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# The Change in Vitamin D Levels during the Early Days of Intensive Care Unit Admission and Factors Affecting Change

## Yoğun Bakıma Yatışın İlk Günlerinde D Vitamini Düzeylerindeki Değişim ve Değişimi Etkileyen Faktörler

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## Abstract

In the last decade, vitamin D has an increasing importance due to its demonstrated pleotropic, antimicrobial, and immunomodulatory effects, and has been a subject of interest especially in critically ill patients due to its role on immune functions. The aim of this study is to determine whether vitamin D levels change during the first days of intensive care unit (ICU) admission and to study factors that may affect these variations. Patients admitted to the ICU between March 2014 and December 2014 were included in the study. Blood samples were obtained on the admission day, and the following three days and 25-hydroxyvitamin D levels were measured by high pressure liquid chromatography (HPLC) technique. Changes over time were analyzed with variance analysis if they were normally distributed, otherwise by Friedman tests. A total of 31 patients were enrolled. Of those, 17 (54.8%) patients were women. On admission median vitamin D level for all was 8.52 (5-37) ng/mL. On the third day median vitamin D level for all was 8.85 (5-35.1) ng/mL. There was no statistically significant difference between vitamin D levels which were measured on the consequent 3 days (p=0.553). There was no significant correlation between daily vitamin D levels and changes in levels of CRP, procalcitonin, and albumin (p=0.061, p=0.61, p=0.179 respectively) or daily fluid balance (first day p=0.31, second day p=0.65, third day p=0.13). Our presented data revealed that repeated measurements of early vitamin D levels on consequent days did not have significant differences and CRP, procalcitonin, albumin levels, and fluid balance parameters do not seem to affect early serum vitamin D levels in critically ill patients.

Keywords: Vitamin D, Inflammation, Critically ill patient, Fluid status.

## Özet

D vitamini son on yılda gösterilmiş pleotropik, antimikrobial ve immünmodülatör etkileri sebebi ile giderek artan bir öneme sahip olup kritik hastalarda özellikle immün fonksiyonlar üzerindeki rolü sebebi ile de ilgi konusu olmuştur. Bu çalışmanın amacı; yoğun bakım ünitesine (YBÜ) kabulün ilk günlerinde D vitamini düzeylerinin değişip değişmediğini belirlemek ve bu değişiklikleri etkileyebilecek faktörleri araştırmaktır. Çalışmaya Mart 2014 ile Aralık 2014 tarihleri arasında yoğun bakım ünitesine alınan hastalar dahil edildi. Hastaların yatış gününde ve takip eden üç gün içinde kan örnekleri alındı ve HPLC (yüksek basınç sıvı kromatografisi) yöntemi ile 25-hidroksivitamin D düzeyleri ölçüldü. Zamana göre değişimler, dağılım normal

ise varyans analizi ile, dağılım normal değilse Friedman testi ile araştırıldı. Toplamda 31 hasta çalışmaya dahil edilmiştir. Bu hastaların 17'si (%54.8) kadındır. Hastaların yoğun bakıma alınması esnasında ortanca vitamin D düzeyi 8.52 (5-37) ng/mL idi. Üçüncü günde ise ortanca D vitamini düzeyi 8.85 (5-35.1) ng/mL olarak saptandı. Hastaların D vitamini düzeylerinde birbirini takip eden üç gün arasında anlamlı bir değişiklik saptanmadı (p=0.553). D vitamininin günlük düzeyleri ile CRP, prokalsitonin ve albümin düzeylerindeki (sırası ile p=0.061, p=0.61, p=0.179) veya sıvı balansındaki (birinci gün p=0.31, ikinci gün p=0.65, üçüncü gün p=0.13) değişimler arasında anlamlı bir korelasyon görülmedi. Çalışma verilerimiz yoğun bakıma yatışın ilk günlerinde tekrarlayan ölçümlerde D vitamini düzeyinin anlamlı bir değişiklik göstermediğini ve kritik hastalığı olan bireylerde CRP, prokalsitonin, albümin ve sıvı balansı parametrelerinin D vitamini düzeylerini etkilemediğini göstermektedir.

Anahtar Kelimeler: Vitamin D, İnflamasyon, Yoğun bakım hastası, Sıvı durumu.

## Introduction

Vitamin D has an increasing importance in the last decades due to its pleotropic, antimicrobial, cardio protective and immunomodulatory effects, which have been shown outside the musculoskeletal system [1]. In addition to controlling calcium metabolism, it is involved in cell growth, proliferation, apoptosis, and immune system regulation [2]. In recent years, in line with retrospective information, it has been reported that there may be a relationship between low vitamin D levels and some types of cancer, immune disorders, diabetes, cardiovascular diseases, hypertension, and metabolic syndrome [3-6].

The role of vitamin D in critically ill patients has not yet been clearly elucidated. Epidemiological studies show in septic patients that vitamin D may be a risk factor for mortality [7,8]. The prevalence of vitamin D deficiency in critically ill patients is between 40 and 70% [9,10] Although most guidelines point to a limit below 20 ng/mL for vitamin D deficiency, there is no established cut-off value for critically ill patients [11,12]. It is thought that organ failure, sepsis, fluid deficit and related treatments may cause changes in vitamin D levels in critically ill patients [13].

Many vitamin deficiencies may occur in critically ill patients. The difference of vitamin D levels is that its blood levels that will give information about vitamin D status can be measured routinely. Despite this, it is recommended that the evaluation of vitamin D should be done carefully, considering that it may be affected by acute fluid changes and inflammatory conditions in critically ill patients.

The aim of the study; was to determine whether there was a change in vitamin D levels in the early stages of the critical disease course in patients admitted to the intensive care unit (ICU) of our university hospital, and to evaluate the factors that may affect the change.

## Material and Method

The ethics committee approval of Ankara University Faculty of Medicine Ethics Committee dated 28.04.2014 and numbered 07-304-14 was obtained. Informed consent was obtained from patients or their relatives.

### *Patients*

All patients with APACHE II score >15 who received inpatient treatment in the Medical ICU between March 2014 and December 2014 were included in this study, after obtaining their or their relatives' approval. The study was designed as a prospective study. Patients with an APACHE II score of <15, who received vitamin D replacement therapy during the last month, who had chronic renal failure, who did not accept the study and who were predicted to stay in ICU for <72 hours were not included in the study.

### *Method*

From patients who do not have exclusion criteria and accepted to participate in the study, 3 cc of blood was taken at the time of admission (Day 0), Day 1, Day 2, and Day 3, and centrifuged for 5 minutes, and stored at -20 degrees for 3 months. 25-hydroxyvitamin D [25(OH)D] levels were studied in Ankara University Faculty of Medicine Endocrinology Laboratory by HPLC (high pressure liquid chromatography) method [14]. 25(OH)D levels were evaluated as sufficient (> 30

mg/mL), insufficient (between 20-30 ng/mL), and deficient (<20 ng/mL) [14].

Patients' age, gender, reasons for hospitalization, accompanying diseases, APACHE II score, SOFA score, albumin, calcium, CRP and procalcitonin levels, and hemodialysis status were recorded in the study database. ICU and hospital outcomes were also recorded in this database.

Data analysis was performed using the Statistical Package for Social Science (SPSS) 15 package program. Descriptive statistics were shown as mean ± standard deviation for variables with normal distribution, median (min - max) for variables with non-normal distribution, and as number of cases and (%) for nominal variables. The change with time was evaluated with the Paired t test if the distribution was normal, and the Wilcoxon test if the distribution was not normal. If the change with time was normal, the variance analysis was used in repeated measures, and the Friedman test was investigated if the distribution was not normal. The significance of the difference between the groups in terms of means was investigated with the t test, and the significance of the difference in terms of median values was investigated with the Mann Whitney test. Nominal variables were evaluated using the Pearson chi-square test. When investigating the relationship between continuous variables, if the distribution was not normal, the Spearman correlation test was used, and if it was normal, the Pearson correlation test was used. Statistical significance was accepted as $p < 0.05$.

## Results

A total of 83 patients were admitted to the medical ICU between March 2014 and December 2014. Because 19 of them had renal failure and 24 of them were using vitamin D, they were not included in the study. Nine patients were excluded because they had APACHE II scores <15. A total of 31 patients participated in the study. Seventeen (54.8%) of these patients were female and 14 (45.2%) were male. Average age was 70.1±13.8 years. The average age of female patients was 71.7±16.9 years and the average age in the male patient group is 68.1±8.1 years. The APACHE II score of patients admitted to ICU was 22.5±7.2 on average, it was 22.4±6.8 in female patients and 22.1±8.9 in male patients (Table 1).

When 25(OH)D vitamin levels during admission to ICU were evaluated 3 patients had sufficient levels, 4 patients had insufficiency, and 24 patients had vitamin D deficiency. Of the patients included in the study, 16 were admitted to ICU with sepsis, 11 with acute respiratory failure due to pneumonia, 2 with acute respiratory failure due to massive pulmonary thromboembolism, 1 with acute respiratory distress syndrome and 1 to acute pulmonary edema.

Median 25(OH)D levels on admission to ICU were 8.52 ng/mL (5-37 ng/mL), 9.2 ng/mL (5-37 ng/mL) in women, and 7.4 ng/mL in men (5-34 ng/mL). No significant difference was found between the vitamin D levels of male and female patients on admission to ICU ($p=0.27$). On the 3rd day, the median vitamin D level was 8.85 ng/mL (5-35.1 ng/mL), 8.8 ng/mL (5-35.1 ng/mL) in women, and 7.1 ng/mL in men (5.4-34.8 ng/mL). No statistically significant difference was present between vitamin D levels of the patients across days ($p=0.553$) (Table 2).

Mean albumin level on ICU admission was 2.53±0.58 g/dL, and it was 2.46±0.45 g/dL on the 3rd day. There was no statistically significant difference between albumin levels on the admission and 3rd days ($p=0.197$). There was no significant correlation between the percentage changes in albumin and the percentage changes between daily vitamin D levels on admission and 3rd days ($r=-0.252$; $p=0.179$) (Table 3 and 4).

The median CRP levels of the patients at the time of ICU admission was 101 mg/dL (3-459 mg/dL) and it was 58 mg/dL (1-248 mg/dL) on 3rd day, with a significant decrease ($p=0.007$). There was no significant correlation between the percentage changes in vitamin D levels and the percentage changes in CRP ($r=0.352$; $p=0.061$) (Table 3 and 4).

Procalcitonin levels on admission to ICU had a median of 1.2 ng/dL (0.03-100 ng/dL), and a median of 0.375 ng/dL (0-50 ng/dL) on the 3rd day. There was a significant decrease in procalcitonin levels between admission and 3rd days ($p=0.01$). When the percentage change in

procalcitonin levels and the percentage changes in vitamin D levels were compared, no significant correlation was found (r=-0.096; p=0.61) (Table 3 and 4). There was no significant difference or correlation in the vitamin D levels of patients who received diuretic or inotropic therapy (p=0.49 and p= 0.58 respectively). It was observed that the need for hemodialysis and the changes in the daily vitamin D levels of the patients did not correlate (p=0.56).

The fluid intake and output of the patients were recorded, including the day of hospitalization. All patients had a positive fluid balance. The median intake minus output in the first 24 hours was 890 cc (5-4,500), the following days it was 1620 cc (20-3,300), 720 cc (50-2,800) and 1010 (20-1,800), respectively. It was revealed that the daily fluid balance of the patients did not correlate with the daily vitamin D levels (Table 5).

**Table 1.** Patient characteristics.

| All patients (n=31) | | | | | |
|---|---|---|---|---|---|
| Age, years (mean ± SD) | | 70.1±13.8 | CRP (mg/dL) Median (min, max) | On admission | 1.2 (0.03-100) |
| APACHE II (mean ± SD) | | 22.5±7.2 | | 3rd Day | 0.4 (0-50) |
| SOFA on admission (mean ± SD) | | 11.9±3.05 | Procalcitonin (ng/dL) Median (min, max) | On admission | 1.2 (0.03-100) |
| SOFA on 3rd day (mean ± SD) | | 12.8±5.2 | | 3rd Day | 0.4 (0-50) |
| 25 (OH) D, ng/mL Median (min, max) | On admission | 8.52 (5-37) | Diuretic therapy, n (%) | | 6 (%19.3) |
| | 1st Day | 8.95 (5.1-39) | Inotropic therapy, n (%) | | 15 (%48) |
| | 2nd Day | 8.45 (5-41) | Fluid Balance (cc) (median (min, max)) | On admission | 890 (50-4,500) |
| | 3rd Day | 8.85 (5-42) | | 1st Day | 1620 (20-3,300) |
| Albumin, g/dL (mean ± SD) | | | | 2nd Day | 720 (50-2,800) |
| | On admission | 2.57 ± 0.58 | | 3rd Day | 1010 (20-1,800) |
| | 3rd Day | 2.46 ± 0.45 | Hemodialysis, n (%) | | 5 (%16) |
| Ca (corrected), mg/dL (mean ±SD) | | 8.9±1.12 | | | |

**Table 2.** Vitamin D levels across days.

| 25(OH)D | Minimum | Maximum | Median | Interquartile ratio (25-75) | P value |
|---|---|---|---|---|---|
| On Admission | 5.0 | 37 | 8.52 | (5.8-24) | 0.553* |
| 1st Day | 5.1 | 39 | 8.95 | (5.65-22.2) | |
| 2nd Day | 5 | 41 | 8.45 | (5.45-20.85) | |
| 3rd Day | 5 | 42 | 8.85 | (5.48-20.2) | |

* Analyzed using the Friedman test.

**Table 3.** CRP, procalcitonin and albumin changes.

| | On Admission | 3rd day | P value |
|---|---|---|---|
| Albumin, g/dL | 2.57 ±0.58 | 2.46±0.45 | 0.197 |
| CRP, mg/dL | 101 (3-459) | 58 (1-248) | 0.007 |
| Procalcitonin, ng/mL | 1.2 (0.03-100) | 0.375 (0-50) | 0.01 |

**Table 4.** Results of correlation analysis of vitamin D with albumin, CRP and procalcitonin.

| | 25(OH)D | | |
|---|---|---|---|
| Albumin (Admission – 3rd day) | r | P | |
| CRP (Admission – 3rd day) | -0.252 | 0.179 | |
| Procalcitonin (Admission – 3rd day) | 0.352 | 0.061 | |
| Albumin (Admission – 3rd day) | -0.096 | 0.610 | |

**Table 5.** Results of correlation analysis of vitamin D with fluid balance of patients over days.

| | 25(OH)D 1st Day | | 25(OH)D 2nd Day | | 25(OH)D 3rd Day | |
|---|---|---|---|---|---|---|
| Fluid balance | r | p | r | p | R | p |
| 1st Day | -0.138 | 0.32 | -2.64 | 0.264 | -0.042 | 0.268 |
| 2nd Day | | | -0.150 | 0.65 | -0.121 | 0.62 |
| 3rd Day | | | | | -0.134 | 0.13 |

## Discussion

In this study, critically ill patients were evaluated for changes in vitamin D levels in the early days of critical illness and whether hyperinflammation or fluid balance had an effect on vitamin D levels. However, in our study group, vitamin D levels were found to be stable over the first three days. The findings of our study oppose the opinion that hyperinflammation or daily fluid balance may affect vitamin D levels in critically ill patients.

When values between 21-29 ng/mL are defined as vitamin D insufficiency and values below 20 ng/mL as vitamin D deficiency; studies have reported a high rate of vitamin D deficiency in critically ill patients [15]. In a study of 130 patients by Arnson et al., 82% of critically ill patients admitted to ICU had vitamin D deficiency and their average vitamin D level was 14.04±6.9 ng/mL [16]. In addition, it has been demonstrated that vitamin D levels decrease over time because of insufficient intake or replacement, lack of ultraviolet B (UVB) rays, malnutrition, decreased 1-alpha hydroxylation, and increase in the conversion of 25 hydroxyvitamin D to 1.25 dihydroxyvitamin D in tissues [16]. Similarly in our study, most critically ill patients had vitamin D deficiency on admission. However, in our study, it was aimed to monitor early vitamin D levels, so information on the long-term progress of vitamin D levels was lacking. Vitamin D deficiency in critically ill patients was described approximately twenty years ago in hypocalcemic patients. In recent studies, vitamin D deficiency has also been documented in normocalcemic critically ill patients, and it has been shown that vitamin D deficiency may have consequences other than hypocalcemia in critically ill patients [15,17]. In our study, none of the patients were hypocalcemic on admission.

Zella et al. hypothesized that changes in serum levels of albumin and VDBP (Vitamin D Binding Protein) may affect vitamin D levels, but they could not reveal a significant difference, similar to our findings [18]. In a study conducted by Krishnan et al., it was revealed that there was a rapid change in albumin levels due to the hemodilution effect in the early period and that vitamin D levels decreased transiently but significantly and simultaneously [13]. It was stated that this effect might be related to the decrease in VDBP levels due to dilution. In a study conducted by Bikle et al., it was revealed that VDBP concentrations were higher than 25(OH)D levels, and VDBPs have a very high affinity for vitamin D metabolites, therefore decreasing VDBP levels may decrease total dihydroxyvitamin D levels but not affect free levels [19]. In our study, no significant difference was found between the changes in the percentage of albumin and vitamin D between the admission and 3rd days of the patients. If it is considered that intensive fluid support is given in patients admitted to ICUs, vitamin D levels would be expected to decrease due to dilution. As a matter of fact, vitamin D levels measured immediately after acute fluid loading during the operation were found to be significantly low in patients who underwent coronary bypass on 19 patients [13]. However, in this study, hemodilution had caused a decrease in the level of vitamin D measured at the fifth minute of acute fluid loading. However, in the twenty-fourth hour measurements, it was observed that the vitamin D level did not change significantly compared to basal. Both our study and this study revealed that the effect of hemodilution is not lasting over days.

The recommended daily dose of vitamin D (cholecalciferol) to protect bones is 600 IU for ages 19-70, 800 IU for ages 71 and older [20] and

higher daily intake in the elderly and people at other risk for vitamin D deficiency, and 200 IU/day should be supplemented in patients receiving TPN [21]. In our study, all of our patients received enteral nutritional support after their clinical condition was stabilized, and patients with a daily calorie requirement of 1,500 kcal received 432 IU vitamin D through commercially available enteral products. In a study involving 22 patients by Van den Berghe et al., 500 IU of vitamin D was administered daily, and vitamin D levels remained low despite this supplementation [22]. Based on this information, higher doses of vitamin D deficiency treatment may suggested in critically ill patients. In the ESPEN (European Society for Clinical Nutrition and Metabolism) guideline published on nutrition of patients in ICUs, the recommendation grade is 0, but a single dose of 500,000 IU vitamin D replacement is recommended for patients with a vitamin D level below 12.5 ng/mL one week after admission to ICU [23]. A meta-analysis of 18 randomized controlled trials involving noncritical patient groups demonstrated that adequate vitamin D supplementation led to a 7% reduction in mortality [24].

There is no common opinion about vitamin D supplementation in critically ill patients. One of the reasons for this, and most importantly, is that the threshold value for the definition of vitamin D deficiency in critically ill patients is yet to be determined. Secondly, there are suggestions that a single measurement may not be enough because fluctuations that can be seen in critically ill patients and hence, it has been argued that repeated measurements are more valuable in determining the vitamin D level [13]. In our study, it was observed that there was no statistically significant difference between the vitamin D levels in the repeated measurements of the patients on the day they were admitted to the ICU and during the following three days.

Krishnan et al. evaluated whether acute fluid changes affected vitamin D levels or not [13]. In their study, they hypothesized that critically ill patients are mostly hypotensive and hypovolemic, and large volumes of fluid that are loaded after admission to ICU may lower vitamin D levels due to hemodilution effect. They studied cardiopulmonary bypass surgery patients, since a standard fluid loading is performed during the procedure and this group would be an ideal group to demonstrate the effect of acute fluid loading on vitamin D levels. The patients' vitamin D levels were measured five minutes before surgery, five minutes after the start of the surgery (immediately after a large volume of fluid was loaded), on returning to ICU after the operation, 24 hours and five days after the operation. A significant decrease with 35% was detected in vitamin D levels taken after fluid loading during the operation. Although the fluid balance of the patients stabilized on the fifth postoperative day, vitamin D levels were not significantly different between admission levels, 24$^{th}$ hour and postoperative fifth day. In our study, it was observed that all of the patients had a positive balance during the first 3 days of their stay. No significant difference was found in vitamin D levels across days when compared with the time of admission. The results of these two studies support the finding that vitamin D levels are relatively stable over time unless there is immediate fluid loading.

In our study, it was also studied whether acute changes in inflammation markers were related to changes in vitamin D levels in the early period of critical illness. Inflammation was monitored with CRP and procalcitonin levels. A significant decrease in CRP and procalcitonin levels was observed during the three days. However, there was no significant correlation between vitamin D changes and CRP and procalcitonin changes.

One of the limitations of our study is that it was conducted in a small number of patient groups. Another limitation of the study is that it is a single center study. In addition, since there was no long-term patient follow-up in the study, no data could be presented on the long-term fluctuations related to vitamin D levels.

## Conclusion

In conclusion, the results of repetitive measurements of vitamin D levels in this study suggest that vitamin D levels do not fluctuate significantly over time and are not affected by inflammation severity, albumin-protein levels,

and daily fluid balance. As well, considering the extent of vitamin D deficiency, it is clear that early replacement therapy may be required in patients admitted to ICUs.

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## References

**1.** Bikle D. Nonclassic actions of vitamin D. J Clin Endocrinol Metab 2009; 94(1): 26-34. [Crossref] [PubMed]

**2.** Gil Á, Plaza-Diaz J, Mesa MD. Vitamin D: Classic and Novel Actions. Ann Nutr Metab 2018; 72(2): 87-95. [Crossref] [PubMed]

**3.** Dou R, Ng K, Giovannucci EL, Manson JE, Qian ZR, Ogino S. Vitamin D and colorectal cancer: molecular, epidemiological and clinical evidence. Br J Nutr 2016; 115(9): 1643-60. [Crossref] [PubMed]

**4.** Nitsa A, Toutouza M, Machairas N, Mariolis A, Philippou A, Koutsilieris M. Vitamin D in Cardiovascular Disease. In Vivo 2018; 32(5): 977-81. [Crossref] [PubMed]

**5.** Latic N, Erben RG. Vitamin D and Cardiovascular Disease, with Emphasis on Hypertension, Atherosclerosis, and Heart Failure. Int J Mol Sci 2020; 21(18): 6483. [Crossref] [PubMed]

**6.** Sacerdote A, Dave P, Lokshin V, Bahtiyar G. Type 2 Diabetes Mellitus, Insulin Resistance, and Vitamin D. Curr Diab Rep 2019; 19(10): 101. [Crossref] [PubMed]

**7.** Rech MA, Hunsaker T, Rodriguez J. Deficiency in 25-hydroxyvitamin D and 30-day mortality in patients with severe sepsis and septic shock. Am J Crit Care 2014; 23(5): e72-9. [Crossref] [PubMed]

**8.** Amrein K, Papinutti A, Mathew E, Vila G, Parekh D. Vitamin D and critical illness: what endocrinology can learn from intensive care and vice versa. Endocr Connect 2018; 7(12): R304-R315. [Crossref] [PubMed]

**9.** Braun A, Chang D, Mahadevappa K, Gibbons FK, Liu Y, Giovannucci E, et al. Association of low serum 25-hydroxyvitamin D levels and mortality in the critically ill. Crit Care Med 2011; 39(4): 671-7. [Crossref] [PubMed]

**10.** Zajic P, Amrein K. Vitamin D deficiency in the ICU: a systematic review. Minerva Endocrinol 2014; 39(4): 275-87. [PubMed]

**11.** Lips P, Cashman KD, Lamberg-Allardt C, Bischoff-Ferrari HA, Obermayer-Pietsch B, Bianchi ML, et al. Current vitamin D status in European and Middle East countries and strategies to prevent vitamin D deficiency: a position statement of the European Calcified Tissue Society. Eur J Endocrinol 2019; 180(4): P23-P54. [Crossref] [PubMed]

**12.** Amrein K, Scherkl M, Hoffmann M, Neuwersch-Sommeregger S, Köstenberger M, Tmava Berisha A, et al. Vitamin D deficiency 2.0: an update on the current status worldwide. Eur J Clin Nutr 2020; 74(11): 1498-513. [Crossref] [PubMed]

**13.** Krishnan A, Ochola J, Mundy J, Jones M, Kruger P, Duncan E, et al. Acute fluid shifts influence the assessment of serum vitamin D status in critically ill patients. Crit Care 2010; 14(6): R216. [Crossref] [PubMed]

**14.** Keyfi F, Nahid S, Mokhtariye A, Nayerabadi S, Alaei A, Varasteh A-R. Evaluation of 25-OH vitamin D by high performance liquid chromatography: validation and comparison with electrochemiluminescence. Journal of Analytical Science and Technology 2018; 9(1): 1-6. [Crossref]

**15.** Lucidarme O, Messai E, Mazzoni T, Arcade M, du Cheyron D. Incidence and risk factors of vitamin D deficiency in critically ill patients: results from a prospective observational study. Intensive Care Med 2010; 36(9): 1609-11. [Crossref] [PubMed]

**16.** Arnson Y, Gringauz I, Itzhaky D, Amital H. Vitamin D deficiency is associated with poor outcomes and increased mortality in severely ill patients. QJM 2012; 105(7): 633-9. [Crossref] [PubMed]

**17.** Mata-Granados JM, Vargas-Vasserot J, Ferreiro-Vera C, Luque de Castro MD, Pavón RG, Quesada Gómez JM. Evaluation of vitamin D endocrine system (VDES) status and response to treatment of patients in intensive care units (ICUs) using an on-line SPE-LC-MS/MS method. J Steroid Biochem Mol Biol 2010; 121(1-2): 452-5. [Crossref] [PubMed]

**18.** Zella LA, Shevde NK, Hollis BW, Cooke NE, Pike JW. Vitamin D-binding protein influences total circulating levels of 1,25-dihydroxyvitamin D3 but does not directly modulate the bioactive levels of the hormone in vivo. Endocrinology 2008; 149(7): 3656-67. [Crossref] [PubMed]

**19.** Bikle DD, Halloran BP, Gee E, Ryzen E, Haddad JG. Free 25-hydroxyvitamin D levels are normal in subjects with liver disease and reduced total 25-hydroxyvitamin D levels. J Clin Invest 1986; 78(3): 748-52. [Crossref] [PubMed]

**20.** Ross AC, Manson JE, Abrams SA, Aloia JF, Brannon PM, Clinton SK, et al. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. J Clin Endocrinol Metab 2011; 96(1): 53-8. [Crossref] [PubMed]

**21.** Kelly DG. Guidelines and available products for parenteral vitamins and trace elements. JPEN J Parenter Enteral Nutr 2002; 26(5 Suppl): S34-6. [Crossref] [PubMed]

**22.** Van den Berghe G, Van Roosbroeck D, Vanhove P, Wouters PJ, De Pourcq L, Bouillon R. Bone turnover in prolonged critical illness: effect of vitamin D. J Clin Endocrinol Metab 2003; 88(10): 4623-32. [Crossref] [PubMed]

**23.** Singer P, Blaser AR, Berger MM, Alhazzani W, Calder PC, Casaer MP, et al. ESPEN guideline on clinical nutrition in the intensive care unit. Clin Nutr 2019; 38(1): 48-79. [Crossref] [PubMed]

**24.** Autier P, Gandini S. Vitamin D supplementation and total mortality: a meta-analysis of randomized controlled trials. Arch Intern Med 2007; 167(16): 1730-7. [Crossref] [PubMed]