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Serum Periostin Level Reflects Progression of Ossification of the Posterior Longitudinal Ligament

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Background: Ossification of the posterior longitudinal ligament (OPLL), characterized by ectopic new bone formation in the spinal ligament, causes neurological impairment due to narrowing of the spinal canal. However, the etiology has not been fully elucidated yet. Several biomarkers may be related to the pathogenesis of OPLL. The present study focused on the serum level of periostin, which is recognized as an important bone formation regulator.

Methods: This study included 92 patients with OPLL and 54 control patients without OPLL. For the case-control analysis, 54 age and sex-matched patients were randomly included in the OPLL group. The serum fibroblast growth factor-23 (FGF-23), creatinine, inorganic phosphate, calcium, alkaline phosphatase, and periostin levels were assessed. Furthermore, the calcium, creatinine, and inorganic phosphate levels in urine and the percentage of tubular reabsorption of phosphate were also analyzed. Moreover, the relationship between the biomarkers and the extent of OPLL was analyzed. The data were compared between patients with OPLL progression (the progression group) and without OPLL progression (the non-progression group).

Results: The mean serum FGF-23 and periostin levels in the OPLL group were higher than that in the control group. The serum inorganic phosphate level in the OPLL group was lower than that in the control group. No correlation was found between any of the biomarkers and the extent of ossification. The serum periostin level in the progression group was higher than that in the non-progression group. No significant difference in the serum FGF-23 level was noted between the progression and non-progression groups. Moreover, no correlation was found between serum periostin and FGF-23 levels.

Conclusions: The serum periostin level is related to OPLL progression.

Level of Evidence: Prognostic Level III. See Instructions for Authors for a complete description of levels of evidence.

Ossification of the posterior longitudinal ligament (OPLL), which occurs following ectopic new bone formation along the spinal ligament, results in the reduction of the spinal canal leading to neurological impairments, such as cervical myelopathy and radiculopathy (Fig. 1)[1,2]. OPLL was reported in the early 1960s. However, the etiology has not been fully elucidated yet. A systemic rise in bone formation activity is observed among patients with OPLL. According to a report by Resnick et al., OPLL may be considered as a partial phenotype of diffuse idiopathic skeletal hyperostosis[3]. Previous studies have revealed that several biomarkers are related to OPLL pathogenesis[4-9]. The serum intact osteocalcin and carboxyterminal propeptide of human type-I procollagen levels are used to characterize the activity of the general ectopic bone formation in OPLL[5]. Moreover, the serum leptin and insulin levels are related to OPLL[6,7]. Serum sclerostin (SOST) levels among men with OPLL are greater than those in control subjects, and a negative correlation exists between SOST and Dickkopf-1 (DKK1) levels in male patients with OPLL[8]. A previous study revealed that the serum hypersensitive C-reactive protein (hs-CRP) level in the OPLL group was greater than that in the control group, and the serum hs-CRP level was also higher in the OPLL progression group compared with the OPLL non-progression group[9]. Bone metabolism may be associated with the pathogenesis of OPLL according to these results, and the occurrence of local inflammation is suggested in patients with OPLL. Furthermore, a recent study found that the level of serum fibroblast growth factor-23 (FGF-23)[10], which is expressed in osteophytes and by osteoblasts in the bone and plays a role regulating phosphate levels in the plasma[10-12],

Disclosure: The **Disclosure of Potential Conflicts of Interest** forms are provided with the online version of the article (http://links.lww.com/JBJSOA/A354).

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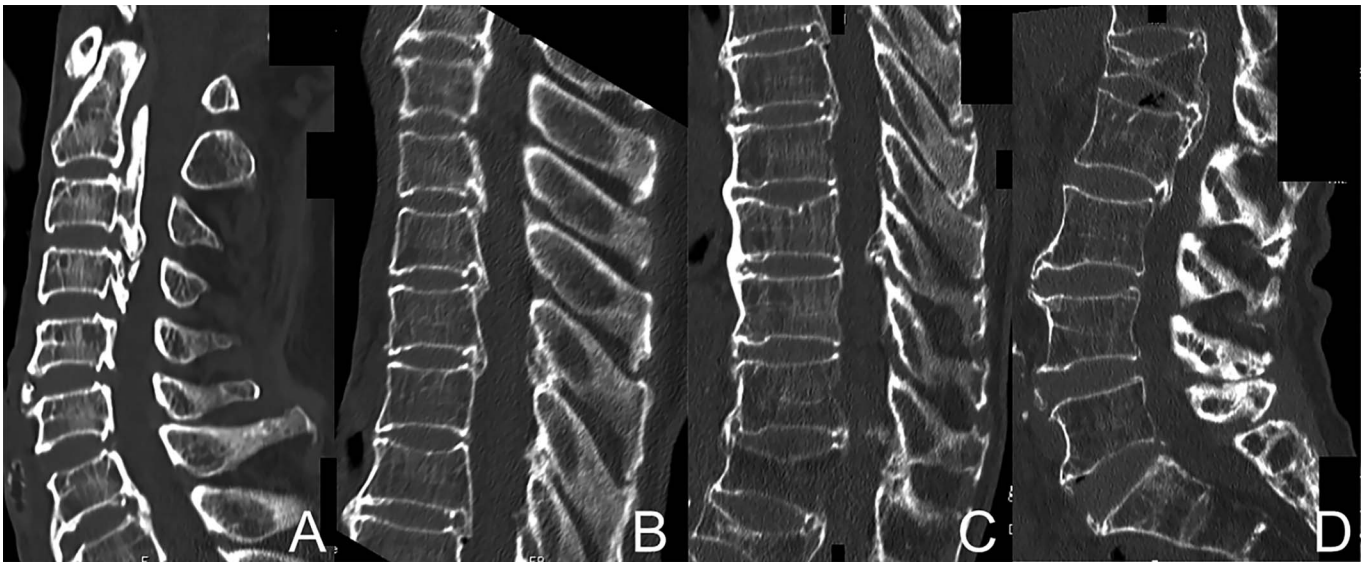

Fig. 1

A 76-year-old woman with OPLL at the cervical spine (**Fig. 1-A**), upper thoracic spine (**Fig. 1-B**), lower thoracic spine (**Fig. 1-C**), and lumbar spine (**Fig. 1-D**). The patient also had ossification of the ligamentum flavum and ossification of the anterior longitudinal ligament at the thoracic spine. The ossification indices of the patient were as follows: OP: 16, OF: 4, OA: 7, and OS: 27.

was higher[13] and the serum inorganic phosphate level was lower in individuals with OPLL compared with control subjects[9,10].

This study investigated the serum periostin level. Periostin, identified in a mouse osteoblastic cell line in 1993, was originally named osteospecific factor 2[14]. It is primarily produced by osteoblasts and osteocytes and is highly secreted by the periosteum[15]. It has been recognized as an important bone formation regulator[16-18]. Periostin is also found in the lung, heart valves, intestines, and skin and contributes to heart valve development, the healing process in wounds, the fibrotic process, and cancer invasion[19-21]. Recent studies have revealed that periostin is a useful biomarker for asthma[22,23], atopic dermatitis[24], interstitial pneumonia[25], systemic sclerosis[26], proliferative diabetic retinopathy[27], and cholangiocarcinoma[28]. With regard to musculoskeletal diseases, periostin is associated with osteoarthritis[29,30]. However, no study has investigated the relationship between periostin and OPLL. Thus, whether periostin can be a positive OPLL biomarker is of great interest. This study was performed to compare the serum periostin levels in individuals with OPLL and their age and sex-matched controls and to evaluate the factors related to the change in the serum periostin levels over time. Our hypothesis was that the concentration of serum periostin increases in individuals with OPLL, whereas it does not in patients without OPLL, referred to as the control group in this study.

TABLE I Demographic Data of the OPLL Group and the Control Group

	OPLL	Control	P Value
Sex*			1
Male	27	27	
Female	27	27	
Age† *(yr)*	71.0 ± 11.2	68.9 ± 13.5	0.32
Height† *(cm)*			
Men	164.7 ± 6.5	164.7 ± 6.7	0.32
Women	153.4 ± 5.9	151.3 ± 7.4	0.26
Weight† *(kg)*			
Men	68.0 ± 13.2	65.7 ± 9.7	0.08
Women	58.4 ± 9.7	56.4 ± 11.0	0.10
BMI† *(kg/m²)*			
Men	25.0 ± 4.4	24.1 ± 2.6	0.08
Women	24.8 ± 3.8	24.5 ± 3.8	0.18

*The values are given as the number of patients. †The values are given as the mean and the standard deviation.

Materials and Methods

Data were collected from 97 individuals with OPLL (59 men and 38 women, with a mean age [and standard deviation] of 69.3 ± 10.4 years) and 61 control patients without OPLL (31 men and 30 women, with a mean age of 69.8 ± 13.3 years). All individuals with OPLL and control patients were recruited from 2014 to 2016 from the outpatient clinic at our hospital. The study protocol was approved by the ethics committee of the university hospital of this study. Informed consent was obtained from all subjects, with a written explanation indicating their freely expressed willingness to be involved in this study. Among these patients, 5 in the OPLL group and 7 in the control group were excluded because they had diseases that may have affected the data. Three patients had cancer, 1 patient had pneumonia, and 1 patient had spondylodiscitis in the OPLL group, whereas 3

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TABLE II Comparison of Biomarkers Between the OPLL Group and the Control Group

Biomarkers	OPLL Group*	Control Group*	P Value
Serum FGF-23 *(pg/mL)*	56.3 ± 29.4	41.1 ± 32.4	0.013
Creatinine *(mg/dL)*			
Serum	0.84 ± 0.27	0.78 ± 0.35	0.3
Urine	84.6 ± 54.1	81.3 ± 65.9	0.8
Inorganic phosphate *(mg/dL)*			
Serum	3.4 ± 0.5	3.6 ± 0.5	0.04
Urine	34.1 ± 24.4	37.7 ± 26.8	0.5
%TRP	89.0 ± 6.2	89.0 ± 6.8	1
Alkaline phosphatase *(U/L)*	251.2 ± 58.8	225.9 ± 77.5	0.06
Calcium *(mg/dL)*			
Serum	9.5 ± 0.3	9.4 ± 0.5	0.2
Urine	11.3 ± 10.7	11.7 ± 10.3	0.44
Periostin *(ng/mL)*	87.8 ± 29.8	69.6 ± 18.7	0.0003

*The values are given as the mean and the standard deviation.

patients had dermatitis, 1 patient had ulcerative colitis, 1 patient had asthma, 1 patient had allergic rhinitis, and 1 patient had gastric cancer in the control group. Therefore, 92 patients with OPLL (56 men and 36 women, with a mean age of 68.8 ± 10.4 years [range, 44 to 90 years]) and 54 control patients without OPLL (27 men and 27 women, with a mean age of 68.9 ± 13.5 years [range, 30 to 88 years]) were included in this study. As for the case-control analysis, 54 age and sex-matched patients were randomly included in the OPLL group. The demographic data of the study subjects are shown in Table I.

OPLL diagnosis was performed using radiographic findings, such as radiographs and computed tomographic (CT) scans of the cervical, thoracic, and lumbar spine. For all patients with OPLL, whole-spine CT images were utilized for the assessment of the ossified OPLL lesions. Metabolic diseases related to OPLL (e.g., hypophosphatemic rickets or osteomalacia and hyperparathyroidism) were excluded on the basis of radiographic and biochemical assessments. The control patients were diagnosed with cervical spondylotic myelopathy (13 patients), cervical spondylosis (1 patient), cervical disc herniation (3 patients), lumbar spondylosis (8 patients), lumbar disc herniation (6 patients), and lumbar spinal stenosis (23 patients). The diseases were diagnosed using imaging studies, including radiographs, CT, and magnetic resonance imaging. The control patients had either cervical or lumbar spinal disease, which was treated at our outpatient clinic, but none of the control subjects were observed to have spinal ossifications based on radiographic studies. Patients with inflammatory disease (e.g., collagen diseases and rheumatoid arthritis), infection, trauma, ischemic heart disease, cerebral infarction, cancer, or renal disease, in either the OPLL group or the control group, were strictly excluded. None of the patients with OPLL or the control patients had severe osteoarthritis requiring joint replacement. The diseases reported to affect the serum periostin level were also strictly excluded.

Blood and urine samples were collected from all participants on the morning of the hospital visit. The serum and urine samples were immediately stored at −80°C until analysis. The serum intact FGF-23 level was assessed using a sandwich enzyme-linked immunosorbent assay (ELISA) kit (Kainos Laboratories) following the manufacturer's instructions. The detection limit indicated by the manufacturer is 2 pg/mL, and the intra-assay and inter-assay coefficients of variation are reported as 2.1% to 3.8%[31]. The concentration of serum periostin was measured using ELISA with 2 purchased rat anti-periostin monoclonal antibodies (clone numbers, SS18A and SS17B; IBL International), as previously reported[25]. Additional laboratory tests, including the assessment of serum creatinine, inorganic phosphate, calcium, and alkaline phosphatase levels, were performed. Furthermore, calcium, creatinine, and inorganic phosphate levels in urine and the percentage of tubular reabsorption of phosphate (%TRP) were also analyzed.

The severity of the ossified lesions in the whole spine was assessed using a previously published index (the ossification [OP] index, which indicates the extent of the ossification area of the OPLL) in patients with OPLL[32,33]. This index is determined by the sum of the number of levels of vertebral bodies and intervertebral discs where OPLL is present. To quantify ossified lesions of the PLL, the distribution of OPLL at each vertebral body and intervertebral disc level was recorded, and the number of levels at which OPLL was present was defined as the OP index. Theoretically, the maximum OP index is 14 in the cervical spine. The OP index in the thoracic spine ranges from 0 to 24, and the OP index in the lumbar spine ranges from 0 to 11. The same index for ossification of the ligamentum flavum (the OF index) and the anterior longitudinal ligament (the OA index) was also determined. The summation of the OP, OF, and OA indices, termed the total ossification index (the OS

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TABLE III Comparison Between the OPLL Progression and Non-Progression Groups

	Non-Progression Group (N = 43)	Progression Group (N = 28)	P Value
Age* *(yr)*	71.4 ± 9.9	67.9 ± 10.1	0.15
Sex†			0.53
Male	26	19	
Female	17	9	
Follow-up* *(yr)*	4.6 ± 2.5	5.4 ± 2.9	0.22
Serum FGF-23* *(pg/mL)*	49.5 ± 23.0	59.9 ± 32.0	0.12
Creatinine* *(mg/dL)*			
Serum	0.8 ± 0.2	0.9 ± 0.3	0.09
Urine	89.1 ± 56.3	112.5 ± 78.2	0.14
Inorganic phosphate* *(mg/dL)*			
Serum	3.4 ± 0.6	3.5 ± 0.6	0.49
Urine	36.5 ± 24.2	48.0 ± 28.2	0.07
%TRP*	88.9 ± 5.8	87.6 ± 5.9	0.36
Alkaline phosphatase* *(U/L)*	239.2 ± 64.2	254.7 ± 75.6	0.36
Calcium* *(mg/dL)*			
Serum	9.5 ± 0.3	9.4 ± 0.3	0.17
Urine	11.7 ± 11.1	8.6 ± 6.5	0.19
Periostin* *(ng/mL)*	72.7 ± 20.4	87.5 ± 30.8	0.017

*The values are given as the mean and the standard deviation. †The values are given as the number of patients.

index), was utilized to indicate the extent of ossification in the spinal ligament.

Of the patients with OPLL, 71 (45 men and 26 women) completed follow-up of ≥2 years with radiographic examinations. The mean patient age was 70.0 ± 10.1 years (range, 45 to 90 years). The mean follow-up length (and thus the interval between the 2 images) was 5.0 ± 2.6 years (range, 2 to 12 years). Radiographs were used in 26 patients and CT images were used in 45 patients to evaluate OPLL lesions and determine OPLL progression. OPLL lengthening of >2 mm during the follow-up was categorized as OPLL progression. Data were compared between patients with OPLL progression (the progression group) and those without OPLL progression (the non-progression group).

The cutoff value of the serum periostin level for evaluating the OPLL and control subjects was identified using a receiver operating characteristic (ROC) curve. The same method was used for the comparison between the progression and non-progression groups. ROC analysis and measurement of the corresponding area under the curve (AUC) were conducted using R version 4.0.3 (The R Foundation for Statistical Computing) and the pROC package.

Statistical Analysis

A chi-square test was used to analyze the difference in sex between the OPLL and control groups. Age, height, weight, and body mass index (BMI), as well as the serum levels of the biomarkers, were presented as the mean and the standard deviation. The differences between groups were analyzed for significance using the Student t test (unpaired). Simple linear regression and Pearson correlation were used to analyze the relationship between the OP, OF, OA, and OS indices and several biomarkers, including FGF-23 and periostin. STATEMATE V software (Nihon 3B Scientific) was used for the analysis, and significance was set at $p < 0.05$.

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Results

Table II compared the serum and urine biomarker levels of the OPLL and control groups. The mean serum FGF-23 level in the OPLL group was higher than that in the control group ($p = 0.013$). Although the serum calcium level did not differ between the 2 groups, the serum inorganic phosphate level in the OPLL group was lower compared with the control group ($p = 0.04$). The creatinine, calcium, and inorganic phosphate levels in urine and the %TRP were similar between the 2 groups. The mean serum periostin concentration in the OPLL group was higher than that in the control group ($p = 0.0003$). When data were divided by sex, the mean serum periostin levels in men

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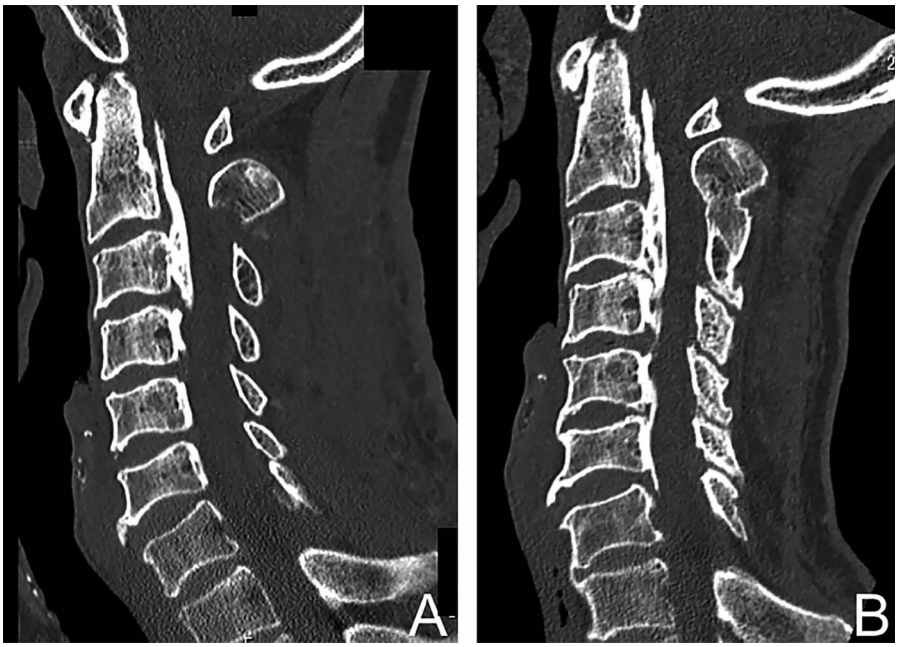

Fig. 2

A 63-year-old man had cervical laminoplasty (**Fig. 2-A**), and OPLL progression was found 6 years after the surgical procedure (**Fig. 2-B**).

(88.5 ± 26.5 ng/mL) and women (87.1 ± 33.3 ng/mL) in the OPLL group were greater than those in men (70.1 ± 19.7 ng/mL) and women (69.2 ± 18.0 ng/mL) in the control group; these differences between groups were significant for both men ($p = 0.006$) and women ($p = 0.017$). No difference in any biomarker between men and women was noted in either the OPLL group or the control group. A positive correlation was found between FGF-23 and inorganic phosphate, although the correlation was weak ($p = 0.037$; $r = 0.2$). However, no significant correlation existed between periostin and FGF-23 or inorganic phosphate.

The mean OP index of the total spine was 10.4 ± 6.5 (range, 2 to 37). The mean OF index was 1.0 ± 1.4 (range, 0 to 6), and the mean OA index was 12.1 ± 12.2 (range, 0 to 48). No correlation was established between the concentration of serum periostin and any of the indices, including the OP, OF, OA, and OS indices. Moreover, no correlation was found between any of the biomarkers and indices.

The data of the OPLL progression and non-progression groups are shown in Table III. Of the 71 patients, 28 (39%) had OPLL progression (Fig. 2). No significant difference was noted in sex, age, or follow-up length between the 2 groups. The serum periostin level was higher in the progression group than in the non-progression group ($p = 0.017$). No significant difference in the serum FGF-23 level was noted between the progression and non-progression groups ($p = 0.12$). None of the other biomarkers showed significant differences between the 2 groups. In addition, the difference in the serum periostin level between the non-progression OPLL and control groups was not significant.

The optimum cutoff value of the serum periostin level for the comparison between the OPLL and control groups was 76.8 ng/mL (Fig. 3). The AUC was 0.684 (95% confidence interval [CI], 0.583 to 0.784). The sensitivity was 0.685 and the specificity was 0.648. In addition, the cutoff value for the comparison of periostin between the OPLL progression and non-progression groups was 72.0 ng/mL (Fig. 4). The AUC was 0.653 (95% CI, 0.521 to 0.786). The sensitivity was 0.821 and the specificity was 0.512.

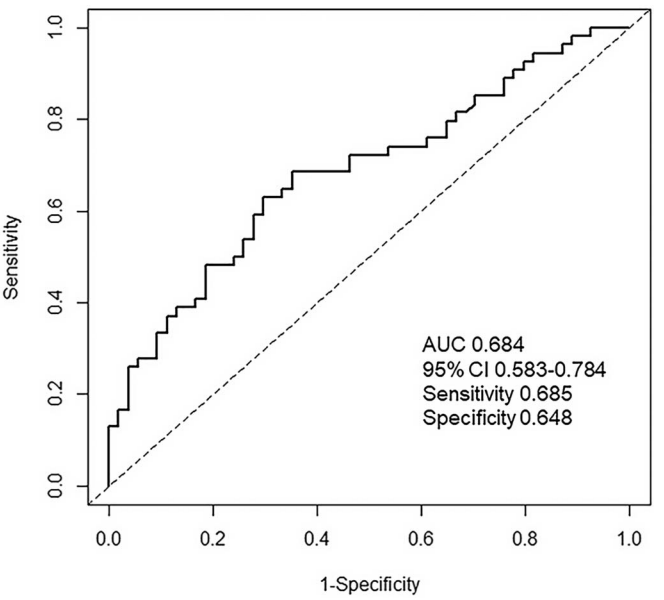

Fig. 3

Identification of the cutoff value for the comparison of periostin between the OPLL group and the control group using an ROC curve. The cutoff value of the serum periostin level was 76.8 ng/mL. The AUC was 0.684 (95% CI, 0.583 to 0.784). The sensitivity was 0.685 and the specificity was 0.648.

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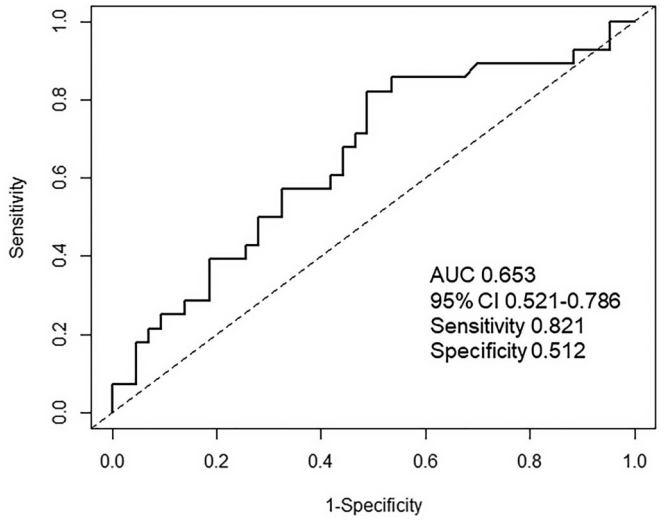

Fig. 4

Identification of the cutoff value for the comparison of periostin between the OPLL progression and non-progression groups using an ROC curve. The cutoff value of the serum periostin level was 72.0 ng/mL. The AUC was 0.653 (95% CI, 0.521 to 0.786). The sensitivity was 0.821 and the specificity was 0.512.

Discussion

Periostin is secreted by the periosteum of the bone and increases bone formation by osteoblasts. Walsh et al. showed that serum periostin levels were higher in adolescents than in elderly subjects, and insulin-like growth factor 1 (IGF-1) was positively correlated with the serum periostin levels[18]. Based on these results, periostin was speculated to have a role in IGF-1-driven cortical bone remodeling and consolidation in young adults. Thus, periostin is recognized as an important bone formation regulator[34].

In this study, the concentration of serum periostin was greater in patients with OPLL than in control patients. This fact may reflect a systemic increase of bone formation activity in OPLL. This is reasonable based on the pathology of OPLL. However, no correlation was found between the serum periostin level and the OS index, which indicates the extent of ossification in the spinal ligaments. The OS index is merely an indicator of the spread of ectopic bone formation in the spinal ligaments. Interestingly, the serum periostin level was higher in the OPLL progression group than in the non-progression group. This may indicate that periostin reflects active bone formation in the spinal ligaments. However, no significant difference was found between the OPLL non-progression group and the control group. This finding is important because some patients have neurological deterioration due to OPLL progression in the long-term follow-up after cervical laminoplasty[35]. Thus, periostin may be a positive biomarker that reflects OPLL progression at the time of follow-up. The cutoff value of the serum periostin level for the comparison between the OPLL progression and non-progression groups might be a useful way to identify those with a tendency for OPLL progression.

Periostin has been found to contribute to the biomechanical properties of connective tissues[36]. OPLL progression is induced by mechanical stress. In vitro data have suggested that mechanical stress plays an important role in OPLL progression through vimentin[37] and connexin 43[38]. Thus, it is reasonable that periostin may induce OPLL progression under mechanical stress in the spinal ligament.

Transforming growth factor-beta (TGF-β) and the bone morphogenetic proteins (BMPs) are widely recognized to play roles in bone formation during mammalian development. Periostin can be induced by TGF-β[15] and BMP-2[39]. In their recent study using a bone defect model in rabbits, Zhang et al.[40] reported that periostin promotes osteogenesis by upregulating Wnt/β-catenin signaling. Using mesenchymal stem cells with soluble periostin modified with cytotoxic T-lymphocyte-associated antigen 4 (CTLA4) may inhibit the glycogen synthase kinase-3β activity and may increase the β-catenin expression by upregulating lipoprotein-related protein-6 phosphorylation in order to promote osteogenic differentiation. However, these effects could be suppressed by blocking integrin αvβ3, a receptor of periostin. The Wnt/β-catenin signaling pathway plays an important role in osteogenesis. SOST and DKK1 are antagonists for the Wnt/β-catenin signaling pathway. Kashii et al. demonstrated that serum SOST levels in men with OPLL were higher than those in the control subjects, and a negative correlation was found between SOST and DKK1[8]. Niu et al. found that the DKK1 level was lower and SOST was higher in patients with diffuse idiopathic skeletal hyperostosis and patients with OPLL than in the control group[41]. Thus, the higher periostin level in patients with OPLL may be due to increased osteogenesis in the spinal ligament through Wnt/β-catenin signaling.

In a previous study and this study, the serum FGF-23 concentration in the OPLL group was demonstrated to be significantly higher compared with the control group, and the serum inorganic phosphate level in the OPLL group was lower than that in the control group[10]. Thus, FGF-23 and inorganic phosphate could also be positive OPLL biomarkers. However, no correlation was found between periostin and FGF-23. Similarly, no correlation was found between periostin and inorganic phosphate. Based on the results, periostin, FGF-23, and inorganic phosphate may be considered independent OPLL biomarkers. Further research should be performed, although the mechanisms are still unclear.

The present study had several limitations. First, a limited number of patients and controls were included in the study. A larger sample size is needed for future studies. Second, patients with severe hip and/or knee osteoarthritis requiring joint replacement were excluded. However, whether slight joint osteoarthritis or spondylosis affects the serum periostin level is unclear. In the future, a precise medical history should be taken in both patients with OPLL and control patients. Third, the evaluation point was a single time point. Multiple measurements over time would be helpful to study the relationship between the periostin level and OPLL progression. Fourth, in vivo analysis with regard to the relationship between periostin and the Wnt/β-catenin signaling pathway should be performed in patients

with OPLL. Furthermore, the relationships between periostin and both SOST and DKK1 should be analyzed. Lastly, the mechanism of the OPLL pathology with regard to periostin, FGF-23, and inorganic phosphate should be examined.

In conclusion, periostin is a positive biomarker that reflects OPLL progression. ■

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