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Original article

Investigating changes in calcium, phosphorus, alkaline phosphatase, and 25-hydroxy Vitamin D after surgical repair of fractures of femur or tibia

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Abstract. *Background:* The recovery of long bones after fracture requires a specific process to restore the natural bone anatomy as well as its proper function. Changes in calcium, phosphorus, alkaline phosphatase and 25-hydroxy vitamin D can be justified either in the fracture process or in the repair procedure. The aim of this sectional study is to investigate changes in all these compounds after the surgical repair of fractures of femur and tibia bones. *Materials and Methods:* A random sample of 68 patients was selected from whom referring to a hospital with fractures of femur or tibia and candidate for repair surgery. The mentioned bone markers were measured at the time after surgery, six and twelve weeks after the surgery with laboratory-specific kits. A p-value, lower than 0.05, was considered to be statistically significant. *Result:* Of the patients, 34 were with fractures of femur and 34 were with fractures of tibia, equally. The patients were aged 2 to 69 with a mean age of 27.93 ± 14.8 years old. The means of calcium ($p = 0.001$) and phosphorus ($p = 0.014$) at three intervals were statistically significant difference. In contrast, the means serum alkaline phosphatase and vitamin D levels did not show any significant changes over time ($p = 0.042$). *Conclusion:* In conclusion, the means of calcium and phosphorus over the follow-up were statistically significant. The observed difference of vitamin D after the surgery, as well the level of alkaline phosphatase for femoral fracture between male and female are one of our important findings. (www.actabiomedica.it)

Keywords: Bone Fractures, Calcium, Phosphorus, Alkaline phosphatase, 25-Hydroxyvitamin D2

Introduction

The recovery of long bones after fracture requires a specific process to restore the natural bone anatomy as well as its proper function (1, 2). Indeed, various systemic and hormonal factors are involved in regulating the recovery process (3). Vitamin D, as a nutrient, contributes to the regulation of calcium homeostasis as well as bone metabolism (4, 5). Vitamin D is a type of fat-soluble vitamin which has various metabolites in human body. The two most important of these metabolites are 25-Hydroxyvitamin D and 1, 25-dihydroxyvitamin D, which are considered as an active storage form of this vitamin (6, 7). Recent studies have shown that 1,25-dihydroxyvitamin D is a form used in the fracture site to produce lost cells (8, 9). In addition, changes at the serum level of vitamin D are predictable both at the time of bone fracture and in the process of bone repair (10, 11). Accordingly, the serum calcium level can also be predictable during the process of bone repair (12). In fact, any change in serum calcium level will modify the calcium's efficacy to transmit, store, metabolize, and repair the bone (13). In addition, various studies have detected the presence of alkaline phosphatase in the process of bone recovery. As a matter of fact, it turned out that shortly after the fracture, the level of alkaline phosphatase decreases significantly, but bounces back to the normal amount within four hours after the fracture (14-18).

In a study, bone changes and calcium metabolic were determined in the patients suffering from hip fracture. Serial changes of biomarkers were evaluated shortly after the fracture until the bone repair. One week after fracture, the average serum level of calcium increased while the serum level decreased in 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D. In the second month, calcium level decreased, but levels of 1,25 dihydroxy-vitamin D increased. At the end of the third month, the calcium level was normalized (19).

In another study on patients with femoral neck fracture intertrochanteric fracture were evaluated, the level of serum calcium was significantly different between the two groups at the time of admission and discharge from the hospital, but there was no meaningful difference in these parameters after the surgery (20).

In a study on patients suffering from femoral shaft and tibia shaft fractures, the levels of serum vitamin D indicated a downward trend (21). In a study, the patients with femoral fracture were examined. The increased amount of alkaline phosphatase levels hit the plateau within a week and returned to baseline in two months thereafter. Phosphorus level gradually reached the peak within 2 to 3 weeks, following a gradual decrease. Calcium level also declined within one month and then increased (22).

With regard to the aforementioned, changes in all these compounds can be justified either in the fracture process or in the repair procedure, but the serum changes of these compounds have not been investigated after the bone surgery or the therapeutic interventions.

The aim of the present study is to evaluate the changes in calcium, phosphorus, alkaline phosphatase and 25-hydroxyvitamin D after the surgical repair of fractures of femur and tibia bones.

Methods

This sectional study aimed to investigate changes in calcium, phosphorus, alkaline phosphatase, and 25-hydroxy Vitamin D after surgical repair of fractures of femur or tibia in patients referring to Rasool Akram Medical Complex in 2017.

For the purpose of the study, a random sample of 68 patients was selected from whom referring to the hospital with fractures of femur or tibia and Candidate for repair surgery, from December 2018 to March 2019. All patients with tibial and femoral fractures during their healing period of fracture were included after obtaining their notified consent. Exclusion criteria was other fracture. Patient demographic information was obtained in an interview. Bone markers including calcium, phosphorus, alkaline phosphatase and 25-hydroxyvitamin D were measured at three points, including the time after surgery, six weeks after the surgery, and twelve weeks after the surgery with laboratory-specific kits. The laboratory method used to measure alkaline phosphatase was DGKC with a normal range of 180-1200. Colorimetric was the laboratory method used to measure calcium and phosphorus in the normal range of 8.5 - 10.5 and 3.7 - 5.6, respectively. The laboratory method used to measure vitamin D was ELFA in the normal range of 100-30.

Statistical analysis

Statistical evaluation of the data was performed using the SPSS for Windows, version 16 (Chicago, Illinois, USA). The results were statistically described as Mean ± SD and range of continuous variables. The normality of continuous variables was checked using the Kolmogorov-Smirnov test. A paired t-test or its nonparametric counterpart (Wilcoxon signed-rank test) were used for the comparison of the before and after mean values. An independent t-test or its nonparametric counterpart (Mann–Whitney U test) was used for the comparison of the mean values between independent variables. A comparison of the mean values for different postoperative times was made using an analysis of variance (ANOVA) test for parametric and using the Kruskal-Wallis test for non-parametric variables. A p-value of fewer than 0.05 was considered significant.

Information of patients was only available to the project manager. They will be explained about the project. Also, the cost of the study will not be imposed on them.

Result

Of the 68 patients (51 men and 17 women) who selected, 34 (50%) were with fractures of femur and 34 (50%) were with fractures of tibia, equally. The patients were aged 2 to 69 with a mean age of 27.93 ± 14.8 years old.

Table 1 presents the mean and standard deviation of serum levels of calcium, phosphorus, alkaline phosphatase and 25-hydroxyvitamin D over follow up in addition p-values of the repeated measurement ANOVA analyses. A p-value, lower than 0.05, was considered to be statistically significant.

The mean level of serum calcium at three intervals, after the surgery, six weeks and twelve weeks after the surgery, was 8.846 (± 0.57) mg/dl, 9.396 (± 0.57), and 9.876 (± 0.39), respectively with a statistically significant difference. P value = 0.00; which shows a steadily upward trend. The mean level of serum phosphorus at three time points after surgery, 6 weeks and 12 weeks after surgery were 3.24 mg/dl (± 1.11), 3.55 (± 0.77), and 3.59 (± 0.45), respectively, which has a meaningful difference (P-value = 0.014). The mean serum alkaline phosphatase levels at three intervals after the surgery, 6 weeks and 12 weeks after the surgery were 293.78 (± 274.79) international units in liters, 314.51 (± 292.00), and 277.00 (± 104.74), respectively, which were no statistically significant (P-value = 0.537).The mean level of serum vitamin D in the three intervals after the surgery, 6 weeks and 12 weeks after the surgery were 26.19 (± 11.08) ng/ml, 21.10 (± 11.08), and 22.94 (± 10.83), respectively, which were statistically insignificant (P value = 0.062).

The serum levels were compared between males and females. Results showed in table 2. In addition, this table provides the mean and standard deviation of the serum levels divided into sex group. As can be seen from the table, there is only a significant difference for 25-Hydroxy Vitamin D after the surgery between the two sex groups.

Based on table 3, comparison of serum levels between two groups of fracture type, there is a statistically difference in alkaline level after 12 weeks between patient with tibia fracture and whom suffering from femural fracture (P value = 0.041).

The results obtained from the comparison of two types of fracture in sex groups are shown in table 4. There is not a significant difference in serum levels for male and female with tibia fracture. In contrast, for patients with femural fracture, the level of calcium serum after 12 weeks is statistically different for male and female (P value = 0.038). Furthermore, the level of

Table 1. Mean and standard deviation of calcium, phosphorus, alkaline phosphatase and 25-hydroxy vitamin D in after surgery, 6 and 12 weeks after surgery, as well p-values of analytical statistics (1. Sphericity test, 2. Greenhouse-Geisser test)

Serum level	time	Mean ±SD	p-value[1]	p-value[2]
Calcium	After surgery	8.85±0.82	0.001	0.001*
	After 6 weeks	9.40±0.82		
	After 12 week	9.88±0.39		
Phosphorus	After surgery	3.24±1.12	0.003	0.014*
	After 6 weeks	3.55±0.77		
	After 12 week	3.58±0.46		
alkaline phosphatase	After surgery	210.00±274.80	0.000	0.537
	After 6 weeks	234.50±292.00		
	After 12 week	241.00±104.74		
25-hydroxy Vitamin D	After surgery	26.19±11.09	0.001	0.062
	After 6 weeks	21.097±11.09		
	After 12 week	22.94±10.83		

Table 2. Mean and standard deviation of calcium, phosphorus, alkaline phosphatase and 25-hydroxy vitamin D in after surgery, 6 and 12 weeks after surgery, as well p-values of U Mann-Whitney test divided into sex groups.

Serum level	time	Sex (Mean ±SD)		p-value
		male	female	
Calcium	After surgery	8.81±0.83	8.93±0.81	> 0.05
	After 6 weeks	9.40±0.56	9.40±0.61	> 0.05
	After 12 weeks	9.87±0.41	9.88±0.35	> 0.05
Phosphorus	After surgery	3.15±1.06	3.50±1.25	> 0.05
	After 6 weeks	3.59±0.80	3.40±0.69	> 0.05
	After 12 weeks	3.58±0.47	3.59±0.40	> 0.05
Alkaline Phosphatase	After surgery	273.78±199.57	353.76±432.03	> 0.05
	After 6 weeks	287.69±160.15	395.00±517.41	> 0.05
	After 12 weeks	283.12±106.14	258.65±101.27	> 0.05
25-HydroxyVitamin D	After surgery	23.38±15.75	34.60±27.62	0.042*
	After 6 weeks	19.68±9.28	25.32±14.83	> 0.05
	After 12 weeks	21.72±8.62	26.59±15.50	> 0.05

Table 3. Mean and standard deviation of calcium, phosphorus, alkaline phosphatase and 25-hydroxy vitamin D in after surgery, 6 and 12 weeks after surgery, as well p-values of U Mann-Whitney test based on type of fracture

Serum level	time	Type fracture		p-value
		Tibia (Mean±SD)	Femur (Mean±SD)	
Calcium	After surgery	8.51±0.75	9.11±0.84	> 0.05
	After 6 weeks	8.85±0.82	9.95±0.86	> 0.05
	After 12 week	9.41±0.39	10.1±0.39	> 0.05
Phosphorus	After surgery	3.14±11.01	3.34±1.13	> 0.05
	After 6 weeks	3.16±0.77	3.94±0.81	> 0.05
	After 12 week	3.21±0.55	3.95±0.74	> 0.05
alkaline phosphatase	After surgery	206.00±101.2	217.00±108.2	> 0.05
	After 6 weeks	214.1±105.6	255.2±109.2	> 0.05
	After 12 week	219.5±105.74	263.5±110.4	0.041*
25-hydroxy Vitamin D	After surgery	24.11±12.09	28.13±10.9	> 0.05
	After 6 weeks	20.14±10.09	21.9±11.1	> 0.05
	After 12 week	20.1±10.25	24.3±11.3	> 0.05

alkaline phosphatase after 6 weeks (P value = 0.028) and 12 weeks (P value = 0.041) are significantly different between two sex groups.

Discussions

As stated in the current study, patients with femur and tibia fractures who were willing to receive fracture repair surgery were examined in the study. Bone markers including calcium, phosphorus, alkaline phosphatase and 25-hydroxyvitamin D were measured with laboratory-specific kits. The changing trends of serum levels for these markers were assessed in twelve weeks after the surgery.

The mean level of serum calcium at three intervals shows a steadily upward trend. Compared to other studies, the results were the same in the short run. As

Table 4. Mean and standard deviation of calcium, phosphorus, alkaline phosphatase and 25-hydroxy vitamin D in after surgery, 6 and 12 weeks after surgery, as well p-values of U Mann-Whitney test according to sex groups and type of fracture

Type of fracture	Serum level	time	Sex (Mean±SD)		p-value
			Male	Female	
Femur	**Calcium**	After surgery	8.9±0.83	8.2±0.74	> 0.05
		After 6 weeks	9.66±0.88	8.2±0.81	> 0.05
		After 12 weeks	10.52±0.97	8.33±0.86	0.038*
	Phosphorus	After surgery	3.18±1.11	3.14±1.01	> 0.05
		After 6 weeks	4.01±0.88	3.85±0.82	> 0.05
		After 12 weeks	3.99±0.85	3.93±0.72	> 0.05
	alkaline phosphatase	After surgery	265.1±107.2	298.2±101.2	> 0.05
		After 6 weeks	285.2±106.2	395±112.5	0.028*
		After 12 weeks	301.1±115.3	256.2±110.4	0.041*
	25-hydroxy Vitamin D	After surgery	30.1±10.9	27.12±10.2	> 0.05
		After 6 weeks	27.5±11.1	25.24±10.4	> 0.05
		After 12 weeks	26.2±10.65	24.2±10.5	> 0.05
Tibia	**Calcium**	After surgery	8.8±0.88	8.1±0.77	> 0.05
		After 6 weeks	9.65±0.87	8.95±0.71	> 0.05
		After 12 weeks	10.1±0.97	9.23±0.86	> 0.05
	Phosphorus	After surgery	3.07±1.01	3.01±9.9	> 0.05
		After 6 weeks	3.88±1.022	3.75±0.95	> 0.05
		After 12 weeks	3.90±0.87	3.88±0.86	> 0.05
	alkaline phosphatase	After surgery	256.1±98.2	293.2±90..2	> 0.05
		After 6 weeks	280.1±102.2	314.5±110.3	> 0.05
		After 12 weeks	288.2±114.3	263.2±103.1	> 0.05
	25-hydroxy Vitamin D	After surgery	29.2±11.2	26.31±9.5	> 0.05
		After 6 weeks	27.1±11.1	25.4±10.2	> 0.05
		After 12 weeks	24.9±9.6	23.5±8.95	> 0.05

stated in a study by Sato et al., one week after fracture, the mean level of serum calcium increased. However, in the long run, the results of various studies were contrasting in a way that the present study indicated a rise of the level of serum calcium after 3 months, while the study by Sato demonstrated a drop after two months and a normal level at the end of the third month (19). In addition, Li et al. (2016) showed that there was a significant difference in the amount of serum calcium between the two groups at the admission and discharge from the hospital, but there was no significant difference after the surgery (20), which is in line with the findings of the present study. Moreover, the study by Nilsson et al. also displayed the increasing trend of serum calcium after one month (22), which is in agreement with the results of this study. The increase of the mean level of serum phosphorus was significant during week 6 which maintained approximately the same value until week 12. The results of the study by Nilsson et al are in line with the reports of the present study; they stated that phosphorus level had gradually increased and peaked after 2 to 3 weeks and then declined gradually (22).

Mean serum alkaline phosphatase levels at three intervals were no statistically significant. Even though, the observed upward trend of mean serum alkaline phosphatase levels was not significant. It is worth noticing that it was not consistent at week 6 and, more importantly, the corresponding amount at week 12 was less than the initial level. In a study by Nilsson

et al., it was observed that the increased alkaline phosphatase level reached the plateau within one week and returned to baseline within two months (22), which confirms our results in this study.

The mean level of serum vitamin D in the three intervals was statistically insignificant. However, our finding point to a noticeable downward trend during the first 6 weeks which slightly bounced back over the next 6 weeks but did not reach the initial value. In comparison to the study by Sato et al (2001) which indicated a decrease of serum vitamin D after one month and its increase in the second month (19), the results are consistent. Furthermore, the falling trend of vitamin D in the first measurement, after one week, and at the end of the third week in the study by Ettehad et al (2014) confirmed the findings of the current study (21).

One of the most advantages of this study compared with previous studies is that the patients have been followed for a longer time. Another is in our comprehensive study more bone markers simultaneously measured and analyzed. However, there are a number of limitations to this study that need to be considered in future research; first of all, having a small number of the patients, secondly, disregarding some effective interventions, such as age, used drugs and supplements, and the success rate of surgery. Further study is necessary to evaluate the exact change in the process of bone healing and remove these limitations.

Conclusion

The means levels of serum calcium and phosphorus over the follow-up were statistically significant. Their upward trends were consistent with previous published studies. The means serum alkaline phosphatase and vitamin D levels did not show any significant changes over time. However, in the light of the curative effect of vitamin D in the formation and mineralization of bones, it is suggested for patients with tebial and femoral fractures to get vitemin D supplement in the curative period.

The observed difference of the serum level of vitamin D after the surgery between male and female is one of our important findings that has been not noticed in the previous studies yet. Another important finding was that the significantly difference of the level of calcium serum and alkaline phosphatase for male and female was only observed in patients with femural fracture. It seems to us that this finding has important implications for developing further studies to investigate the impact of sex on the changes in these compounds after the surgical repair of fractures of femur bone.

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