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Course and Rate of Post-Fracture Bone Healing in Correlation with Bone-Specific Alkaline Phosphatase and Bone Callus Formation

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A B S T R A C T

Alkaline phosphatase (ALP) and bone-specific alkaline phosphatase (S-bone ALP) activities may serve as markers of the course and rate of bone healing after sustained fractures. The aim of this study was to examine whether the assessment of S-bone ALP as a biochemical parameter in the early posttraumatic phase may indicate the course of fracture healing. To date, the methods used to monitor the bone healing process have been based on the patients' assessment and the radiographic findings. In view of the fact that patient opinion is highly subjective, that the radiographic findings depend on the radiologist's experience and that the monitoring of bone healing is a long-lasting process, measurements of biochemical parameters appear to be the only objective evidence of the changes occurring during bone regeneration. In this study, the activity of bone-specific alkaline phosphatase was measured in the serum of 41 patients who had sustained long bone fractures. The participants included 26 males and 15 females, aged 15 to 80 years. All patients were treated surgically. The activity of S-bone ALP was assessed every seven days over a period of 4 weeks. The study patients were followed up radiologically for several months. Our research showed that the increase of alkaline phosphatase correlated with an increase of S-bone ALP levels. In addition, changes in ALP levels on days 7 and 14 as compared to those on day 1 post injury were associated with changes in S-bone ALP levels on the same day. Likewise, the callus volume correlated with the decrease, no change or increase in the levels of ALP and S-bone ALP in the same way. Based on these results, it may be concluded that monitoring changes in the biochemical parameters alkaline phosphatase and bone-specific alkaline phosphatase allows early detection of fracture healing rates. A minor increase in the activity or no change in the level of the biochemical parameters ALP and S-bone ALP in the period of the first two weeks indicates successful fracture fixation, rapid bone healing and the formation of a minimal or insignificant callus. A major increase in the activity of the biochemical parameters ALP and S-bone ALP in the period of the first two weeks indicates inadequate fracture fixation, delayed bone healing and the formation of a visible and significant callus.

***Key words**: alkaline phosphatase, callus, healing rate*

Introduction

It is a well-known fact that the alkaline phosphatase (ALP) enzyme plays an important role in the biological processes of ossification. Recent research has shown that one of its isoenzymes in particular, the so-called bone-specific alkaline phosphatase (S-bone ALP), has a major part in the ossification processes[1,2].

Experimental and clinical studies have proved that the amount of S-bone ALP in osteoblast cells and bone is commensurate with collagen formation[3–6]. Regardless of the treatment option (conservative or surgical), an increase of total ALP occurs, with a peak around day 21 post injury[7,8]. On day 7 post injury, changes in S-bone

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ALP levels occur along with change sin the total ALP level, in a correlation of 1.1%[8]. The highest post-injury ALP increase was measured in conservatively treated patients, while the smallest increase occurred in patients treated by stable osteosynthesis. ALP activity depends on the stability of post-fracture bone fragments. As unstable osteosynthesis results in an increased bone fragment mobility as compared to stable osteosynthesis, the measured ALP levels in these cases are higher. Conservative treatment is associated with the greatest bone fragment mobility, and accordingly results in the highest measured ALP levels[9]. In their studies on S-bone ALP increase in relation to various types of traumatic injury, Laurer et al.[10] have concluded that the initial S-bone ALP increase is not only a consequence of the bone response to trauma, but also the result of the total stress the patient experiences in relation to the injury itself as well as the surgery. Only the later S-bone ALP increase, occurring along with the applied osteosynthesis, is associated with the fracture localisation. A marked increase of both total ALP and, analogously, S-bone ALP, accompanied by the formation of a large callus, has been observed only in patients with long tubular bone fractures in combination with traumatic brain injuries[11–13]. In these patients, just like in those without traumatic brain injuries, the biochemical levels continuously grow from day 1 to day 21 post injury[8,12]. Research by Bowles et al.[5] does not show a continuous increase of either total ALP or S-bone ALP between days 1 and week 10 post injury. The results of their measurements indicate a marked decrease in both total ALP and S-bone ALP levels until day 4 post injury, a return to day 1 levels on day 8, followed by a steady increase up to week 10 post injury. Later findings by the same authors[14] in their research on tibial fractures also report a decrease in S-bone ALP levels in the first week post injury, followed by an increase in the second week. Total alkaline phosphatase levels did not decrease, but showed an increase from day 1. These results differ from the findings of Leung et al.[15] and Oni et al.[2], who did not observe an initial decrease in ALP levels.

Although the role of S-bone ALP in bone healing has been much researched, measurements of its activity in the first postoperative weeks and the correlation of the measured levels with ALP activity during the same period, as well as the volume of the formed callus after the bone healing has been completed, and the possible pre-

TABLE 1A

PARAMETERS ACCORDING TO TYPE OF FRACTURE AND IN TOTAL FOR VARIABLES 1D ALP, 7D ALP, 14D ALP, 21D ALP

Group		1D ALP	7D ALP	14D ALP	21D ALP
T	N	33	33	9	5
	Median	109.0000	119.0000	172.0000	219.0000
	$\overline{X}$	124.2727	130.6667	181.2222	246.2000
	SD	92.3161	53.1693	71.9093	116.5406
	Min.	54.00	74.00	107.00	145.00
	Max.	508.00	317.00	340.00	429.00
F	N	7	7	1	
	Median	139.0000	137.0000	–	
	$\overline{X}$	146.0000	135.7143	119.0000	
	SD	65.9444	42.8397	–	
	Min.	41.00	59.00	119.00	
	Max.	265.00	195.00	119.00	
H	N	1	1		
	Median	–	–		
	$\overline{X}$	125.0000	152.0000		
	SD	–	–		
	Min.	125.00	152.00		
	Max.	125.00	152.00		
Total	N	41	41	10	5
	Median	110.0000	122.0000	158.5000	219.0000
	$\overline{X}$	128.0000	132.0488	175.0000	246.2000
	SD	86.8245	50.5049	70.5943	116.5406
	Min.	41.00	59.00	107.00	145.00
	Max.	508.00	317.00	340.00	429.00

N – number of subjects, Median – central value, $\overline{X}$ – arithmetic mean, SD – standard deviation (measure of dispersion from the arithmetic mean), Min. – minimum value, Max. – maximum value.

TABLE 1B
PARAMETERS ACCORDING TO TYPE OF FRACTURE AND IN TOTAL FOR VARIABLES 1D S-BONE ALP, 7D S-BONE ALP, 14D S-BONE ALP, 21D S-BONE ALP

Group		1D S-BONE ALP	7D S-BONE ALP	14D S-BONE ALP	21D S-BONE ALP
T	N	33	33	9	5
	Median	27.0000	13.0000	53.0000	64.0000
	$\overline{X}$	45.0606	29.5152	54.0000	106.4000
	SD	81.5122	47.7403	41.3491	100.0540
	Min.	2.00	2.00	14.00	30.00
	Max.	368.00	253.00	148.00	278.00
F	N	7	7	1	
	Median	58.0000	23.0000	–	
	$\overline{X}$	57.4286	48.8571	2.0000	
	SD	38.9737	60.5294	–	
	Min.	6.00	5.00	2.00	
	Max.	126.00	178.00	2.00	
H	N	1	1		
	Median	–	–		
	$\overline{X}$	11.0000	28.0000		
	SD	–	–		
	Min.	11.00	28.00		
	Max.	11.00	28.00		
Total	N	41	41	10	5
	Median	28.0000	16.0000	42.5000	64.0000
	$\overline{X}$	46.3415	32.7805	48.8000	106.4000
	SD	74.8153	49.2694	42.3105	100.0540
	Min.	2.00	2.00	2.00	30.00
	Max.	368.00	253.00	148.00	278.00

dictive role of the measured levels on the rate of bone healing, have not been fully investigated to date. The aim of this paper is to establish the extent to which it is possible to estimate the rate of bone healing after fractures, as well as to predict the callus size upon the completion of bone healing, on the basis of S-bone ALP and total ALP activity measurements within the first weeks after long bone surgery.

Patients and Methods

Patients

S-bone ALP activity was measured from the serum of 41 patients with long bone fractures treated surgically. 26 of them were male and 15 female, aged 15 to 80 years. Thirty-three of them (80.4%) had sustained tibial and seven of them (17.1%) femoral fractures, and there was only one (2.4%) with a humoral fracture. After their histories were taken, the patients had laboratory tests done (CBC, total bilirubin, AST, ALT, GGT, ESR and urine) to establish that their findings were within the reference values and thus made them eligible for our study. The authors want to emphasise that in this research they used hematological laboratory findings as well as X-ray pictures that were made as part of the regular clinical procedure during the patients' hospital stay and within the routine follow-up investigations after their discharge. All of the patients underwent successful surgical treatment. S-bone ALP activity was assessed from the serum on a weekly basis during a period of four weeks. ALP measurements from which S-bone ALP levels were calculated were done on an OPTON PM2DL flow-through spectrophotometre, 1-cm cuvette, at 405-nm wavelength and a temperature of 37 °C.

The study patients were radiologically observed through periodic follow-ups in the course of several months. The control group was comprised of 100 healthy adults (70 men and 30 women) aged 25–60 years. S-bone ALP levels were calculated from their serum by means of the same procedure that was used for the study group.

Methods

Clinical work-up

The bone healing process was clinically and radiologically followed during the course of the study patients' hospitalisation, as well as after discharge until full recovery. Based on the rate of bone fracture healing, the subjects were assigned into two groups: a rapid bone-healing group (full weight-bearing 6 weeks post surgery) and a slow bone-healing group (full weight-bearing 10 weeks post surgery).

Laboratory work-up

The laboratory work-up of samples included two different methods: assessment of alkaline phosphatase and assessment of S-bone ALP activity[16].

The Da Foncesca-Wolheim method[17] was used for assessing total serum ALP activity, followed by the assessment of S-bone ALP activity by the method described by S.B. Rosalki and A.Y. Foo in 1984[18].

Radiological work-up

From parameters measured from the X-ray images, the fracture fissure and callus sizes were mathematically calculated. Radiography was done by standard method based on the bone type, with the use of standard film (EFKE, Fotokemika Zagreb) and standard exposition. The films were developed in an automatic processor.

Statistics

The quantitative data were compared by Mann-Whitneyevim U test, while the Friedman and Wilcoxson Z tests were used for the comparison of data obtained on different days, i.e. for longer time span[19,20].

Results

Table 1 comprises two tables. Table 1a shows alkaline phosphatase levels for the parameter of type of fracture (T – tibia, F – femur and H – humerus) in correlation with the variable of time expressed in days post injury (day 1 – 1D, day 7 – 7D, day 14 – 14D and day 21 – 21D). S-bone ALP levels for the parameter of type of fracture (T – tibia, F – femur and H – humerus) in correlation with the variable of time expressed in days post injury (day 1 – 1D, day 7 – 7D, day 14 – 14D and day 21 – 21D) are shown in Table 1b. Table 1a shows that in group T, the mean value of the ALP variable on day 21 was significantly higher than on days 1, 7 and 14, and that ALP was significantly higher on day 14 as compared to days 1 and 7. In group F, mean ALP levels on day 7 did not show a significant change as compared to day 1. Group H was not significant due to the fact that it comprised only one subject. Thus, it is clearly visible that, regardless of the type of fracture, the mean value of the ALP variable on day 21 was significantly higher than on days 1, 7 and 14, and that ALP was significantly higher on day 14 as compared to days 1 and 7. Table 1b shows that in group T the mean value of the S-bone ALP variable on day 21 was significantly higher than on days 1, 7 and 14, and that S-bone ALP was significantly higher on day 14 than on days 1 and 7, while it was significantly lower on day 7 than on day 1. In group F, on day 7 the mean value was significantly lower as compared to day 1. Group H was not significant due to the fact that it comprised only one subject. Thus, it can be seen that, regardless of the type of fracture, the mean value of the S-bone ALP variable on day 21 was significantly higher than on days 1, 7 and 14, and that S-bone ALP was significantly higher on day 14 as compared to days 1 and 7, while it was significantly lower on day 7 than on day 1.

Testing for differences in total alkaline phosphatase on the chosen days by Wilcoxon rank test showed a statistically significant difference between days 21 and 7 (Z=2.023; p=0.043) in the ALP variable, whereas in the S-bone ALP variable a statistically significant difference was found between days 7 and 1 (Z=1.997; p=0.046) as well as between days 21 and 7 (Z=2.023; p=0.043).

The callus volume values expressed in cm^3 according to type of fracture and in total are shown in Table 2. It needs to be noted that in the callus volume measurements a 10-cm^3 callus was taken as mean value. All callus values in the range of 5–10 cm^3 were considered small, those between 0–5 cm^3 were insignificant, and values of 0 cm^3 signified that the callus was non-existent. Callus volume values between 10 and 15 cm^3 were considered significant, and values exceeding 15 cm^3 were specially significant. Table 2 shows that the mean callus volume

TABLE 2
PARAMETERS ACCORDING TO TYPE OF FRACTURE AND IN TOTAL FOR THE CALLUS VOLUME (cm^3) VARIABLE

Type of fracture		Callus volume (cm^3)
T	N	33
	Median	3.9600
	$\bar{X}$	8.6373
	SD	14.7950
	Min.	0.00
	Max.	72.45
F	N	7
	Median	29.0600
	$\bar{X}$	40.1314
	SD	41.3293
	Min.	0.00
	Max.	113.04
H	N	1
	Median	–
	$\bar{X}$	25.5300
	SD	–
	Min.	25.53
	Max.	25.53
Total	N	41
	Median	5.2800
	$\bar{X}$	14.4263
	SD	24.0352
	Min.	0.00
	Max.	113.04

TABLE 3A
PARAMETERS ACCORDING TO DECREASE, NO CHANGE AND INCREASE OF ALP LEVELS ON THE CHOSEN DAYS, THE DIFFERENCES IN ALP LEVELS BETWEEN THE CHOSEN DAYS, AND CALLUS VOLUME

ALP		1D ALP	7D ALP	14D ALP	Difference ALP 7D – ALP 1D	Difference ALP 14D – ALP 1D	Callus volume (cm^3)
Decrease	N	7	7	2	7	2	7
	Median	145.00	122.00	229.50	–23.00	–157.00	3.96
	$\overline{X}$	242.71	156.14	229.50	–86.57	–157.00	4.00
	SD	163.60	81.56	156.27	89.11	15.56	4.05
	Min.	109.00	86.00	119.00	–215.00	–168.00	0.00
	Max.	508.00	317.00	340.00	–8.00	–146.00	9.68
No change	N	25	25	3	25	3	25
	Median	98.00	118.00	113.00	5.00	15.00	3.89
	$\overline{X}$	101.60	118.32	118.33	16.72	9.67	9.18
	SD	32.31	41.51	14.74	47.74	9.24	16.65
	Min.	41.00	59.00	107.00	–40.00	–1.00	0.00
	Max.	162.00	290.00	135.00	217.00	15.00	80.42
Increase	N	9	9	5	9	5	9
	Median	123.00	152.00	187.00	33.00	73.00	25.56
	$\overline{X}$	112.11	151.44	187.20	39.33	82.20	37.10
	SD	22.60	32.78	30.33	31.65	49.57	35.85
	Min.	79.00	102.00	145.00	–21.00	22.00	0.00
	Max.	139.00	195.00	218.00	96.00	139.00	113.04
Total	N	41	41	10	41	10	41
	Median	110.00	122.00	158.50	5.00	18.50	5.28
	$\overline{X}$	128.00	132.05	175.00	4.05	12.60	14.43
	SD	86.82	50.50	70.59	67.65	101.11	24.04
	Min.	41.00	59.00	107.00	–215.00	–168.00	0.00
	Max.	508.00	317.00	340.00	217.00	139.00	113.04

value was significantly lower in group T than in group F. Table 3a shows the parameters according to the decrease, no change or increase of ALP levels, according to days post injury and according to the differences in ALP levels between the chosen days, as well as the callus volumes. Table 3b shows the same parameters for S-bone ALP. It may be concluded that a decrease in ALP values on the chosen days corresponded with the formation of an insignificant (mean 4.00 cm^3) callus. Further, it can be seen that insignificant changes in ALP levels measured on the chosen days resulted in the formation of a small (mean 9.18 cm^3) callus, while an increase in ALP levels on the chosen days corresponded with the formation of a specially significant (mean 37.10 cm^3) callus (Table 3a). A decrease, as well as insignificant changes, in S-bone ALP levels on the chosen days, resulted in the formation of small calluses of mean volumes of 7.98 cm^3 and 7.11 cm^3, respectively, whereas an increase in S-bone ALP levels on the chosen days corresponded with the formation of a significant (mean 35.81 cm^3) callus (Table 3b). A comparison of the differences in ALP and S-bone ALP levels on the chosen days therefore indicates an almost equivalent behaviour of the observed parameters in relation to either a decrease, no change or increase of ALP and S-bone ALP levels on the one hand, and callus formation on the other.

A comparison of the mean values, mean values ±SD, and the minimum and maximum ALP and S-bone ALP values on the chosen days (Tables 3a and 3b), shows that an increase in alkaline phosphatase levels is associated with a corresponding increase in bone-specific alkaline phosphatase levels. Tables 3a and 3b also show that only a significant increase of both ALP and S-bone ALP resulted in the formation of significant mean callus volumes of 37.10 cm^3 and 35.81 cm^3, respectively.

By correlating callus volume in relation to ALP and S-bone ALP values on the chosen days, a statistically significant association was found between callus volume and S-bone ALP on day 7 post injury ($r=0.371$; $P=0.017$), while the correlation found for the other chosen days was not proved to be statistically significant.

Table 4 shows the results of the measured total alkaline phosphatase and bone-specific alkaline phosphatase levels in relation to the bone healing outcome (rapid – slow) in the tibia group. It can be seen that the arithme-

TABLE 3B
PARAMETERS ACCORDING TO DECREASE, NO CHANGE AND INCREASE OF S-BONE ALP LEVELS ON THE CHOSEN DAYS, THE DIFFERENCES IN S-BONE ALP LEVELS BETWEEN THE CHOSEN DAYS, AND CALLUS VOLUME

S-BONE ALP		1D S-BONE ALP	7D S-BONE ALP	14D S-BONE ALP	Difference S-BONE ALP 7D – S-BONE ALP 1D	Difference S-BONE ALP 14D – S-BONE ALP 1D	Callus volume (cm^3)
Decrease	N	15	15	3	15	3	15
	Median	45.00	16.00	32.00	–34.00	–69.00	0.00
	$\overline{X}$	89.73	28.00	60.67	–61.73	–97.33	7.98
	SD	111.41	33.22	77.11	83.11	86.07	20.44
	Min.	9.00	2.00	2.00	–294.00	–194.00	0.00
	Max.	368.00	128.00	148.00	–6.00	–29.00	80.42
No change	N	16	16	2	16	2	16
	Median	11.50	11.50	38.00	–1.00	13.50	3.99
	$\overline{X}$	15.25	13.50	38.00	–1.75	13.50	7.11
	SD	10.64	9.37	26.87	11.14	10.61	8.40
	Min.	2.00	3.00	19.00	–23.00	6.00	0.00
	Max.	37.00	29.00	57.00	21.00	21.00	29.06
Increase	N	10	10	5	10	5	10
	Median	31.00	45.50	53.00	18.50	19.00	25.55
	$\overline{X}$	31.00	70.80	46.00	39.80	23.00	35.81
	SD	16.16	81.37	26.48	71.70	32.75	33.58
	Min.	4.00	2.00	14.00	–20.00	–8.00	5.50
	Max.	58.00	253.00	75.00	213.00	71.00	113.04
Total	N	41	41	10	41	10	41
	Median	28.00	16.00	42.50	–6.00	0.50	5.28
	$\overline{X}$	46.34	32.78	48.80	–13.56	–15.00	14.43
	SD	74.82	49.27	42.31	72.52	73.33	24.04
	Min.	2.00	2.00	2.00	–294.00	–194.00	0.00
	Max.	368.00	253.00	148.00	213.00	71.00	113.04

TABLE 4
DESCRIPTIVE STATISTICS FOR TOTAL ALKALINE PHOSPHATASE AND BONE-SPECIFIC ALKALINE PHOSPHATASE LEVELS FOR THE TIBIA GROUP ACCORDING TO HEALING OUTCOME AND CHOSEN DAYS

Tibia Bone healing outcome		Total alkaline phosphatase				Bone-specific alkaline phosphatase			
		1D	7D	14D	21D	1D	7D	14D	21D
Rapid	N	14	14	2		14	14	2	
	$\overline{X}$	130.64	120.50	110.00		55.43	19.43	25.50	
	Median	109.00	116.50	110.00		28.50	11.00	25.50	
	SD	87.77	37.80	4.24		91.58	19.49	9.19	
	Lowest value	73.00	74.00	107.00		7.00	2.00	19.00	
	Highest value	426.00	211.00	113.00		368.00	74.00	32.00	
Slow	N	19	19	7	5	19	19	7	5
	$\overline{X}$	119.58	138.16	201.57	246.20	37.42	36.95	62.14	106.40
	Median	99.00	122.00	187.00	219.00	22.00	14.00	57.00	64.00
	SD	97.63	62.08	68.68	116.54	74.89	60.33	43.79	100.05
	Lowest value	54.00	76.00	135.00	145.00	2.00	2.00	14.00	30.00
	Highest value	508.00	317.00	340.00	429.00	342.00	253.00	148.00	278.00

TABLE 5
DESCRIPTIVE STATISTICS FOR TOTAL ALKALINE PHOSPHATASE AND BONE-SPECIFIC ALKALINE PHOSPHATASE LEVELS FOR THE FEMUR GROUP ACCORDING TO HEALING OUTCOME AND CHOSEN DAYS

Bone healing outcome		Total alkaline phosphatase			Bone-specific alkaline phosphatase		
		1D	7D	14D	1D	7D	14D
Rapid	N	1	1	1	1	1	1
		265	139	119	71	23	2
Slow	N	6	6		6	6	
	$\bar{X}$	121.33	140.00		37.83	70.50	
	Median	130.00	146.00		34.00	51.50	
	SD	41.47	47.26		25.21	68.90	
	Lowest value	41.00	59.00		6.00	5.00	
	Highest value	162.00	195.00		73.00	178.00	

TABLE 6
CALLUS VOLUME (cm^3) PARAMETER DESCRIPTION ACCORDING TO FRACTURE LOCATION, BONE HEALING OUTCOME AND IN TOTAL

Callus volume (cm^3)							
Fracture location	Bone healing outcome	N	$\bar{X}$	Median	SD	Lowest value	Highest value
Tibia	rapid	14	1.31	0.00	2.67	0.00	9.05
	slow	19	14.04	9.42	17.65	1.37	72.45
Femur	rapid	1	0.00				
	slow	6	46.82	31.25	40.91	11.23	113.04
Humerus	slow	1	25.53		.		

tic mean of the measured total alkaline phosphatase and bone-specific alkaline phosphatase levels showed a moderate decrease over the chosen days in the rapid-healing group, whereas the arithmetic mean of the same parameters gradually increased in the slow-healing group.

Table 5 shows parameter values for total alkaline phosphatase and bone-specific alkaline phosphatase according to the measurements made on the chosen days and the healing outcome (rapid – slow). It can be noticed that rapid fracture healing is correlated with a decrease in total alkaline phosphatase and bone-specific alkaline phosphatase on days 7 and 14 post injury as compared to the levels measured on day 1 post injury. It can also be seen that slow fracture healing is in correlation with an increse in total alkaline phosphatase and bone-specific alkaline phosphatase measured on days 7 and 14 as compared to the levels measured on day 1 post injury.

For every subject, the callus volume was calculated upon completion of treatment and successful bone healing of the surgically treated fractures. Table 6 shows the callus volumes according to fracture location and bone healing outcome in relation to the parameter of rate of healing (rapid – slow).

In the rapid-healing tibia group of patients, the correlation between callus volume and total and bone-specific alkaline phosphatase was calculated according to the chosen days. Thus a statistically significant correlation was established between callus volume and total alkaline phosphatase on day 1 ($r=0.849$; $p<0.01$), and between callus volume and total bone-specific alkaline phosphatase on day 1 ($r=0.785$; $p=0.001$) and day 7 ($r=0.0564$; $p=0.036$). In the slow-healing group, no statistically significant correlation was found between callus volume and total and bone-specific alkaline phosphatase according to the chosen days.

By calculating the correlation between callus volume and total and bone-specific alkaline phosphatase according to the chosen days, a statistically significant correlation was found between callus volume and bone-specific alkaline phosphatase on day 7 ($r=0.944$; $p=0.001$).

These results indicate that measurements of alkaline phosphatase and bone-specific alkaline phosphatase levels on days 1, 7 and 14 post injury may serve as an indicator of the outcome and rate of bone fracture healing.

Discussion

According to the criterion of treatment, patients with long bone fractures can be classified into two groups – the conservative treatment group and the surgical treatment (osteosynthesis) group. It has been established that patients in the former group undergo a slower healing process characterised by a marked increase of alkaline phosphatase and bone-specific alkaline phosphatase levels on day 14 post injury, as well as a markedly increased callus volume upon completion of treatment. Based on the results of research[21,22], patients from the surgical treatment group fall into two categories: those with a successful stable osteosynthesis and an observed small or minimum increase of alkaline phosphatase and bone-specific alkaline phosphatase and callus volume, and those in whom a completely stable osteosynthesis could not be achieved, with an observed marked increase in alkaline phosphatase and bone-specific alkaline phosphatase as well as a corresponding callus. This confirms with the findings of other authors[9,21]. Our research showed that the measured changes of total and bone-specific alkaline phosphatase were consistent on all of the chosen days. Upon completion of the study, in the rapid-healing group a statistically significant and positive correlation between callus volume and total and bone-specific alkaline phosphatase was established for day 1, and between callus volume and bone-specific alkaline phosphatase for day 7. According to our research, total and bone-specific alkaline phosphatase increased evenly and continuously from day 1 to day 7, which is consistent with the findings of Leung[15] and Oni[2].

The results of a number of comparative researches show that the amount of bone-specific alkaline phosphatase is proportional to callus size[8,11,23,24]. The results of our measurements of total and bone-specific alkaline phosphatase are consistent with these findings. A continuous marked increase in total and bone-specific alkaline phosphatase characterises the slow-healing group for all fracture locations, indirectly indicating an outcome with the formation of a larger callus.

According to our research, alkaline phosphatase levels reached a peak on day 14 post injury, which differs from researches[10,15] where peak levels of alkaline phosphatase were not measured before day 21. In our research, there was no initial decrese in total alkaline phosphatase levels like in the findings of Bowles[14], Jingush[25] and Joerring[6]. Bowles considered this initial descrese to be the consequence of a systemic inflammatory response.

The differing results of the above-mentioned authors may be explained by the complexity of factors affecting the blood levels of alkaline phosphatase, as well as by the differences in the research itself, involving the effects of the circadian rhythm of bone remodelling, seasonal effects, as well as the patient's age, sex and ethnicity[26–28].

The differing results of a number of researches in this field may well be explained by taking into consideration all the above factors, i.e. the considerable biological variables of the biochemical indicators of bone remodelling. Thus the need arises for a more thorough investigation of the causes associated with the circumstances and the time of observation of individual patients or patient groups. In this way, adverse effects of factors which increase variation and consequently mask the investigated clinical sign or change could be avoided, and results would be easier to compare.

Our research of long bone fracture healing showed corresponding and parallel changes in total and bone-specific alkaline phosphatase during the whole course of observation. It should be specially emphasised that, depending on the outcome, an increase in total and bone-specific alkaline phosphatase occurred as early as on day 7 in the slow-healing group of patients, whereas a decrease occurred in the rapid-healing group. This is an important result insofar as it indicates the possible prognostic value of this clinical biochemic parameter for the assessment of successful surgical long bone fracture healing. The results of our research indicate that early changes in these enzymes correspond with the success and quality of the surgical procedure itself.

Conclusion

Changes in alkaline phosphatase activity are accompanied by almost identical changes in bone-specific alkaline phosphatase levels. Callus volume is commensurate with the increase in the activity of the discussed parameters. Our research results indicate the possible prognostic value of total and bone-specific alkaline phosphatase activity measurements in surgucally treated long bone fractures, owing to the fact that a difference between slow and rapid healing in this patient group could be observed as early as on day 7. In slow fracture healing, a continuous increase in total and bone-specific alkaline phosphatase occured. In rapid fracture healing, after day 7 there was a decrease in the activity of total and bone-specific alkaline phosphatase[29].

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TIJEK I BRZINA KOŠTANOG CIJELJENJA OVISNO O VRIJEDNOSTI KOŠTANOG IZOENZIMA I VOLUMENU STVORENOG KALUSA

S A Ž E T A K

Aktivnosti alkalne fosfataze i koštanog izoenzima mogu predstavljati indikatore u procjeni tijeka i brzine cijeljenja koštanih prijeloma. Svrha našeg istraživanja je pokazati da li i u kolikoj mjeri određivanje koštanog izoenzima, kao biokemijskog parametra, u ranoj posttraumatskoj fazi, ukazuje na slijed događanja u sanaciji koštanog prijeloma. Stav bolesnika je subjektivan način vrednovanja, a radiološki način ovisan o iskustvu radiologa i i praćenje dugotrajno, mjerenje biokemijskih parametara predstavlja jedini objektivan dokaz promjena koštane pregradnje. Aktivnost koštanog koenzima AP određivana je u serumu 41 bolesnika s prijelomom dugih kostiju. Od 41-og obrađenog bolesnika, 26 bili su muškarci, a 15 žene, starosne dobi od 15–80 godina.. Svi pacijenti kirurški su sanirani. Aktivnost alkalne fosfataze i koštanog izoenzima određivana je u serumu bolesnika svakih sedam dana tijekom četiriju tjedana. Isti bolesnici su praćeni i radiološki periodičnim kontrolama tijekom više mjeseci. Naša istraživanja su pokazala da porast vrijednosti alkalne fosfataze prati odgovarajući porast vrijednosti koštanog izoenzima. Također, vidljivo je da promjene vrijednosti alkalne fosfataze sedmog i četrnaestog dana u odnosu na prvi dan ozljeđivanja, prate promjene koštanog izoenzima istog dana. Jednako tako volumen kalusa prati pad vrijednosti, bez promjene vrijednosti i porast vrijednosti alkalne fosfataze i koštanog izoenzima na identičan način. Iz navedenih rezultata može se zaključiti da je praćenjem promjena biokemijskih parametara alkalne fosfataze i koštanog izoenzima moguće rano otkrivati dinamiku sanacije koštanog prijeloma. Manje pove**ć**anje aktivnosti ili izostanak promjene biokemijskih parametara (alkalna fosfataza i koštani izoenzim) do četrnaestog dana je oznaka za dobro izvedenu osteosintezu, brzu sanaciju koštanog prijeloma sa stvaranjem minimalnog odnosno beznačajnog kalusa, dok je veće povećanje aktivnosti biokemijskih parametara (alkalna fosfataza i koštani izoenzim) do četrnaestog dana oznaka za lošije izvršenu steosintezu, usporenu sanaciju koštanog prijeloma sa stvaranjem vidljivo zanačajnog volumena kalusa.