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Serum Vitamin D Levels in Sheep with Caseous Lymphadenitis

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In this study, in sheep with caseous lymphadenitis, serum 25(OH) vitamin D levels were studied for hematologic and biochemical parameters. Material of the study is composed of blood serum and abscess samples taken from 10 healthy (control group) sheep and 14 sheep that had caseous lymphadenitis, all of whose breeding conditions were the same. It is determined that serum 25(OH) Vitamin D level of sheep with caseous lymphadenitis was significantly lower than the control group (P<0.001).

Key Words: *Caseous lymphadenitis, Corynebacterium pseudotuberculosis, vitamin D, sheep.*

Kazeöz Lenfadenitisli Koyunlarda Serum D Vitamini Düzeyleri

Bu çalışmada kazeöz lenfadenitis belirlenen koyunlarda hematolojik ve biyokimyasal parametreler ile serum 25(OH) D vitamin düzeylerinin araştırılması amaçlanmıştır. Çalışmanın materyalini besleme şartları aynı olan 10 adet sağlıklı (kontrol grubu) ve 14 adet kazeöz lenfadenitisli (hasta grubu) olmak üzere toplam 24 adet koyundan alınan kan serumları ve apse içerikleri oluşturdu. Kazeöz lenfadenitisli koyunlarda serum 25(OH) vitamin D seviyesinin istatistiki olarak anlamlı derecede kontrol grubundan düşük olduğu belirlendi. (P<0.001)

Anahtar Kelimeler: *Kazeöz lenfadenitis, Corynebacterium pseudotuberculosis, D vitamini, koyun.*

Introduction

Vitamin D is synthesized from provitamins of vitamin D with the effect of ultraviolet light on skin. All forms of vitamin D are transported by binding to vitamin D binding protein in serum (1). Besides bone tissue, receptors of active vitamin D are determined in many tissues such as hypophysis, overs, skin, stomach, pancreas, thymus, breast, kidney, parathyroid glands and lymphocyte (2). Finding vitamin D receptors in different tissues shows that vitamin D has some different functions in addition to the -already known- effect of it on bone and calcium metabolism. Vitamin D ensures maturation, differentiation and migration in dentritic cells, it suppresses activation in T1 cells, it stimulates regulator T cells, activates cells of myeloid and erythroid; all these show that vitamin D is a significant immune modulator and it controls some processes that are formed against infections (1-3).

It is known that vitamin D ligands increase the production of cathelicidin, which is anti microbial effective peptides that increase fagositic activity of macrophage through a series of inflammation occured by bacteria, viruses and funguses (1). When monocytes and macrophages are exposed to lipopolysaccharide they upregulate Vitamin D receptor (VDR) gene and CYP27B1 alpha hydroxylase gene. Increased 1.25(OH) vitamin D production ends with cathelicidin synthesis, which is an antimicrobial effective peptide. 25(OH) Vitamin D insufficiency means that there is not sufficient substrate for CYP27B1 hydroxylase. This case decreases binding of 1.25(OH) vitamin D to VDR in macrophages, limits activation of 1.25(OH)D-VDR towards antimicrobial genes and decreases phagocytosis power monocyte and macrophages against infection agents (1, 4).

It is reported in the studies that vitamin D deficiency increase tendency to tuberculosis, otitis media, influenza and upper respiratory system infection in human; on the other hand, it was also reported that serum 25(OH) vitamin D level of cattle decrease in case of diseases such as tuberculosis and malignant catarrhal fever (5-8).

Caseous lymphadenitis in sheep and goat is mostly caused by *Corynebacterium pseudotuberculosis;* it is a chronic course disease and characterized by abscess in lymph nodules and internal organs (9).

The disease occurs in two forms as external and visceral. It is stated that caseous lesions in the external form are formed in superficial lymph nodule while caseous lesions in visceral form are formed internal organs and their lymph nodules. In external

organs and their lymph nodules. In external form, abscess in superficial lymph nodules decrease the quality of skin. In visceral form, body weight of animal, wool yield and quality and fertility decrease. It is also significant to mention that these negative effects of the disease cause economic loss (9).

The aim of this study was to investigate serum 25(OH) vitamin D levels in sheep with caseous lymphadenitis with hematologic and biochemical parameters and to determine the effect of 25(OH) vitamin D in the emergence of caseous lymphadenitis

Materials and Methods

The material of the study was consist of a total of 24 sheep average 2 old. Control group included, 10 healthy sheep while the diseased group included 14 sheep with caseous lymphadenitis.

Lymph nodule contents of sheep diagnosed with caseous lymphadenitis were taken into sterile injector and microbiologic examination of them were carried out. Lymph nodule contents taken with sterile injector were brought to laboratory with cold chain; they are placed to 5 % blood agar, Mac-Conkey and EMB agar in two series; while one series was incubated at 37 °C in aerobic atmosphere, the other series was incubated at the same temperature in micro aerobic atmosphere. Cultures were checked every day for growth. From growth of bacterial cultures, passage was done on blood agar according to single colony culture and left for incubation again. Colonies that growth as pure culture were analyzed macroscopically and microscopically and their biochemical tests were done. For this aim, passages are made to Triple Sugar Iron *Agar* (TSIA), urea agar, Sulfate Indole Motility (SIM) agar, Eosin Methylene Blue (EMB) agar and Oxidation Fermentation (O/F) medium; then cultures were identified by taking coloration, morphologic and biochemical features into consideration.

Glucose, cholesterol, creatine, aspartat aminotransferase (AST), lactate dehydrogenase (LDH), calcium (Ca), phosphorus (P), albumin (Alb) from serum biochemical analyses were made by auto analyzer (Mindray BS 200; PRC). Serum 25(OH) vitamin D levels are determined through chemiluminescence method (Abott I 2000; USA). Hematological examinations (hematocrit neutrophils, eosinophil, basophil, lymphocyte and monocyte) were made with the methods by Eksen 1991 (10).

SPSS 14.0 (SPSS for Windows Release 2005) package program was used for analyzing changes in serum biochemical values and hematological parameters, determining groups variances homogeneity with levene test and comparing groups with Student t test. In statistical evaluations, $P<0.05$ level is accepted to be the lowest indicator for significant difference

Results

Microbiological agent isolation from 10 of 14 sheep, which are clinically determined to have caseous lymphadenitis in head and neck lymph nodules; 8 of these isolated agents are called *Corynebacterium pseudotuberculosis* 57.12% while 2 of them are called *Staphylococcus aureus* 14.28%.

C. pseudotuberculosis growth small colonies that formed 0.5-1 mm in diameter, grayish-white colored, small zone of β-haemolysis in 5-7% blood agar. In microscopic analysis made by gram staining, it is determined that these bacteria have Gr (+), rood, pleomorphic, nonsporing, noncapsulated morphology. It is also determined that isolated agents are oxidase (-) motility (-), catalase (+), glucose (+), lactose (-), gas (-), H_2S (+), urease (+), indole (-).

S. aureus identification, plantations to mannitol salt agar is made to the colonies that produced round, convex, 1-4 mm diameter, β haemolysis in 5% blood agar. The ones that produced yellow colonies at 37 °C are seperated for identification. Catalase test which is in accordance with gram staining and morphologic features and lam and tube plasma coagulase test are determined as positive, isolates whose oxidase activities are (-), are identified as *S. aureus*.

Serum biochemical values that were determined in sheep with caseous lymphadenitis and healthy sheep are presented in Table 1, hematological values are presented in Table 2.

Table 1. Serum biochemical values in healthy and with caseous lymphadenitis sheep

Test	Healthy		Caseous Lymphadenitis		P
	n	± SE	n	± SE	
Glucose (mg/dL)	10	50.25±2.57	14	50.19±1.18	0.980
Cholesterol (mg/dL)	10	60.75±3.38	14	57.50±4.08	0.610
Creatine (mg/dL)	10	1.83±0.04	14	1.77±0.05	0.414
AST(IU/L)	10	113.88±2.59	14	133.38±4.52	0.008
LDH (IU/L)	10	411.38±16.06	14	477.38±14.50	0.010
Calcium (mg/dL)	10	8.78±0.36	14	7.59±0.19	0.004
Phosphorus (mg/dL)	10	4.75±0.25	14	4.45±0.28	0.495
Albumin (g/dL)	10	2.72±0.09	14	2.81±0.10	0.554
25(OH)vitamin D	10	41.13±1.20	14	29.56±0.79	0.001

Table 2. Hematological findings in healthy and with caseous lymphadenitis sheep

Test	Healthy		Caseous Lymphadenitis		P
	N	± SE	n	± SE	
Hematocrit %	10	32.88±0.83	14	32.44±0.68	0.043
Neutrophil %	10	42.13±0.64	14	50.81±0.89	0.001
Lymphocytes %	10	52.25±0.53	14	43.00±0.88	0.001
Eosinophil %	10	3.08±0.18	14	3.19±0.21	0.020
Monocytes %	10	2.25±0.16	14	2.38±0.24	0.732
Basophile %	10	1.00±0.27	14	0.63±0.18	0.249

It is determined that there are statistically insignificant changes in hematocrit, eosinophil leucocyte, basophilic leucocyte, glucoses, cholesterol, creatin, LDH, phosphor and albumin levels of control and diseased groups; on the other hand, it is determined that there is a statistically significant difference between groups in terms of neutrophil leucocyte ($P<0.001$), lymphocyte ($P<0.001$), calcium ($P<0.01$) and 25(OH) vitamin D ($P<0.001$) levels.

Discussion

Similar with the findings of microbiologic studies on determining the etiology of caseous lymphadenitis in sheep (11), in this study *C. pseudotuberculosis.* and *S. aureus* are isolated from abscess contents of sheep with caseous lymphadenitis.

On the other hand, it is determined that serum biochemical values both in sheep with caseous lymphadenitis and healthy sheep are between ranges that are normal for sheep; it is also determined that the difference between control and diseased groups in terms of glucose, cholesterol, creatine, lactate de hydrogenase, phosphor and albumin values are statistically not significant. Although measured serum calcium levels are in normal ranges in both groups (12), the level of serum calcium in sheep with caseous lymphadenitis is significantly lower than healthy sheep; based on this data, it is thought that low serum level of 25(OH) vitamin D, which is one of the factors that regulate calcium metabolism, is related with this lowness.

It is determined in the study that hematological values in diseased and control groups are between the ranges which are accepted to be normal for sheep (13); in addition to this, it is determined that there was a statistically significant decrease in lymphocyte and increase in neutrophile ratio of sheep with caseous lymphadenitis when compared to control group ($P<0.001$). It is interpreted that the decrease in lymphocyte number in diseased group can be related to the lysis in lymph nodules in which abscess is formed, and increase in neutrophile number can be related to bacterial infection.

Vitamin D receptors are identified in all of the immune system cells, primarily the ones that present especially antigen such as T and B lymphocytes, macrophages and dentritic cells (1). In case of vitamin D deficiency, oscillation of pro inflammatory cytokine increases according to a more Th1 answer. In this case, leucocyte chimiotaxy is influenced, immune answer is destroyed and infection tendency increases (1). It is also known that monocytes and macrophages have key roles in activating natural immunity against invasive features of infection agents (1, 4). Consistent with the results of the studies in the literature, it is determined in this study that, there is not a statistically significant difference between monocyte number of sheep with caseous lymphadenitis and healthy group; but, on the other hand there is a significant difference between healthy group and diseased group in terms of 25(OH) vitamin D levels. These findings show that, for the activation of monocytes, 25(OH) vitamin D level should be sufficient. This result can be interpreted as; 25(OH) vitamin may have a significant role in preventing infections.

Through cell cultures made of mouse epidermis cells, it has been shown by the researchers (14) that, vitamin D affect to differentiation and proliferation of keratinocytes. Studies show that vitamin D analogs activate genes that control oscillation of peptides that have antimicrobial effects on skin and they have significant role in continuation of healthy structure of skin and they decrease sun damage (15). Information reported in these studies show that when pathogenesis of caseous lymphadenitis, which is known to be formed with the formation of abscesses that have typical lamellation in lymph nodules as a result of the transmission of agents, which enter body from portantre in damaged skin, to local lymph nodules, is combined with the findings of this study, vitamin D's significant becomes clearer.

It is determined that sheep with caseous lymphadenitis has significantly lower 25(OH) vitamin D level when compared to control group ($P<0.001$). As a result, it is determined that low serum 25(OH) vitamin D level in sheep with caseous lymphadenitis may negatively affect protecting animals from chronic infections.

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