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Changes in Phosphorus, Calcium and Alkaline Phosphatase Levels in Patients with End-stage Renal Disease on Long-term Hemodialysis Therapy

Kovacevic Ajla[1], Hatkic Alen[2*], Softic Adaleta[2], Smajlovic Aida[2], Dautovic Esmeralda[2], Halilcevic Dalila[2], Šaric Asja[2] and Srabovic Nahida[2]

[1]*Faculty of Medicine, Health Studies Department, University of Tuzla, Tuzla, Bosnia and Herzegovina.*

[2]*Faculty of Pharmacy, Biochemistry Department, University of Tuzla, Tuzla, Bosnia and Herzegovina.*

Corresponding Author:** ***Alen Hatkic, *Faculty of Pharmacy, Biochemistry Department, University of Tuzla, Tuzla, Bosnia and Herzegovina.* ***Email:*** *alen.hatkic@untz.ba*

Abstract

Vascular calcification is one of the most common comorbidities in patients with end-stage renal disease (ESRD). Hyperphosphatasia and increased expression of alkaline phosphatase (ALP) are among the significant risk factors for the development of vascular calcification. The aim of this study is to assess changes in phosphorus, calcium and ALP levels in patients with ESRD on long-term hemodialysis therapy in relation to age, gender and duration of hemodialysis therapy. The research was conducted as a "case control" study in which 40 patients with ESRD on long-term hemodialysis therapy were included. In order to determine the influence of the duration of hemodialysis therapy on the serum levels of calcium (Ca), phosphorus (P) and ALP, the values of the mentioned parameters were determined before the start of hemodialysis treatment and measured during the last routine biochemical treatment of the same patients. Obtained results showed significant increase in concentration of Ca and ALP in relation to long-term hemodialysis therapy. However, the results of Spearman's correlation test showed that only activity of ALP significantly correlates with the duration of hemodialysis therapy. Furthermore, the mutual correlation of Ca, P and ALP levels was tested, as well as the correlation of the investigated parameters with the age and the gender of the patients, and no statistically significant correlation was found. Our research may certainly contribute to a better understanding of the complex changes in bone mineral metabolism of ESRD patients on long-term hemodialysis.

Keywords: *Alkaline phosphatase; Calcium; End-stage renal disease, Hemodialysis, Phosphorus.*

1. INTRODUCTION

End-stage renal disease (ESRD) is the most advanced stage of chronic kidney disease and requires renal replacement therapy, such as hemodialysis (HD) which is the most frequently used dialysis modality performed in more than 80 % patients with ESRD [1,2]. Numerous studies have attempted to identify risk factors for mortality and morbidity in this population. Most have shown important relations among demographic factors (e.g. older age, male gender, white race) and mortality, as well as comorbid conditions (e.g. diabetes, cardiovascular disease) with mortality and morbidity [3-5]. The most common death causing comorbid condition in patients with chronic kidney disease (CKD) is cardiovascular disease (CV), and extraosseous vascular calcification as a proven risk factor [6,7]. The risk of vascular calcification corresponds with the deterioration of renal function and is highest in ESRD [8,9]. Vascular calcification is mainly caused by disturbance of mineral metabolism, calcium based therapies, chronic inflammation in a uremic milieu, and the active process of osteogenesis in vascular smooth muscle cells. Among all that factors, hyperphosphatemia promotes vascular calcification accompanied with the expression of alkaline phosphatase (ALP) [10]. For this reason, it is very important to continuously monitor calcium, phosphorus and ALP in patients with ESRD on long-term hemodialysis therapy.

The primary aim of this study is to assess changes in phosphorus, calcium and ALP levels in patients with ESRD on long-term hemodialysis therapy in relation to age, gender and duration of hemodialysis therapy.

2. Materials & Methods

2.1. Data Source

The research was conducted as a "case control" study in which 40 patients with ESRD on long-term hemodialysis therapy in the hemodialysis center of the General Hospital "dr. Mustafa Beganović" Gračanica, Bosnia and Herzegovina, were included. Blood samples and preparation of serum for the determination of calcium, phosphorus and ALP were taken at the last routine biochemical treatment of the patient. Concentrations of calcium, phosphorus and ALP were determined using a Indiko™ Plus biochemical analyzer (Thermo Scientific™). The serum values of calcium, phosphorus and ALP determined before the start of the therapy for each patient included are taken from the medical records and were used as control sample.

The research was previously approved by the Ethics Committee of the General Hospital "Dr. Mustafa Beganović" Gračanica, Bosnia and Herzegovina (01-890-3/21 dated 07/19/2021).

2.2. Statistical Analyses

For statistical data processing, after determining the nature of the distribution of the obtained results with the help of a distribution histogram and checking the stepwise linear distribution test (P-P-plot and graph of detrended values), we used appropriate statistical tests (Wilcoxon's test for a dependent sample, Mann-Whitney test for independent sample and Spearman's correlation test) applying SPSS/WIN program (Release 26.0, SPSS Inc., Chicago, IL, USA.). In all tests, a value of $p \leq 0.05$ was considered statistically significant.

3. Results

Out of a total of 40 patients included, 19 (47.5%) were female, and 21 (52.5%) were male. The average age of the patients was 60.425 ± 2.2153, where the oldest patient was 85 years old, and the youngest was 23 years old. The average duration of hemodialysis therapy expressed in months was 58.975 ± 7.69744 months, with the longest period of treatment being 172 months and the shortest being 9 months.

In order to determine the influence of the duration of hemodialysis therapy on the serum levels of Ca, P and ALP, the values of the mentioned parameters were determined before the start of hemodialysis treatment and measured during the last routine biochemical treatment of the same patients. Results of Wilcoxon's test for a dependent sample showed statistically significant increase in the concentrations of Ca (Z=-3.130; p=0.002) and ALP (Z=-2.448; p=0.014) compared to the start of the therapy (Table 1).

Table 1. *Changes in serum levels of Ca, P and ALP in ESRD patients before start of the hemodialysis therapy and at the last routine biochemical examination of the same patient. Statistic indicators: *p ≤ 0.05.*

	N	**A)** Mean ± st.error min - max	**B)** Mean ± st.error min - max	**Wilcoxon Z, p ≤0 .05***	
Ca (*mmol/L*)	40	1.9588 ± 0.47756 0.9 - 2.7	2.2325 ± 0.23467 1.6 - 2.8	Z = -3.130	**p = 0.002***
P (*mmol/L*)	40	1.506 ± 0.43692 0.81 - 2.3	1.6102 ± 0.35402 1 - 2.8	Z = -1.110	p = 0.267
ALP (*U/L*)	31	88.9677 ± 40.87092 37 - 199	130.4 ±1 39.7661 12 - 711	Z =-2.448	**p = 0.014***
A) Serum levels determined before the start of the hemodialysis treatment B) Serum levels determined at the last routine biochemical treatment of the patient					

The association between the duration of hemodialysis therapy and Ca, P and ALP was tested using Spearman's correlation test and a statistically significant correlation was found only between the duration of therapy and ALP activity in the serum (rho=0,498, p=0,001). Furthermore, the mutual correlation of Ca, P and ALP levels was tested, as well as the correlation of the investigated parameters with the age of the patients, and no statistically significant correlation was found. Also, using Mann-Whitney U test, no stattically significant difference was found in the levels of Ca, P and ALP between men and women included in the research.

4. Discussion

As serum calcium, phosphorus and ALP are already well-established risk factors for the development of vascular calcification [11], we examined the serum levels of those parameters in ERSD patients on

long-term hemodialysis therapy. Although, numerous studies have shown the importance of monitoring Ca, P and ALP in ESRD patients and their role in the development of vascular calcification. However, there are very few results showing the association between the duration hemodialysis therapy and the those parameters. We showed a significant increase in the serum levels of Ca (p= 0,002) and ALP (p=0,014) in ESRD patients on long-term hemodialysis therapy, and a statistically significant correlation between the duration of hemodialysis therapy and ALP (p=0,001), but obtained results have not showed statistically significant change in phosphorous level or significant correlation between Ca and P with duration of the therapy. Our results are partly understandable especially if we take into consideration the fact that vascular calcification is the pathological deposition of mineral in the vascular system which is associated with atherosclerosis, diabetes, certain heredity conditions and kidney disease, especially CKD, and also patients with vascular calcification are at higher risk for adverse cardiovascular events [12]. Although age is the strongest predictor of coronary artery disease [13], coronary artery calcification is most prevalent and more severe in CKD patients than in general population. In patients not yet on dialysis, over 50% have coronary artery calcification [14] whereas 70% - 90% of prevalent dialysis patients have significant coronary artery calcification [15,16] Abnormal mineral metabolism has been recognized as nontraditional risk factor in developement of vascular calcification in CKD patients and is associated with increased mortality in both pre-dialysis and dialysis patients [17,18]. Furthermore, hyperphosphatemia is associated with prevalence and progression of vascular calcification in dialysis patients [19]. Several studies have demonstrated that the use of non-calcium-based as compared with calcium-based phosphate binders attenuated vascular calcification and mortality in dialysis patients [20,21]. Also, hypercalcemia is also associated with development of vascular calcification in CKD population [18]. In addition, the use of calcium containing phosphate binders which induce positive calcium balance is associated with increased arterial calcification in the majority of studies [22,23]. Our results showed significant increase of serum levels of Ca but not serum levels of P which is understandable especially If we take into consideration the fact that the patients were treated with the calcium containing phosphate binders during hemodialysis therapy, which is widely used and prescribed in patients undergoing hemodialysis, aiming to control the levels of calcium and phosphate [24]. On the other hand, our results showed significant increase in ALP levels in patients on long-term hemodialysis and significant correlation with duration of hemodialysis therapy as we expected because numerous studies suggest that ALP is strongly associated with vascular calcification and all-cause mortality in hemodialysis patients [25]. Serum ALP is originally a hydrolitic enzyme that removes phosphates from various molecules such as protein and nucleotides [26]. It is an indicator for checking liver and bone disorders and is commonly evaluated in clinical practice. For ESRD patients on hemodialysis therapy, Kidney Disease: Improving Global Outcomes recommends monitoring serum ALP activity to evaluate mineral bone disorder [27]. But, there are studies with conflicting results. Namely, Zhut at al. [28] found that serum ALP levels were not associated with increase death risk in a cohort of prevalent HD patients, over a 5-year interval. Accordingly, the association of ALP and vascular calcification and consequently increasing death risk in ESRD patients on long-term hemodialysis therapy needs to be investigated more in future by studies on a larger-scale and with longer observational period.

Although the association between APL, Ca and P was determined in earlier studies [29], our results did not show their mutual association, which can be explained by the complexity of the mechanisms of bone mineral metabolism disorders that occur in patients with ESRD, which were reviewed by Chen NX et al. [11] The results our study would have been much clearer if we had included a larger number of patients, if we had monitored other parameters of bone mineral disorders such as parathyroid hormone, and if we had had insight into possible bone mineral disorders that patients developed during hemodialysis therapy. These are the main limitations of the study.

5. CONCLUSION

In conclusion, the results showed the association of Ca and ALP with hemodialysis therapy, but their relationship with each other and with P levels was not determined. However, if we consider that elevated Ca, P and ALP are well established risk factors for the development of vascular calcification [11], the obtained results may certainly contribute to a better understanding of the complex changes in bone mineral metabolism of ESRD patients on long-term hemodialysis but further investigations are needed

especially in order to better understand and reduce vascular calcification-cause mortality risk in hemodialysis patients.

CONFLICT OF INTEREST

The authors declare no potential conflicts of interest with respect to research, authorship and/or publication of this article.

ETHICS APPROVAL

This study was performed in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the General Hospital "Dr. Mustafa Beganović" Gračanica, Bosnia and Herzegovina (approval number 01-890-3/21 dated 07/19/2021).

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