



Changes in vitamin D and calcium-phosphorus metabolism in patients with severe acute pancreatitis

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ABSTRACT

Objective: The problem of predicting the course of acute pancreatitis and early diagnosis of its complications remains unresolved. This study aimed to determine changes in vitamin D and calcium-phosphorus metabolism in patients with severe acute pancreatitis.

Material and Methods: There were examined 72 people divided into two groups as healthy persons (comparison group) - males and females without pathology of the gastrointestinal tract and any other conditions or diseases that could affect the state of calcium-phosphorus metabolism (n= 36) and patients with acute pancreatitis (main group, n= 36). In addition, in order to determine the prognostic criteria for the severity of the disease, patients in the main group were divided into two subgroups. The first subgroup included patients with severe disease (n= 18), the second (n= 18) - with mild and moderate disease.

Results: Serum calcium value was lower in patients with severe acute pancreatitis comparison to healthy persons: 2.18 (2.12; 2.34) vs 2.36 (2.31; 2.43) mmol/L ($p < 0.0001$), and the decrease of calcium levels was associated with an increase in the severity of acute pancreatitis. Therefore, hypocalcemia can be considered a reliable predictor of the severity of the disease. In patients with acute pancreatitis, the level of vitamin D was significantly low than in the healthy persons and was 13.8 (9.03; 21.34) and 28.4 (21.8; 32.3) ng/mL, respectively ($p < 0.0001$).

Conclusion: For patients with acute pancreatitis, serum vitamin D levels ≤ 13.28 ng/mL can be considered as a significant predictor of severe disease (sensitivity 83.3%, specificity 94.4%) regardless of calcium level.

Keywords: Acute pancreatitis, vitamin D, calcium-phosphorus metabolism

INTRODUCTION

Acute pancreatitis is a polyetiological disease that remains one of the most pressing problems of modern medicine. According to the literature, more than 140 causes of this disease are known, most of which lead to a violation of the passage of pancreatic secretion. The leading factors in the occurrence of acute pancreatitis are alcohol abuse in 17-65% of cases, gallstone disease and pathology of the biliary system in 21-58% of cases, neoplasm or cicatricial stenosis of the large duodenal papilla, a intraductal or parenchymal neoplasia (1). Other causes include mechanical trauma to the pancreas (including iatrogenic damage), hypertriglyceridemia, tissue ischemia (atherosclerotic vascular disease), virsungolithiasis, chemotherapy (drug pancreatitis), hyperparathyroidism, and others (2).

The role of calcium in the genesis of acute pancreatitis remains debatable. It is known that calcium is a powerful release of peptides that stimulate the secretion of the pancreas, as well as intracellular secondary stimulators of the synthesis and secretion of pancreatic enzymes (3).

On the one hand, hypercalcemia in the case of prolonged hyperparathyroidism (4), genetic disorders or cancer (5) can cause the development of acute pancreatitis; however, currently, the detection of high calcium levels in patients with acute pancreatitis without concomitant pathology could explain the need to expand the diagnostic search and examination of the patient, in particular for hyperparathyroidism.

On the other hand, the development of multiple organ failure in acute pancreatitis leads to hypocalcemia (6), and several authors suggest considering the content of calcium in serum as a criterion for the severity of acute pancreatitis (7). One of the probable mechanisms of reducing serum calcium may be its accumulation in aci-

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nar cells, which explains the relationship between hypocalcemia and the severity of acute pancreatitis (8).

Another factor that is closely related to calcium and parathyroid hormone is vitamin D. Previously, it has been considered exclusively in terms of skeletal diseases; however, the discovery of receptors for vitamin D in various organs and tissues has indicated a much wider impact on the human body. Today, the role of vitamin D in the genesis of autoimmune, oncological, chronic inflammatory diseases, and etc. is being actively studied, and only isolated studies have found a link between vitamin D and acute pancreatitis (9).

A feature of acute pancreatitis is a high risk of complications with a mortality rate of 5.5%. In severe cases, this figure varies between 40-70% and is accompanied by a large number of clinical manifestations with options of unpredictable course (10). Therefore, accurate methods of diagnosing the nature of the pathomorphological process and the severity of multiorgan disorders are extremely important in order to identify them in a timely manner and provide adequate treatment.

It should be noted that many clinical prognostic markers of adverse course of acute pancreatitis have been proposed to date. In particular, the age of the patient and the timing of the onset of early multiple organ failure significantly affect mortality rate. Obesity is also an important risk factor for severe acute pancreatitis. The level of hematocrit at hospitalization $\geq 47\%$, which does not decrease during the day, is a predictor of necrotic pancreatitis. In addition, an increase in the concentration of C-reactive protein above 150 mg/L during the first 48 hours after admission to the hospital is a marker of severe prognosis of acute pancreatitis (11,12). However, the sensitivity and specificity of these indicators are low (13).

Thus, despite the progress of modern surgical science, the problem of predicting the course of acute pancreatitis and

early diagnosis of its complications remains unresolved and requires further development, finding new diagnostic markers, taking into account various pathogenetic factors of the disease.

This study aimed to determine changes in vitamin D and calcium-phosphorus metabolism in patients with severe acute pancreatitis.

MATERIAL and METHODS

The study was conducted by the staff of the Department of Surgery No: 2 Bogomolets National Medical University. All patients were examined between January and December 2021 and signed informed consent to participate in this study and/or treatment at the clinic. Approval for this study was granted by the Local Commission on Bioethical Expertise and Ethics of Scientific Research at the Bogomolets National Medical University (Decision No: 5/3, Date: 17.12.2020).

Study Design

Seventy-two patients aged 26-83 years were examined and divided into two groups as healthy persons (comparison group) - males and females without gastrointestinal pathology and any other conditions or diseases that could affect the state of calcium-phosphorus metabolism (n= 36) and patients with acute pancreatitis (main group, n= 36). Patients of the comparison group were examined on an outpatient basis. All patients of the main group were hospitalized at the clinic of the Department of Surgery No: 2. According to the etiological factor, acute pancreatitis of alcoholic etiology occurred in 24 (66.7%) patients and biliary pancreatitis in 12 (33.3%) patients. Patients in the two groups did not differ significantly in age and sex (Table 1). In addition, in order to determine the prognostic criteria for the severity of the disease, patients in the main group were divided into two subgroups. The first subgroup included patients with severe acute pancreatitis (n= 18) and the second (n= 18) with mild and moderate course of acute pancreatitis.

Table 1. Demographic and clinical characteristic of the patients

Demographic data	Healthy subjects (n= 36)	Patients with acute pancreatitis (n= 36)	p
Sex			
Male	31 (86.1%)	30 (83.3%)	>0.05
Female	5 (13.9%)	6 (16.7%)	>0.05
Age (year)	51.8 ± 17.8	51.2 ± 18.1	>0.05
Etiological factors:			
Alcoholic		24 (66.7%)	
Biliary		12 (33.3%)	
Severity of acute pancreatitis:			
Mild and moderate		18 (50%)	
Severe		18 (50%)	

Blood sampling in patients of the comparison group was performed on an empty stomach and patients in the main group, it was performed during hospitalization before infusion therapy. Determination of total calcium, albumin, total phosphorus, alkaline phosphatase in serum was performed on a biochemical automatic analyzer A15 (BioSystems, Italy). The level of albumin-adjusted calcium was determined by the following formula: Adjusted calcium (mmol/L) = total Ca (mmol/L) + 0.02 [40 - serum albumin (g/L)]. Determination of vitamin D (25(OH) D) and parathyroid hormone was performed using the electrochemiluminescent method on the Sobas analyzer (Roche Diagnostics, Germany).

Patient Selection

The diagnosis of acute pancreatitis in patients of the main group was established by the presence of two of the following three criteria: clinical (upper abdominal pain), laboratory (serum amylase or lipase level 3 times higher than the maximum normal value), imaging (CT, MRI, Ultrasound) criteria. The study used the classification proposed by the Acute Pancreatitis Classification Working Group and the International Association of Pancreatology/American Pancreatic Association in 2012 (14). The severity of the course was determined using the APACHE II scale (severe course - 8 points or more). The diagnosis of mild acute pancreatitis was established in the absence of reliable signs of pancreatic necrosis based on a typical set of clinical and laboratory data, moderate in the presence of transient multiorgan failure or local/systemic complications without organ failure, and severe in the presence of persistent multiorgan failure.

Exclusion criteria for both groups were any chronic disease affecting calcium-phosphorus metabolism, mental illness, recent surgery, glucocorticoids, calcium or vitamin D for three months before enrollment.

Statistical Analysis

Normality of data distribution was determined by the Shapiro-Wilk test. The difference between the groups was established using Student's t-test for independent samples and Mann-Whitney U test. Differences in sample distribution were assessed

using the χ^2 test criterion. Correlation analysis was performed using Pearson correlation for parametric and Spearman correlation for nonparametric data distribution. The relation between the indicators was determined using ROC analysis. The results were presented as mean values and their standard deviation ($M \pm SD$) in the case of parametric distribution and as median and quartile [Me (Q1; Q3)] in the case of non-parametric data distribution. Differences between indicators were considered significant at $p < 0.05$.

Statistical analysis was performed using Statistica 10, IBM SPSS Advanced Statistics 22.0 and MEDCALC® (open source internet resource, <https://www.medcalc.org/calc/>).

RESULTS

Changes in vitamin D and calcium-phosphorus metabolism in the individuals of both groups are shown in Table 2. Significantly lower levels of albumin, total and albumin-corrected calcium were also reported, but parathyroid hormone levels did not differ significantly and alkaline phosphatase levels were significantly higher.

Decreased albumin levels and increased alkaline phosphatase are known as direct laboratory manifestations of acute pancreatitis. The decrease in serum total calcium in albumin-bound form is also due to a decrease in albumin. Therefore, in patients with acute pancreatitis, it is necessary to determine albumin-corrected calcium, an indicator that takes into account the content of albumin. Because albumin-corrected calcium was normal in patients with pancreatitis, no increase in parathyroid hormone levels was expected, as would be expected with true hypocalcemia.

Significant changes were also found in total calcium and albumin and in the content of albumin-adjusted calcium and other indicators of calcium-phosphorus metabolism (alkaline phosphatase, phosphorus, parathyroid hormone) significantly did not differ (Table 3).

The decrease in total calcium can be considered a criterion for the severity of acute pancreatitis, but a significant decrease in albumin content leads to the retention of albumin-corrected

Table 2. Vitamin D and calcium-phosphorus metabolism indices in healthy subjects and patients with acute pancreatitis

Indices	Range	Healthy subjects (n= 36)	Patients with acute pancreatitis (n= 36)	p
Total calcium, mmol/L	2.15-2.58	2.36 (2.31; 2.43)	2.18 (2.12; 2.34)	<0.0001
Albumin, g/L	35-50	43.2 (40.1; 45.6)	32.6 (27.2; 35.6)	<0.0001
Albumin-adjusted calcium, mmol/L	2.15-2.58	2.37 (2.31; 2.43)	2.34 (2.21; 2.42)	0.35
Alkaline phosphatase, U/L	26-117	76.6 (63.7; 91.0)	127.5 (102.5; 147.8)	<0.0001
Vitamin D, ng/mL	30-50	28.4 (21.8; 32.3)	13.8 (9.03; 21.34)	<0.0001
Parathyroid hormone, pg/mL	15.0-65.0	46.3 (39.5; 55.9)	39.3 (20.6; 56.2)	0.06
Phosphorus, mmol/L	0.81-1.45	1.17 (1.01; 1.27)	1.07 (0.8; 1.4)	0.39

Table 3. Calcium-phosphorus metabolism indices in patients with acute pancreatitis

Indices	Severe acute pancreatitis (n= 18)	Mild and moderate acute pancreatitis (n= 18)	p
Total calcium, mmol/L	2.13 (2.09; 2.18)	2.33 (2.17; 2.41)	<0.001
Albumin, g/L	29.9 (25.8; 31.5)	34.2 (32.9; 37.2)	<0.05
Albumin-adjusted calcium, mmol/L	2.31 (2.19; 2.38)	2.39 (2.32; 2.44)	0.09
Alkaline phosphatase, U/L	131.9 (101.2; 157.6)	119.1 (103.4; 133.9)	0.23
Vitamin D, ng/mL	9.17 (7.38; 12.10)	20.4 (15.1; 24.1)	<0.0001
Parathyroid hormone, pg/mL	41.7 (33.2; 46.9)	24.1 (17.6; 73.1)	0.86
Phosphorus, mmol/L	1.11 (0.9; 1.34)	0.95 (0.72; 1.46)	0.49

calcium within normative values, and therefore, patients do not develop the clinic of hypocalcemia and do not increase the content of parathyroid hormone. This should be taken into account when deciding on additional calcium intake in such patients.

Significantly lower levels of vitamin D in patients with severe acute pancreatitis are currently unclear. The possible connection with both calcium metabolism and the inflammatory process is discussed.

The content of total calcium (Figure 1) and vitamin D (Figure 2) was also analyzed according to the severity of acute pancreatitis according to the APACHE II scale.

The study found that a decrease in total calcium levels is associated with an increase in the severity of acute pancreatitis, but the relation between disease severity and vitamin D content is closer. No association was found between calcium and vitamin D, indicating the independence of these predictors.

In our opinion, the decrease in the level of total calcium in serum may be due to both a decrease in the level of albumin and the impregnation of calcium with damaged cells and is not associated with changes in phosphorus-calcium metabolism.

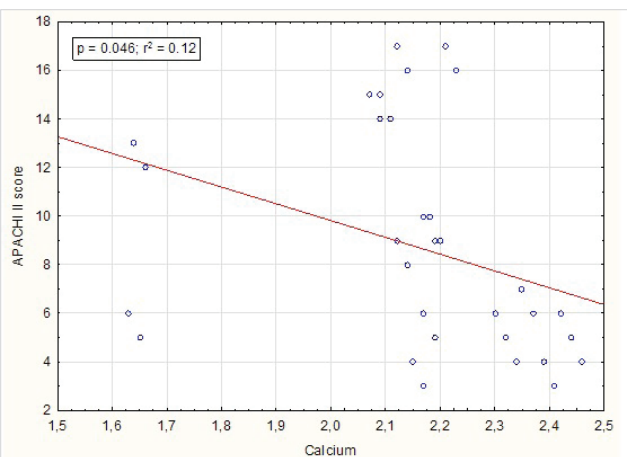


Figure 1. The association between total calcium levels and the severity of acute pancreatitis.

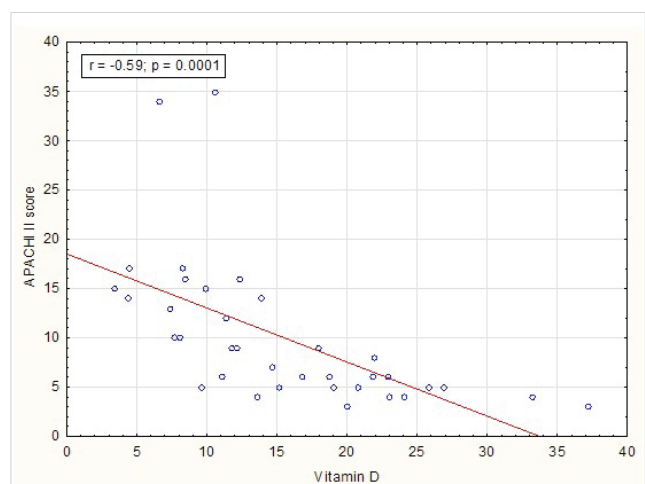


Figure 2. The association between vitamin D levels and the severity of acute pancreatitis.

That is why a link was not found between calcium and vitamin D and parathyroid hormone. The decrease in calcium and vitamin D in these patients depends on the severity of acute pancreatitis.

The next step in the study was to set a threshold value for serum vitamin D that can be used as a criterion for predicting the severity of acute pancreatitis. For this purpose, ROC analysis was used (Figure 3).

According to the results of the analysis, the area under the ROC curve (AUROC) was 0.907 (95% CI= 0.807-0.99; p= 0.001), the cut-off point corresponded to 13.28 ng/mL, Youden index was 0.667, that is for patients with acute pancreatitis, serum vitamin D level ≤13.28 ng/mL can be considered as a significant predictor of severe course (sensitivity 83.3%, specificity 94.4%).

Currently, there are different classifications of vitamin D conditions in the world with different points of difference. Different recommendations define the proposed cut-off points, and no consensus has been reached so far. Therefore, we defined our own cut-off point as a criterion for the severe course of acute pancreatitis.

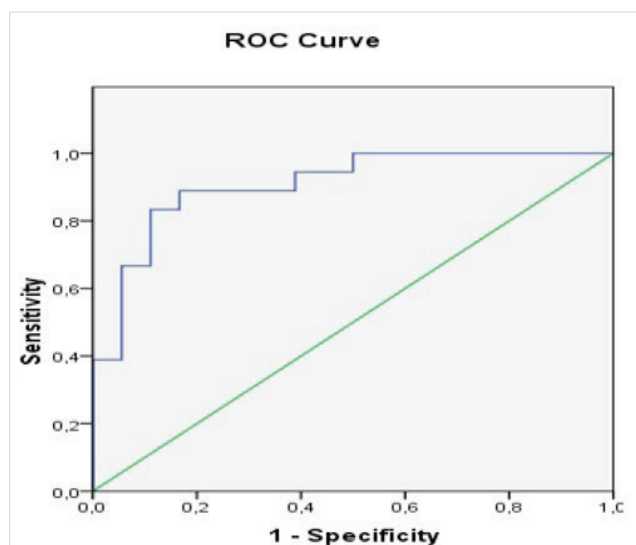


Figure 3. ROC curve for vitamin D levels in predicting the severity of acute pancreatitis.

DISCUSSION

In recent years, the place of the universal trigger in the occurrence of acute pancreatitis is given to the growth in the cytoplasm of the acinar cell content of free calcium ions and, consequently, changes in the nature of the calcium signal at its apical pole (15). To date, it is believed that the physiological stimulus causes a series of calcium oscillations. For many cell types, including acinar cells, an increase in calcium concentration is a trigger for exocytosis of proteins and enzymes (16). It is proven that most of the etiological factors that cause acute pancreatitis (alcohol, hypoxia, hypercalcemia, hyperlipidemia, some pharmacological drugs) also contribute to abnormally high fluctuations in calcium levels (17). Therefore, we can assume that in acute pancreatitis, calcium is a common trigger for various etiological factors that trigger the pathological process.

However, according to the literature, a decrease in serum calcium below 1.97 mmol/L is a criterion for multiorgan failure as a complication of acute pancreatitis (sensitivity 89.7%, specificity 74.8%) (18).

In our study, hypocalcemia was significantly more common in people with severe acute pancreatitis, and a decrease in calcium levels was associated with an increase in APACHE II scores, so hypocalcemia should be considered a predictor of severe disease.

Although new data, which have recently been published, suggest a possible link between vitamin D deficiency and an increased risk of acute and chronic disease, only a few studies with acute pancreatitis have been found, which do not contain reliable evidence of such a connection (19). According to the results of our study, it was found that the content of vitamin

D was lower in patients with acute pancreatitis. It is unclear whether low level vitamin D is a predictor or consequence of acute pancreatitis. On the one hand, pancreatic cells have the ability to extrarenal synthesis of 1.25 (OH) 2D regulated by CYP27B1 and in the case of an aggressive inflammatory process, can affect the level of vitamin D, on the other hand, the most common causes of acute pancreatitis (alcoholism and gallstone disease) can be caused by vitamin D deficiency due to lifestyle changes and reduced insolation and eating disorders.

The treatment of patients with acute pancreatitis remains a difficult task due to the variety of clinical manifestations, the difficulty of timely early diagnosis of complications and prediction of the course of the disease. Thus, patients with acute pancreatitis of mild and moderate severity are hospitalized in the surgical department, while patients with severe disease require comprehensive treatment in the intensive care unit. Timely assessment of the severity of acute pancreatitis in patients with hospitalization is an important component of diagnosis and comprehensive treatment of this pathology, so the use of scales and markers that predict the severity of the disease and determine the degree of inflammation significantly affects the end results of comprehensive treatment. Thus, in order to predict the course of acute pancreatitis, many systems for assessing the severity of patients have been developed and implemented in clinical practice, among which the most common are the Ranson scale and APACHE II. It should be noted that the sensitivity and specificity of these scales in predicting the development of severe acute pancreatitis is 88.6% and 70.4% and 91.4% and 92.6%, respectively (20). In addition, such scales are quite complex and cumbersome for use in clinical practice.

These changes in vitamin D in the serum of patients with acute pancreatitis necessitate their monitoring, starting from the stage of hospitalization in order to early predict the severity of the disease and prevent its fatal complications. The sensitivity and specificity of the serum vitamin D content ≤ 13.28 ng/mL as a predictor of severe acute pancreatitis was 83.3% and 94.4% respectively.

Like any other study, our study also has limitations. It should be noted that there are many factors that determine the severity of acute pancreatitis. In this study, we did not analyze other factors, but found only a correlation between the level of vitamin D in the serum of patients and the severity of acute pancreatitis, which can be considered as a relation rather than a causal relation/prediction, especially since the sample size is small to test any hypothesis. Therefore, we agree that a much more detailed analysis is needed to test the hypothesis that "vitamin D is a predictor of severe acute pancreatitis." It was a one-center study with its own limitations.

CONCLUSION

Serum calcium value was lower in patients with severe acute pancreatitis when compared to healthy persons: 2.18 (2.12; 2.34) vs 2.36 (2.31; 2.43) mmol/L ($p < 0.0001$), and the decrease of calcium levels was associated with an increase in the severity of acute pancreatitis, therefore, hypocalcemia can be considered a reliable predictor of the severity of the disease. In patients with acute pancreatitis, the level of vitamin D was significantly low compared to that of healthy persons and was 13.8 (9.03; 21.34) and 28.4 (21.8; 32.3) ng/mL respectively ($p < 0.0001$). For patients with acute pancreatitis, serum vitamin D levels ≤ 13.28 ng/mL can be considered a significant predictor of severe disease (sensitivity 83.3%, specificity 94.4%) regardless of calcium level.

Ethics Committee Approval: Approval for this study was granted by the Local Commission on Bioethical Expertise and Ethics of Scientific Research at the Bogomolets National Medical University (Decision No: 5/3, Date: 17.12.2020).

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Conflict of Interest: The authors have no conflicts of interest to declare.

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**ORJİNAL ÇALIŞMA-ÖZET**

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Ağır akut pankreatitli hastalarda D vitamini ve kalsiyum- fosfor matebolizmasındaki değişiklikler

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ÖZET

Giriş ve Amaç: Akut pankreatitin seyrini tahmin etme ve komplikasyonlarının erken teşhisi sorunları çözülmemiştir. Bu çalışma, şiddetli akut pankreatitli hastalarda D vitamini ve kalsiyum-fosfor metabolizmasındaki değişiklikleri belirlemeyi amaçladı.

Gereç ve Yöntem: İki gruba ayrılan 72 kişi incelendi: Birinci grup (karşılaştırma grubu) gastrointestinal sistem patolojisi olmayan ve kalsiyum-fosfor metabolizmasının durumunu etkileyebilecek diğer koşullar veya hastalıkları olmayan erkek ve kadınlardan (n= 36) ve ikinci grup (ana grup) akut pakreatitli hastalardan oluştu (n= 36). Ayrıca hastalığın şiddetine yönelik prognostik kriterlerin belirlenmesi amacıyla ana gruptaki hastalar iki alt gruba ayrıldı. Birinci alt grup, şiddetli hastalığı olan (n= 18), ikinci hafif ve orta hastalığı olan hastalar içerdi.

Bulgular: Şiddetli akut pankreatitli hastalarda sağlıklı kişilerle karşılaştırıldığında serum kalsiyum değeri daha düşüktü: 2,18 (2,12; 2,34) ve 2,36 (2,31; 2,43) mmol/L ($p < 0,0001$) ve kalsiyum düzeylerindeki azalma, akut pankreatitin şiddetinin artması ile ilişkiliydi. Bu nedenle hipokalsemi, hastalığın şiddetinin güvenilir bir göstergesi olarak kabul edilebilir. Akut pankreatitli hastalarda D vitamini düzeyi sağlıklı kişilere göre anlamlı derecede düşüktü ve sırasıyla 13,8 (9,03; 21,34) ve 28,4 (21,8; 32,3) ng/mL idi ($p < 0,0001$).

Sonuç: Akut pankreatitli hastalarda serum D vitamini düzeyleri $\leq 13,28$ ng/mL, kalsiyum düzeyinden bağımsız olarak ciddi hastalığın (duyarlılık 83,3%, özgüllük 94,4%) anlamlı bir öngördürücüsü olarak kabul edilebilir.

Anahtar Kelimeler: Akut pankreatit, D vitamini, kalsiyum fosfor metabolizması

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