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Bone remodeling biomarkers and lumbar vertebrae plasticity in cows with hypophosphatemia

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Abstract

Although hypophosphatemia is a major metabolic disorder in cattle, a clinically useful method has not been proposed for its rapid screening in cattle. This study aimed to evaluate bone biomarkers and lumbar vertebrae plasticity in cows with hypophosphatemia. Among referral cows suspected of hypophosphatemia, 27 hypophosphatemic and 10 healthy cows were recruited. Firstly, the plasticity of transverse processes of lumbar vertebrae was determined. Serum levels of calcium, magnesium, vitamin D, bone sialoprotein (BSP), and ALP activity were assayed. Results revealed a significant increase in serum ALP activity and a significant decrease in vitamin D in the hypophosphatemic compared with the healthy cows. Serum BSP had an insignificant increment in the patients than the healthy group. The patients were segregated into 3 subgroups of severe, moderate and mild hypophosphatemia. Serum ALP activity was higher in severe hypophosphatemic than the other subgroups. A strong negative correlation was observed between serum phosphorus concentration and severity of lumbar vertebrate plasticity. In conclusion, hypophosphatemia was associated with significant changes in bone remodeling biomarkers. Since degree of lumbar vertebrate plasticity was directly related to the intensity of hypophosphatemia, it can be used for clinical screening and determining the degree of hypophosphatemia in cattle.

Keywords: ALP activity, Bone sialoprotein, Hooves deformity, Vertebrate plasticity

Introduction

Phosphorus is essential for maintaining cell membrane integrity, rumen micro-flora activity and bone mineralization, and its deficiency can lead to bone abnormalities (Constable et al., 2017; Goff, 2006). The normal serum phosphorus level in cattle was reported to range between 5.6 to 6.5 mg/dl (Constable et al., 2017).

Rickets and osteomalacia are the major metabolic bone problems with the most common causes include mineral insufficiency, imbalance of dietary calcium/phosphorus ratio, nutritional vitamin D deficiency, and abnormal parathyroid hormone (PTH) activity in farm animals. Renal impairment and genetic abnormalities have also been rarely ascribed to metabolic bone disease in farm animals (Smith, 2015). The pathogenesis of these disorders is well described (Carlson and Weisbrode, 2012), but a few pertaining published scientific reports are available in

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large animals. Animals with osteomalacia are poor in body condition and may exhibit shifting lameness, fractures in ribs and long bones and limb deformities (Kahn et al., 2010).

Bone sialoprotein (BSP) is a glycoprotein that forms almost 10% of non-collagenic bone tissue proteins (Wollheim, 1999) and has been known as a biochemical marker in the bone resorption process (Shaarawy et al., 2001; Nakamura et al., 2003), as well as a biochemical bone extracellular matrix marker. (Gorski et al., 2004). Serum Alkaline phosphatase (ALP) activity has been known as a clinically useful marker of abnormal bone remodeling in humans (Kang et al., 2015; Chen et al., 2018) and dairy cows (Vojtic, 2002; Kaya et al., 2008). However, no attempt has been made to investigate serum markers of bone degradation related to hypophosphatemia as a prominent cause of rickets and osteomalacia in large animals.

According to the author's observations and what has been addressed in the past, a palpable softening was noted in the bilateral transverse process of the lumbar vertebrae in cows with hypophosphatemia, which has been simply investigated in a previous study (Nikvand et al., 2014). Therefore, in the present study, the repeatability of this finding and its relationship with some serum indicators pertaining to bone remodeling were investigated.

Serum bone metabolism indices have not yetbeen paid enough attention in hypophosphatemic cattle. On the other hand, so far, a confirmed clinically relevant and easy to use method has not yet been suggested for detecting and screening hypophosphatemia in cattle. Therefore, this study aimed to the following objectives. 1. To evaluate relationship between hypophosphatemia and serum bone remodeling factors in affected cows. 2. To investigate association of lumbar vertebrate plasticity with serum phosphorus level in study cows.

Materials and Methods

Animals and study design

This cross-sectional clinical-laboratory study was performed on 27 cross-breed cows with hypophosphatemia (all were female) and 10 healthy cows as control that were referred to the Veterinary Teaching Hospital of the Shahid Chamran University of Ahvaz, from May to December 2018. After careful physical and laboratory examinations, 27 hypophosphatemic cows (serum phosphorus level less than 3.5 mg/dl) with a range of 2.5 to10 years old were served as the hypophosphatemic group. Some clinical findings including difficulty walking or lameness, poor body condition, and lumbar vertebrae plasticity were also observed in the hypophosphatemic cows. Hooves and legs deformity were also seen in some patients. None of the included hypophosphatemic cases had a history of postpartum hemoglobinuria, as well as had not a coexistent disease. Then, according to the serum phosphorus levels, the hypophosphatemic cows were segregated into 3 subgroups of severe (serum P level $\leq$ 2, n= 6), moderate (2 < serum P level $\leq$ 3, n= 13) and, mild (3 < serum P level $\leq$ 3.5 mg/dl, n= 8) hypophosphatemia. Since the included cows were in late lactating or dry period; the groups were also matched for physiological states. Ten healthy cows without any aforementioned abnormal clinical findings, with normal transverse processes of lumbar vertebrae, and normal serum phosphorus concentrations were also selected as the control group. The mean age of the hypophosphatemic and control groups were 4.5 ± 2.1 and 5.1 ± 1 years, respectively. Regarding details of the rations, although the hypophosphatemic patients were referred from the different semi-industrial or native farms, similar poor diets were notable in history taking that consisted mainly of wheat straw, dry bread, and free grazing in poor pastures.

Compliance with Ethical Standards

This study was performed conforming the requirements of an ethical committee in the faculty of veterinary medicine, Shahid Chamran University of Ahvaz, Iran (approval No. EE/97.24.3. 70182/scu.ac.ir).

Sampling

Following clinical examination, a blood sample of 10 ml from each animal was collected via the jugular vein into a plain tube (without anticoagulant). After clotting and centrifuging, sera were isolated and stored at -70 °C until analysis.

Serum biomarkers assay

Serum levels of Ca, P, Mg, and ALP activity were determined using the colorimetric assay methods and the diagnostic kits (Pars Azmun, Tehran, Iran) using biochemistry auto analyzer (BT-1500, Biotechnica, Rome, Italy). Serum BSP concentration was analyzed using the immunoassays method by a bovine ElISA kit (BioassayTechnology, Shanghai, China) with assay range of 0.5 - 150 ng/ml and sensitivity of 0.31 ng/ml. With an Agilent 1100 series high-performance liquid chromatography (HPLC) set (Gumtree India Analytical, India) that equipped with a UV detector, serum 25-(OH) vitamin D concentration was measured at a wavelength of 264 nm.

Plasticity of transverse processes of lumbar vertebrae and BCS

To evaluate the texture status of vertebral process, bilateral transverse processes of L3, L4 and L5 were palpated and moved in dorso-ventral directions (Figure 1). Then, to improve objectivity for evaluating the degree of plasticity, the vertebral processes were carefully measured with a scaled thumb finger which measured by a ruler. Because the majority of hypophosphatemic patients had a BCS of less than 2.5, supportive muscle tissues did not interfere with plasticity evaluation. The hypophosphatemic patients segregated into three subgroups based on the amount of flexibility in length of transverse processes. In the other words, based on the severity of lumbar vertebrate plasticity, the patients were divided into 3 subgroups of grade (+, n = 7), grade (++, n = 15) and grade (+++, n = 5) plus. 1. Subgroup grade (+): 1 cm in length from the tip of transverse processes of L3, L4 and L5 were flexible. 2. Subgroup grade (++): 1 to 2 cm in length from the tip of transverse processes of L3, L4 and L5 were flexible. 3. Subgroup grade (+++): more than 2 cm in length from the tip of transverse processes of L3, L4 and L5 were flexible (Nikvand et al., 2014). In order to reduce the bias in determining the severity of plasticity, examination was simultaneously performed by two researchers. The body condition of the patients was scored based on a five-point scale of 1 (very poor) to 5 (fat) (McNamara, 2011).

Data analysis

With using the Shapiro-wilk test, except for BSP, all data had normal distributions. Independent sample t-test was used to compare the mean of normal data between the control and patient groups. Mann-Whitney U test was used to compare the mean of BSP values between the two groups. Further, One-way ANOVA and post-hoc Tukey tests were used to compare the mean of the normal data among the three subgroups of hypophosphatemia and with the control group. Kruskal Wallis test was also employed to compare the mean of BSP values among the three subgroups of hypophosphatemic cows. Additionally, a Spearman correlation was calculated to indicate association between serum P concentration and severity of PTLV in the patient group. The data are presented as the mean ± SD and the level of significance was set at $P < 0.05$. All the analyses were done using SPSS statistic software (SPSS statistics for windows, SPSS Inc, version 21. Chicago, USA).

Results

The major complaints in the hypophosphatemic patients were difficulty

walking and weight loss, so that 80% of the patients had a poor body condition, which had BCS less than or equal to 2.5. There was also apparent deformity of the limbs (valgus deformity in 11% and hooves deformity in 40% of the cases (Figure 2). Comparative results of the serum biochemical findings of the hypophosphatemic patients and healthy cows are presented in Table 1. Biochemical findings showed a significant increase in serum ALP activity and a significant decline in vit D in the hypophosphatemic patients compared with the healthy cows ($P < 0.01$). Serum BSP was slightly higher in the patients than the healthy group (Figure 3). No significant difference was found in serum Ca and Mg levels between the hypophosphatemic and healthy cows ($P>0.05$).

Serum ALP activity was significantly higher in severe hypophosphatemic than the other subgroups and healthy cows ($P<0.01$). In addition, the highest level of BSP was found in the severe hypophosphatemic subgroup ($P>0.05$). Serum vitamin D level was significantly lower in the mild hypophosphatemic subgroup than that in the control cows ($P<0.01$), (Table 2, Figure 4).

A strong negative correlation was observed between serum phosphorus concentration and the severity of lumbar vertebrate plasticity in the patients (r=-.672, $P<0.01$). Serum ALP activity in the patient cows with grade three (+++) of vertebrate plasticity was significantly higher compared with the healthy cows ($P<0.01$). Serum vitamin D level in the patients with grade two (++) (9.8±3.8ng/ml) was significantly lesser than that in the healthy cows (16.7±4 ng/ml), ($P<0.01$). Further, the lower serum P concentration was found in the patient cows with grade three of lumbar vertebrate plasticity compared to the other subgroups ($P<0.05$). (Table 3). The lean transverse processes of lumbar vertebrae were visually detected in some hypophosphatemic cows (Figure 5).

Table 1: Serum biochemical and radiological parameters in hypophosphatemic patients and healthy cows

	Healthy cows (n = 10)		Hypophosphatemic patients (n = 27)		*P*-value
Parameters	Mean ± SD	Range	Mean ± SD	Range	
P (mg/dl)	5.1 ± 0.9	4.1 - 6.16	2.7 ± 0.8	0.7 - 3.5	< 0.01
Ca (mg/dl)	9 ± 0.9	8 - 11.1	9.1 ± 3.6	5.5 - 12.7	> 0.05
Mg (mg/dl)	2.1 ± 0.8	1.2 - 3	1.8 ± 0.6	0.9 - 2.9	> 0.05
Vit D (ng/ml)	16.7 ± 4	10.3 - 21.8	11.6 ± 4.8	4 - 20.6	< 0.01
BSP (ng/ml)	7.4 ± 6.1	2.3 - 17.2	8.3± 6.5	2.5 - 24.1	> 0.05
ALP (iu/l)	154 ± 64	64 - 301	261 ± 216	63 - 881	< 0.01

BSP: bone sialoprotein, Vit D: 25-(OH) vitamin D, ALP: Alkaline phosphatase.

Table 2: Comparative results of serum biochemical parameters (Mean ± SD) in the subgroups of hypophosphatemic patients and healthy cows

Group	P (mg/dl)	Ca (mg/dl)	Mg (mg/dl)	Vit D (ng/ml)	ALP (iu/l)	BSP (ng/ml)
Healthy (n = 10)	5.1 ± 0.9*	9 ± 0.9*	2.1 ± 0.8*	16.7 ± 4*	154 ± 64*	7.4 ± 6.1*
Mild (n = 8)	3.3 ± 0.2**	8.5 ± 1.3*	1.7 ± 0.6*	9.9 ± 4.9**	219 ± 122*	6.4 ± 4.9*
Moderate (n = 13)	2.4 ± 0.3**	9.6 ± 1.3*	1.8 ± 0.6*	12.8 ± 4.3*	170 ± 93*	8.9 ± 7.3*
Severe (n = 6)	1.5 ± 0.5***	9.6 ± 2.2*	1.9 ± 0.4*	11.3 ± 6.5*	484 ± 343**	10.9 ± 8.7*

* Different numbers of asterisk in each column denote significant difference between groups. BSP: bone sialoprotein, Vit D: 25-(OH) vitamin D, ALP: Alkaline phosphatase, Mild: 3 < serum P level ≤ 3.5, Moderate: 2 < serum P level ≤ 3, Severe: serum P level ≤ 2 mg/dl.

Table 3: Comparative results of serum biochemical parameters (Mean ± SD) in the healthy and patient cows based on grade of lumbar vertebrate plasticity

Group	P (mg/dl)	Ca (mg/dl)	Mg (mg/dl)	Vit D (ng/ml)	ALP (iu/l)	BSP (ng/ml)
Healthy (n = 10)	5.1 ± 0.9*	9 ± 0.9*	2.1 ± 0.8*	16.7 ± 4*	154 ± 64*	7.4 ± 6.1*
G (+) (n = 7)	2.9 ± 0.6**	8.9 ± 0.8*	1.9 ± 0.7*	15.8 ± 5*	184 ± 142*	4.5 ± 2.2*
G (++) (n = 15)	2.6 ± 0.8**	9.4 ± 1.4*	1.8 ± 0.5*	9.8 ± 3.8**	179 ± 87*	9.1 ± 7.1*
G (+++) (n = 5)	1.9 ± 0.8***	9.8 ± 2.2*	1.8 ± 0.2*	12.2 ± 5*	665 ± 263**	8.6 ± 7.4*

* Different numbers of asterisk in each column denote significant difference between groups. BSP: bone sialoprotein, Vit D: 25-(OH) vitamin D, ALP: Alkaline phosphatase, G (+): Grade one, G (++): Grade two, G (+++): Grade three.

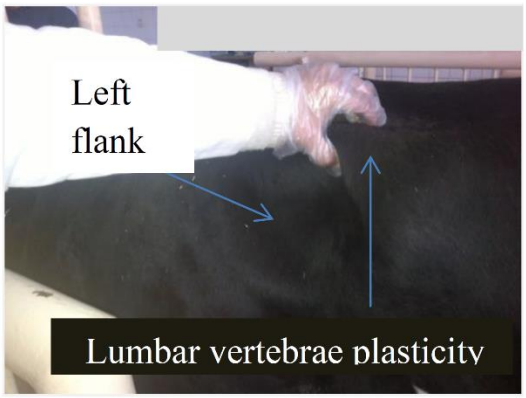

Figure 1: A hypophosphatemic cow with plasticity of transverse processes of lumbar vertebrae.

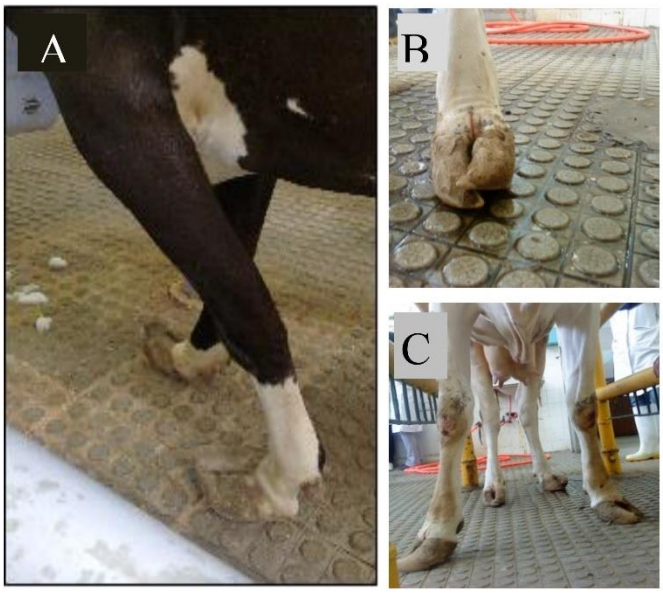

Figure 2: Deformity of limbs and hooves in the hypophosphatemic cows. (A) and (B) Hooves deformity. (C) Valgus legs and hooves deformity.

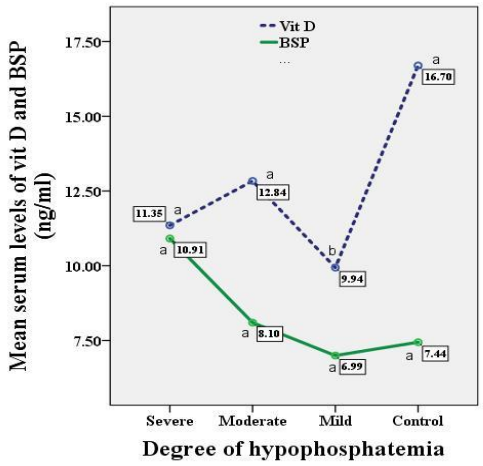

Figure 3: Comparison of serum BSP and vitamin D values in the three subgroups of hypophosphatemia and control group. BSP: bone sialoprotein, Vit D: 25-(OH) vitamin D. Different letters (a, b) on each column donate the significant differences between the groups.

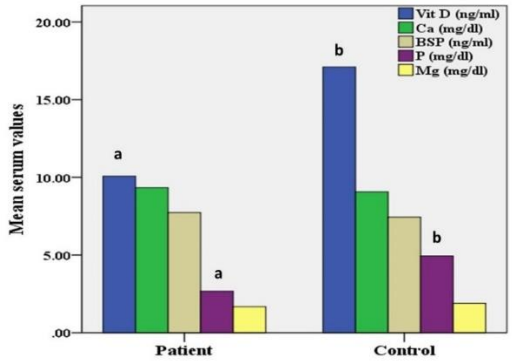

Figure 4: Comparison of serum parameters in the hypophosphatemic and control cows. Different letters (a, b) on bars show the significant differences between the groups. BSP: bone sialoprotein, Vit D: 25-(OH) vitamin D.

Figure 5: Lumbar vertebrate plasticity which visually detected in a severe hypophosphatemic cow with serum phosphorous level of 1 mg/dl. The arrows indicate the direction curvature of transverse processes of lumbar vertebrae.

Discussion

Hypophosphatemia in farm animals is associated with disorders such as PPH, reduced milk production, pica, rickets, and osteomalacia (Zongping, 2005; Radwan and Rateb, 2007; Smith, 2015). A wide range of 3.5 to 8.5 mg/dl has also been reported for serum phosphorus levels in cattle, which makes it difficult to interpret the results (Kurek et al., 2010). Radwan and Rateb (2007) reported a serum phosphorus level of 3.6 ± 0.6 mg/dl in buffaloes with clinical signs of hypophosphatemia. Osteomalacia in buffaloes has been shown to be associated with serum phosphorus levels of less than 3 mg/dl (Heure and Bode, 1998). According to the above-mentioned studies, in the present work, cows with serum phosphorus concentration below 3.5 mg/dl were considered as hypophosphatemic cases.

In the present study, the hypophosphatemic cases had significantly lower serum levels of vitamin D, as well as higher level of ALP activity compared to the healthy group. Also, an elevated serum ALP activity (261±216 IU/l) was found in the hypophosphatemic cases which was greater than the previously reported normal range (0 to 200 IU/l) in cattle (Constable et al., 2017). A significant increase in ALP along with hypophosphatemia in cattle which proposed by Kurek et al. was consistent with the present study (Kurek et al., 1998). The results obtained showed that serum BSP had an insignificant increase in the patients compared with the control group, so that the highest and the lowest levels of BSP were found in the severe and mild hypophosphatemic subgroups, respectively. An increased serum BSP level has been shown in human beings with metabolic bone disease (Seibel et al., 1996). No relevant study was found that investigated serum BSP changes in the context of bone disorders in veterinary medicine.

In our study, serum levels of vitamin D in the cows with hypophosphatemia (11.6 ± 4.8 ng/mL) was much lower than the several past reported values of 20-50 ng/ml (Horst et al., 1994) and 13.8 ± 3.7 ng/mL (Spakauskas et al., 2006) in cattle. In the current study, serum vitamin D levels in the mild hypophosphatemic subgroup (9.9 ± 4.9 ng/ml) were the lowest among all subgroups. Norman (2008) showed that a serum vitamin D concentration lesser than 10 ng/ml indicates severe vitamin D deficiency in cattle. Overall, three subgroups of hypophosphatemic patients appear to have significant vitamin D deficiency.

Authors believe that the usual signs of hypophosphatemia in cattle occur when blood phosphorus level falls below 2 - 2.1 mg/dl (Cheng et al., 1998; Jubb and Crough, 1998). Findings of the present study in the severe and moderate hypophosphatemic subgroups in which 40% of cases had apparent hooves and leg deformities, as well as 80% had low BCS as clinical signs associated with hypophosphatemia, are similar to those reports. Regulation of blood calcium/phosphorus homeostasis is tightly related to the blood vitamin D activity. Although decreased serum activity of vitamin D has been shown to cause hypophosphatemia by decreased renal phosphorus absorption (Uhi, 2018), given that the serum calcium levels of the hypophosphatemic cows were in normal range, it does not appear the hypophosphatemia to be secondary to vitamin D deficiency.

Lumbar vertebrate plasticity was found to be a remarkable clinical sign in cows with hypophosphatemia which, little attention has been given to it, so far. Based on our experiences, the lumbar vertebrate plasticity finding was not observed in healthy cows. Evaluation of lumbar vertebrate plasticity in the hypophosphatemic patients showed that cows with moderate vertebral softness had the lowest and highest serum vitamin D and BSP levels, respectively. The highest serum ALP activity (665 ± 263 IU/L) was also observed in the severe subgroup of plasticity. Regarding a strong negative correlation between serum phosphorus level and the rate of lumbar vertebrate plasticity in the patients, it seems to be an appropriate finding for early diagnosis of bone changes and hypophosphatemia. For the future studies, it would be interesting to see whether degree of plasticity able to measure the length of the P deficiency period and able to use to monitor the correction of P deficiency.

In conclusion, hypophosphatemia at level of 2.8 ± 0.7 mg/dl may be associated with significant bone changes and vitamin D deficiency in cattle. It also seems that evaluation of lumbar vertebrate plasticity in the three grades can be useful as a clinical indicator of hypophosphatemia in cow. So, it can be used to screen and determine the grade of hypophosphatemia in cattle.

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Conflict of interest

The authors declare that they have no conflict of interest.

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شاخص‌های زیستی تخریب و بازسازی استخوان و نرمی مهره‌های کمری در گاوهای مبتلا به هیپوفسفاتمی

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چکیده

اگرچه هیپوفسفاتمی یک اختلال متابولیکی مهم در گاو است، یک روش مفید بالینی برای تشخیص اولیه‌ی آن در گاو پیشنهاد نشده است. این مطالعه با هدف ارزیابی شاخص‌های زیستی استخوانی و نرمی مهره‌های کمری در گاوهای هیپوفسفاتمی انجام شد. از بین گاوهای ارجاعی مظنون به هیپوفسفاتمی، ۲۷ رأس گاو مبتلا به هیپوفسفاتمی و نیز ۱۰ رأس گاو سالم انتخاب شدند. در ابتدا، نرمی زوائد عرضی مهره‌های کمری تأیید گردید. سپس مقادیر سرمی کلسیم، منیزیم، ویتامین D، سیالوپروتئین استخوانی و فعالیت سرمی الکالین فسفاتاز اندازه‌گیری شد. نتایج، افزایش معنی‌داری در میزان فعالیت سرمی الکالین فسفاتاز و کاهش معنی‌داری در میزان سرمی ویتامین D در گاوهای بیمار در مقایسه با گاوهای سالم آشکار ساخت. غلظت سرمی سیالوپروتئین استخوانی یک افزایش غیر معنی‌دار در گاوهای هیپوفسفاتمی در مقایسه با گاوهای سالم داشت. بیماران هیپوفسفاتمی به سه زیرگروه شدید، متوسط و خفیف تقسیم شدند. فعالیت سرمی الکالین فسفاتاز در زیرگروه هیپوفسفاتمی شدید در مقایسه با دیگر زیرگروه‌ها به طور معنی‌داری بالاتر بود. یک همبستگی منفی قوی بین غلظت سرمی فسفر و درجه نرمی مهره‌ای در بیماران یافت شد. نتیجه‌گیری کلی این که، هیپوفسفاتمی با تغییرات قابل توجهی در شاخص‌های زیستی تخریب و بازسازی استخوانی همراه بود. از آنجایی که درجه نرمی مهره‌ای ارتباط مستقیمی با شدت هیپوفسفاتمی داشت، می‌توان از آن برای غربالگری بالینی و تعیین درجه هیپوفسفاتمی در گاو استفاده کرد.

کلمات کلیدی: فعالیت الکالین فسفاتاز، سیالوپروتئین استخوانی، بدشکلی سُم‌ها، نرمی مهره‌ای

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