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

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A B S T R A C T

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.