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Evaluation of the serum ionic fluoride concentration as a biomarker of bone metabolism post-spinal fusion surgery

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ABSTRACT

Background: Bone union after spinal fusion surgery with instrumentation has been determined only with imaging studies. We evaluated the usefulness of the serum ionic fluoride (SIF) concentration as a biomarker of the bone union status.
Methods: We enrolled 25 patients who underwent spinal surgery in our institution, and we divided patients into three groups with and without instrumentation (G1, G2, and G3). We collected the fasting serum level preoperatively and on day 1 (D1), week 1 (D7), week 2 (D14), month 1 (D30), month 3 (D90), and month 6 (D180) postoperatively, and measured SIF concentrations using the flow injection method with an ion-selective electrode.
Results: Although preoperative SIF concentrations were similar among the 3 groups, postoperative SIF concentrations were different among the groups. SIF concentrations in groups with instrumentation (G2 and G3) increased between D14 and D90 postoperatively and decreased at D180 postoperatively. SIF concentrations in the group without instrumentation (G1) decreased between D30 and D180 postoperatively.
Conclusions: An SIF concentration that is higher postoperatively than preoperatively may indicate unstable bone union, whereas a lower SIF concentration postoperatively than preoperatively may indicate stable bone union. We concluded that the SIF concentration may be useful for diagnosing bone union.

1. Introduction

Spinal fusion surgery with instrumentation is rapidly developing because the estimated number of patients with a spinal disorder associated with mechanical instability or deformity has increased [1]. Bone grafting is performed at the fusion site, and the corrected position can be maintained by acquiring bone union. The confirmation of bone union is extremely important for combined postoperative treatment and instruction in activities of daily living. However, bone union is presently assessed comprehensively based on morphological information from modalities such as diagnostic imaging (radiography, computed tomography [CT], and magnetic resonance imaging) [2,3].

These morphologic modalities result in expose to radiation and expensive medical costs. Therefore, a useful biomarker for assessing bone union is desirable. We focused on serum ionic fluoride (SIF) concentrations as a possible biomarker. Fluoride has a high affinity for bone materials [4,5]. Hence, fludeoxyglucose positron emission

tomography is used to assess bone metabolism and bone union [6,7]. We previously reported that in osteoporotic patients, SIF concentrations were significantly reduced following treatment in an alendronate treatment group, indicating that the SIF concentration may reflect bone metabolism [8]. We hypothesized that SIF concentrations might increase in unstable bone union but decrease in stable bone union.

2. Materials and methods

2.1. Subjects

One hundred eighteen patients who underwent spinal surgery in the Department of Orthopedic Surgery, Iwate Medical University Hospital between March and December 2016 were screened for study inclusion. Forty-two patients who received inhalational anesthetics were excluded [9]. In addition, patients who were unable to be followed for 6 months postoperatively; and those with malignant tumors, metabolic bone

disease, renal dysfunction (estimated glomerular filtration rate [eGFR] < 30 ml/min/1.73 m^2) were excluded [10]. Finally, 25 patients were enrolled in the study for analysis.

The study patients were divided into three groups as follows: group 1 (G1) included patients who underwent surgery without the use of instrumentation; group 2 (G2) comprised patients who underwent surgery with single-segment or two-segment interbody fusion; and group 3 (G3) included patients who underwent surgery with multi-segmental (≥ 3 segments) interbody fusion and posterior fusion of ≥ 5 segments.

The present study was approved by the Institutional Review Board of Iwate Medical University. All patients provided written consent after receiving a sufficient explanation of the study. All researchers who engaged in the present study acted in accordance with the Declaration of Helsinki and the Ethical Guidelines for Medical and Health Research Involving Human Subjects.

2.2. Collection of blood samples

All blood samples were collected after 9 h of fasting [11]. Five milliliters of blood was collected preoperatively and 1 day (D1), 1 week (D7), 2 weeks (D14), 1 month (D30), 3 months (D90), and 6 months (D180) postoperatively. Collected blood samples were allowed to clot at room temperature and then centrifuged at 1500 rpm for 10 min. After separation, the serum samples were stored at −80 °C until analysis.

2.3. Measurements

To measure the SIF concentration, a flow injection analyzer (FIA) using a fluoride ion electrode as a detector was used [12]. The FIA system uses a Teflon tube with an inner diameter of 0.4 mm, and 3 double plunger backflow pumps (Uniflose) were used to pump the buffer solution. In order to stabilize the fluoride ion electrode (Model Orion 94–09, Thermo Scientific), we used purified water for the buffer solution, and the electrode (Model 4400, DKK) had a flow rate of 1.0 ml/min. After the electrode was stabilized, the sample was injected, and the measurement was performed. Purified water was prepared using the Milli-RQ and Milli-Q system (Millipore). The standard fluoride solution was prepared by diluting a stock solution (Wako) of 52.6 mmol/l (1000 mg/l). Buffers were prepared as follows. First, 136 g of sodium acetate trihydrate, 117 g of sodium chloride, 2.5 g of sodium dihydrogen phosphate dihydrate, and 2.5 g of trans-1,2-diaminocyclo-hexane-N, N, N, N-tetraacetic acid monohydrate CyDTA (Wako) were placed and dissolved in about 900 ml of purified water. Thereafter, the pH was adjusted to 5.30 with concentrated acetic acid and diluted to 1000 ml with purified water. The solution was filtered through a filter with a pore size of 0.45 µg, and finally, 1 g of Triton X-100 was added to the solution. A 1.2-ml diluted solution (0.05 mol/l sodium acetate solution, pH 5.0) was mixed with each serum sample (0.3 ml), and the pH was adjusted to 5.4 ± 0.2 with 0.1 or 0.5 mol/l hydrogen chloride and sodium hydroxide. The sample solution was injected into the FIA system with a 1-ml syringe. All standard solutions and sample solutions were measured twice. The curve of the height of the potential difference with respect to the serum fluorine concentration was calculated.

2.4. Surgery

The subjects in G1 underwent decompression of 1–2 segments. The extent of and operative procedures for instrumented fusion were as follows. For single-segment fusion, posterior lumbar interbody fusion (PLIF) was performed in 2 patients, transforaminal lumbar interbody fusion (TLIF) was performed in 2 patients, and a combination of lateral lumbar interbody fusion (LLIF) and percutaneous pedicle screwing (PPS) was performed in 3 patients. For two-segment fusion, PLIF was performed in 1 patient, while a combination of LLIF and PPS was performed in 3 patients. In the group of patients who underwent ≥ 3-

segment anterior fusion and multisegmental fusion of ≥ 5 segments for anterior interbody fusion, LLIF was performed in 7 patients. For posterior fusion, TLIF was performed at the L5/S1 level, PPS was performed at the lumbar site, and posterolateral fusion was performed at the thoracic site. In 1 patient, a combination of PLIF and pedicle subtraction osteotomy was performed.

2.5. Biochemical tests

Blood samples were collected from patients before breakfast on the day after admission; levels of albumin (Alb), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and creatinine (Cre) were compared among the groups. The aforementioned laboratory parameters were measured using an automated analyzer (Hitachi High-Technologies). The Alb level was measured using the BCP method (Kainos). The Cre level was measured using the enzymatic method (Sino-test). The eGFR was calculated using the originally established equation for Japanese subjects: eGFR = 194 × serum Cre level − 1.094 × Age − 0.287 × 0.739 [13].

2.6. Statistical analysis

One-way analysis of variance (ANOVA) was performed to compare variables among the three groups, and the Bonferroni test was used to conduct multiple comparisons. Repeated-measure ANOVA was performed to compare the SIF concentration among sampling periods in each group. The Dunnett test was performed to compare the SIF concentrations between the sampling periods of blood samples and when SIF concentrations were 0 µmol/l preoperatively. The Tukey test was performed to compare SIF concentrations based on the sampling periods. The significance probability was 0.05 in two-sided tests. Statistical calculation software SPSS 24.0 J for Mac (SPSS Japan) was used for all analyses.

3. Results

Table 1 shows the characteristics of the 25 subjects by groups. The numbers of subjects (percentages of men) in G1, G2, and G3 were 6 (66.6%), 11 (27.3%), and 8 (36.0%), respectively. The average ages of the groups were 56.5, 61.6, and 69.0 y, respectively. No statistically significant differences were observed among the three groups with respect to body mass index, systolic blood pressure, diastolic blood pressure, AST and ALT activities, and eGFR. There were significant differences in the Alb levels among the three groups (p = 0.031). The operative time and blood loss were different among the 3 groups.

Table 2 shows the average SIF levels of each group preoperatively and postoperatively. SIF concentrations (µmol/l) were similar in the 3 groups and ranged from 0.811 to 0.826 µmol/l. In G1, SIF

Table 1
Baseline characteristic of subjects.

|	Group 1	Group 2	Group 3	Trend P
Number of subjects	6	11	8	
Male (%)	4 (66.6)	3 (27.3)	2 (25.0)	< 0.001
Age (y)	56.5 (14.7)	61.6 (15.2)	69.0 (9.4)	NS
BMI (kg/m^2)	26.4 (4.0)	24.4 (3.9)	25.1 (4.6)	NS
SBP (mmHg)	123 (15)	121 (11)	119 (17)	NS
DBP (mmHg)	67 (10)	73 (9)	70 (10)	NS
Alb (g/dl)	4.3 (0.3)	4.1 (0.4)	3.8 (0.2)	0.031
AST (U/l)	29 (19)	24 (18)	22 (19)	NS
ALT (U/l)	25 (13)	31 (40)	22 (19)	NS
eGFR (ml/min/1.73 m^2)	67.4 (22.6)	79.9 (15.6)	77.4 (20.6)	NS
Operative time (min)	111 (57)	156(96)	516(134)	< 0.001
Blood loss (mg)	63 (89)	168 (2.2)	1698 (1383)	0.001

Data are expressed as means (standard deviation) or percentages.
Abbreviation: SBP, systolic blood pressure; DBP, diastolic blood pressure.

Table 2
Average serum ionic fluoride concentrations.

Group	No. of subjects	SIF concentrations (μmol/L)						
		Before	1	7	14	30	90	180
1	6	0.826(0.16)	0.789(0.25)	0.863(0.35)	0.863(0.22)	0.837(0.22)	0.784(0.21)	0.563(0.23)
2	11	0.811(0.29)	0.668(0.25)	0.795(0.16)	0.847(0.34)	0.953(0.37)	0.890(0.22)	0.700(0.18)
3	8	0.816(0.21)	0.811(0.30)	0.663(0.16)	0.847(0.26)	0.905(0.28)	0.963(0.29)	0.763(0.13)
total	25	0.816(0.23)	0.742(0.26)	0.768(0.28)	0.847(0.27)	0.911(0.30)	0.889(0.24)	0.684(0.19)

SIF concentrations are expressed as means (SD).
Abbreviation: SIF, serum ionic fluoride.
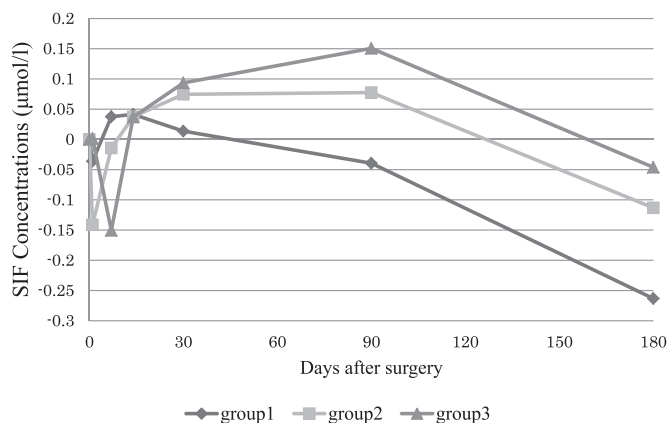
Fig. 1. Changes over days of serum ionic fluoride concentrations. Changes were expressed as average differences from before surgery.
Abbreviation: SIF, serum ionic fluoride.

concentrations postoperatively were as follows: 0.863 μmol/l at D7 and D14, 0.837 μmol/l at D30, 0.784 μmol/l at D90, and 0.563 μmol/l at D180. In G2, SIF concentrations postoperatively were as follows: 0.668 μmol/l at D1, 0.795 μmol/l at D7, 0.847 μmol/l at D14, 0.953 μmol/l at D30, 0.890 μmol/l at D90, and 0.700 μmol/l at D180. In G3, SIF concentrations postoperatively were as follows: 0.811 μmol/l at D1, 0.663 μmol/l at D7, 0.847 μmol/l at D14, 0.905 μmol/l at D30, 0.963 μmol/l at D90, and 0.763 μmol/l at D180.

Fig. 1 shows changes in the SIF concentration postoperatively compared to those preoperatively in each group. The changes were shown as relative SIF levels when the SIF concentration preoperatively was 0 μmol/l. In G1, SIF levels were slightly decreased at D1, slightly increased at D7 and D14, and decreased from D30 to D180. The SIF level at D180 was significantly lower than the levels at D7 ($p = 0.026$), D14 ($p = 0.024$), and D30 ($p = 0.05$).

In G2, SIF levels decreased to -0.141 μmol/l at D1, increased to -0.0142 μmol/l and 0.774 μmol/l from D7 to D90, respectively, and decreased to -0.113 μmol/l at D180. SIF levels at D1 and D180 were significantly lower than those at D30 ($p = 0.011$ and $p = 0.042$, respectively) and D90 ($p = 0.010$ and $p = 0.037$, respectively).

In G3, the SIF level at D1 was the same as that preoperatively. The SIF level at D7 decreased to -0.151 μmol/l, increased to 0.0369 μmol/l and 0.151 μmol/l from D14 to D90, respectively, and decreased to 0.0463 μmol/l at D180. The SIF level at D7 was significantly lower than that at D90 ($p = 0.034$).

4. Discussion

In this study, we confirmed that SIF concentrations increased between D14 and D90 postoperatively with instrumentations compared to preoperatively. SIF concentrations decreased at D180 postoperatively with and without instrumentation.

SIF concentrations transiently decreased at D1 in G1 and G2, and at

D7 in G3 postoperatively. Larger decreases in the levels were observed in G2 and G3 compared with G1, and the decreases in G3 were delayed compared to those in G1 and G2. We cannot explain the mechanism of the phenomenon of these transient decreases. However, we speculated that the rapid bone formation postoperatively might contribute to these decreasing SIF levels. The larger areas of invasion might contribute to the larger decreasing levels in G2 and G3 than in G1 because bone formation occurs in larger areas. The considerable large areas of invasion might contribute to the delayed decrease in SIF levels at D7 in G3.

In G1, SIF levels increased at D7 and D14 after the subtle, transient decreases. In G2 and G3, larger increases of SIF concentrations were observed between D14 and D90 after the transient decreases. It was expected that these increases in the SIF concentrations in G2 and G3 were caused by the wear of bone materials by instruments in the areas of invasion [14–16]. Immediately postoperatively, the fusion of bone materials with instruments was not obtained. Therefore, fluoride contained in bone materials was released in the blood by micro-movement of the instruments [17,18].

At D180 postoperatively, SIF concentrations decreased in G2 and G3. In G1, SIF concentrations decreased from D30 postoperatively. In G2 and G3, fusion of the bone with instruments was obtained at D180 postoperatively; therefore, the release of fluoride in the bone to the blood did not occur. Furthermore, fluoride in the blood was absorbed by the bone materials because of active bone formation postoperatively. Consequently, resultant decreases in SIF concentrations at D180 postoperatively in G2 and G3 were observed. In G1, while slight changes of SIF concentrations were observed until D14, clear decreases were observed from D30. This suggests that bone formation occurred at D30 and continued until D180 postoperatively in G1.

There are a couple of limitations in this study: the small number of cases and the short observation period. However, the method used in this study to measure SIF concentrations has high sensitivity and specificity. This is the merit of this study.

5. Conclusions

Despite the limitations, we concluded that the SIF concentration may be useful for diagnosing bone union. An SIF concentration that is higher postoperatively than preoperatively may indicate unstable bone union, whereas a lower SIF concentration postoperatively than preoperatively may indicate stable bone union. Further studies are needed to confirm the usefulness of SIF concentrations as a biomarker of spinal fusion surgery with instrumentation.

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