs characterized by skeletal [1] and cardiac muscle degenerations [2]. Deficiencies of vitamin E and selenium (Se) play a significant role in etiology of the disease [3-5]. Deficiency of Se and vitamin E induce lipoperoxidation in tissues, which results in muscle degeneration

İletişim (Correspondence)
+90 432 7122637
skozat@hotmail.com

and calcification [2]. Prior to the first clinical signs of NMD in lambs, there is a certain increase in blood CK, AST, LDH, and ALP concentrations, indicatives of muscle degeneration [1,6-8]. On the other hand, activity of GSH-Px enzyme is slightly decreased [9].

Homocysteine is a sulfur-containing amino acid which is found in blood and produced in the metabolism of the essential aminoacid methionine [10]. Epidemiological studies have shown that too much Hcy in the plasma is related to a higher risk of coronary heart disease, stroke and peripheral vascular disease [11,12]. Other evidence suggests that Hcy may have an effect on atherosclerosis by damaging the inner lining of arteries and promoting blood clots [13,14]; nevertheles, a direct causal link hasn't been established, yet. Studies have also suggested that increased Hcy concentrations are associated with an increased rate of stroke, coronary artery disease, peripheral vascular disease, and deep venous thrombosis [15,16].

In recent years, several studies have reported that cTn I have especially become important in early diagnosis of myocardial damage [17]. Moreover, cardiac troponin level is a more specific marker than CK and isoenzim kreatin kinaz-MB (CK-MB) for diagnosing myocardial necrosis [18,19].

Nitric oxide plays an important role on regulating cardiovascular functions including the control of vascular tone, anti-inflammatory properties of the endothelium, cardiac contractility, and thrombocyte activation and aggregation [20]. Numerous experimental data support the view that NO not only acts via cyclic guanosine monophosphate (cGMP)-dependent mechanisms but also modulates protein function by nitrosation, nitrosylation, glutathiolation, and nitration, respectively [20,21].

Although several studies have evaluated the interrelationship among selenium status, homocysteine, and nitric oxide levels [17] and cardiovascular disease in man, there is no report of such probable relationship in disorders affecting the cardiovascular system of large animals [22].

For all these findings, this study aims to study the plasma concentration of Hcy, serum concentrations of cTn I and NO in lambs affected with subclinical nutritional myodegeneration.

MATERIAL and METHODS

This study was conducted on a flock of lambs (including 150 lambs) with suspected on NMD. These lambs, which were Akkaraman, aged between 2 and 6 weeks. Blood samples were collected from the jugular vein of all lambs in flock numbered at day 0. Blood samples were centrifuged at 1700 g for 15 min at room temperature to obtain sera. Levels of serum concentration of AST, ALT, ALP, CK, and LDH were measured in all lambs, and diagnosis of subclinical NMD and healthy lambs were done according to these data. According to enzyme analysis, lambs were divided into two as subclinical NMD (n=20) and healthy lambs (n=10) groups. Treatment of lambs with NMD were applied a commercial preparation (Yeldif®-Ceva DIF/TURKEY) subcutaneously which containing 1 mg sodium selenite and 60 mg vitamin E, and 40 mg vitamin B_1 for per lamb at the beginning of the study. Throughout the study, the lambs were maintained in the same pen under field conditions. They were allowed to suckle their mothers but not receive extra nutritional supplements. The blood samples were firstly taken at the onset of the disease and then 3 and 10 days after treatment. These samples were taken into plain vacutainer tubes and tubes with lithium heparin from the jugular vein. The serum and plasma were separated by centrifugation at 1700 g for 15 min at room temperature. Values of serum AST, ALT, ALP, CK, and LDH were measured with Integra 800 auto-analyzer (Roche-Cobes, Switzerland). Levels of serum cardiac troponin I were determined by ELISA equipment (ELISA Reader®-DAS Italy) and calculated with commercial test kit (ng/ml) as instructed by the manufacturer (Troponin I kit-DRG Diagnostic). Serum nitric oxide (nitrit) levels were measured by ELISA using commercial test kit (Nitrate/Nitrite Colorimetric Assay Kit-Cayman Chemicals). Erythrocyte package was obtained from the plasma received from the centrifuged tube with anticoagulant. The activity of erythrocyte GSH-Px in creating erythrocyte package was determined by commercial kits (RANSEL® - RS505/Randox/UK) using spectrophotometer (Photometer 5010®, Boehringer - Mannheim/Germany). Plasma Hcy levels were determined by ELISA using com-mercial kit (Homocysteine AXIS, Catalog no: 802865065).

Descriptive statistics for each parameter were determined as mean and standard error. Comparison of two independent groups was performed using "t test". Also, "paired t-test" was used to compare two paired groups [23].

RESULTS

Plasma concentration of homocysteine and serum concentrations of NO, cTn I and activity of CK, AST ALT, ALP, LDH, and values of erythrocyte GSH-Px varied between healthy lambs and those lambs with NMD as presented in *Table 1*. Before treatment, plasma Hcy, serum NO, cTn I, AST, ALT, ALP, CK, and LDH values of lambs with NMD were higher than those of healthy ones ($P<0.001$), while GSH-Px activity of lambs with NMD are lower than those of healthy ($P<0.001$). After ten days of treatment no differences were found significant between treated for lambs with NMD and healthy lambs.

DISCUSSION

Nutritional Myodegeneration disease, an subclinic or

Table 1. *Plasma homocysteine concentrations and biochemical variables in healthy lambs and lambs with subclinical nutritional myopathy*
Tablo 1. *Sağlıklı ve subklinik beyaz kas hastalikli kuzularin homocystein ve biyokimyasal değerler*

Parameters	Control Group (n=10)	NMD group (n=20)		
		Before Treatment (Day 0)	After Treatment (3rd Day)	After Treatment (10th Day)
Hcy (µmol/L)	5.10±3.33[a]	17.22±7.42[b]	9.50±2.77[b]	5.19±3.42
NO (µM)	5.27±1.08[a]	8.63±1.28[b]	7.23±1.35[b]	5.59±1.23
cTn I (ng/ml)	0.47±0.21[a]	9.14±2.16[b]	6.54±1.13[b]	0.62±0.20
CK (U/L)	146.6±46.9[a]	579.4±274.3[b]	309.4± 99.0[b]	155.8±48.9
AST (U/L)	66.5±13.5[a]	252±217[b]	135.2±87.5[d]	85.5±36.1
ALT (U/L)	10.91±3.36[a]	82.9±34.8[b]	32.9±11.6[d]	14.55±5.73
ALP (U/L)	497±119[a]	972±196[b]	716±120[b]	548±116
LDH (U/L)	668±111[a]	1024±192[b]	812±66[c]	680±87
GSH-Px (IU/g Hb)	77.6±21.3[a]	28.0±12.9[b]	43.8±18.6[b]	55.3±13.5[c]

Data are presented as mean ± standard deviation. Mean with different superscripts in the same row differ significantly. Statistical importance between control and NMD groups; [ab] *P< 0.001,* [ac] *P<0.01,* [ad] *P<0.05*

clinic form of metabolic disorder of lambs [6], is characterized clinically by locomotor disturbance and circulatory failure, and pathologically by degeneration and necrosis of skeletal and cardiac muscle [24,25]. In lambs with white muscle disease, levels of selenium in blood decreased [3,5] activity of erythrocyte GSH-Px reduced and activity of CK increased in comparison to healthy lambs [7]. Some researchers have reported that early diagnosis is important in WMD as appropriate treatment and prophylactic protocols are applied [1,6,7]. As values of serum enzyme including ALT, AST, ALP, LDH and CK are higher in lambs with NMD than healthy lambs [1,7]. Similarly, in the present study, serum CK, AST, ALT, ALP, and LDH concentrations in subclinical NMD were higher than those of healthy lambs. Our results are consistent with those of researchers [1,6,7,26]. In the present study, the subcutaneous injection of a preparation containing vitamin E, vitamin B_1, and selenium to lambs with subclinical NMD resulted in significant decreases in their serum enzyme levels when compared to healthy lambs. After treatment, levels of serum CK, AST, ALT, ALP, and LDH were brought back to normal values, identical to those of the controls.

It has been reported that glutathione peroxidase activity in erythrocytes correlates directly with blood selenium concentration in ruminants, horses, and rats [27]. Or et al.[7]. noted erythrocyte GSH-Px activity is lower in lambs with WMD than GSH-Px activity of healthy lambs. Yusuf et al.[28]. noted that selenium is an essential component of glutathione peroxidase. In case of selenium deficiency, glutathione peroxidase is inactive, resulting in biochemical and functional abnormalities of erythrocytes. In this study, statistical analysis revealed that erythrocyte GSH-Px activity of lambs with subclinical NMD were significantly lower than those of control group (P<0.001). These results are supported by researches [7,26,28].

Troponins are specific cardio proteins used as marker for miyocardial injures in humans and animals. In recent years, it has been reported that troponins are used as to more right and early diagnosis of cardiac diseases in man [29]. Several investigators reported that levels of serum concentration of cTn I rise in damaged myocardial cells [17]. In current study, serum cTn I concentrations were significantly higher in lambs with subclinical NMD than in healthy lambs. Results of study supported to results of researches [17,30]. NO is a key regulator of cardiovascular functions including the control of vascular tone, anti-inflammatory properties of the endothelium, cardiac contractility, and thrombocyte activation and aggregation [20]. NO in studies related to nitric oxide in skeletal muscle injury is the result of vasodilatation and the increase in NO release was reported [31]. Yet another study reported that levels of NO and cTn I in patients increase with cardiomyopathy [29]. In this study, levels of serum NO were significantly higher in lambs with subclinical NMD than healthy lambs. In this study, the increase in the amount of nitric oxide or release of nitric oxide as an antioxidant or white muscle disease are caused by muscle damage and may be due to vasodilatation. NO augments in lambs with subclinical NMD that result from NO produced by iNOS in infiltrating macrophages and cardiac myoctes may be a trigger for the apoptotic response of cardiac myocytes [17].

The fact that Se plays an important role in providing protection against oxidative damage and effects of dietary selenium on redox status of homocysteine and cysteine have been determined [22]. Furthermore, selenium deficiency has been associated with cardiomyopathy and congestive heart failure [22,28]. It has been reported that reducing glutathione peroxidase activity, a selenoprotein, in mice with selenium deficiency has also been implicated in the development of myocarditis [32]. Studies showed that increased plasma and heart tissue homocysteine concentrations could be considered as a risk factor in myocardium damage in condition associated with oxidative stress [22]. Several meta-analyses have shown an association between total plasma homocyst(e)ine

concentration and cardiovascular disease [33]. In another study, it reported that Hcy concentrations in cardiac form of lambs with acute selenium deficiency were significantly higher than those of healthy lambs, but in the skeletal muscle form the concentrations were notably lower than those of healthy lambs. In addition, there was an inverse relationship between blood and plasma homocysteine levels in lambs affected with cardiac form [22]. According to another study homocysteine was found to be associated with accelerated arteriosclerosis in two individuals with different enzymatic disorders of sulfur amino acid metabolism. Administering homocysteine to rabbits resulted in vascular lesions [34]. In our study, homocysteine concentration in lambs with subclinical NMD were significantly higher than those of healthy lambs *(Table 1)*. We can interpret that elevated homocysteine concentration in lambs with subclinical NMD was resulted from selenium deficiency of myocardial disorders, because selenium deficiency can affect the metabolism of homocysteine and methionine [22]. The elevated concentration of Hcy in selenium-deficient lambs may be due to myocardial damage of lambs with sublinical nutritional myodegenration.

To sum up, the evaluation of the parameters, plasma Hcy, serum cTnI and NO would be useful for determining diagnosis and prognosis of subclinical NMD in lambs. Myocardial disorders related to the work which would be done to the shed light on these parameters, have been concluded.

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