Biomarker Research Approach to the Pathogenesis of Ossification of the Spinal Ligament: A Review

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Abstract:

The ossification of the spinal ligaments (OSL) is characterized by ectopic new bone formation in the spinal ligament. However, the etiology of OSL has not yet been fully elucidated. This review paper summarizes the contents of previous reviews, introduces recent advances in the study of OSL and discusses future perspectives. A review of the literature that investigated the biomarkers involved in OPLL was published in 2019. The review cited 11 reports in which a calcium phosphate metabolism marker, bone turnover markers, sclerostin, dickkopf-1, secreted frizzled-related protein-1, fibroblast growth factor-23, fibronectin, menatetrenone, leptin, pentosidine, and hypersensitive C-reactive protein were examined as markers. Data published in 2021 noted that non-coding RNAs might be useful biomarkers for OSL. In addition, triglycerides, uric acid, gene expression levels of interleukin-17 receptor C, chemokine (C-X-C motif) ligand 7 (CXCL7) in the serum reportedly are biomarkers of OSL. However, several issues have been raised in previous studies. Therefore, biomarkers have yet to be conclusively investigated. Research using biomarkers is very important in clarifying pathomechanisms. Results for studies using biomarkers might also be useful for the treatment of patients with OSL in the near future.

Keywords:

biomarkers, ossification of spinal ligaments, pathogenesis

Spine Surg Relat Res 2022; 6(3): 224-232

dx.doi.org/10.22603/ssrr.2021-0229

1. Introduction

The ossification of the spinal ligaments (OSL) causes neurological symptoms, such as cervical myelopathy and/or radiculopathy, owing to the narrowing of the spinal canal. Some patients' neurological impairment results in quadriplegia and/or severe disability, impacting the activities of daily living. Clinical Practice Guidelines for Ossification of Spinal Ligaments were published in Japanese in 2019 and were translated into English in 2021[1]. OSL consists of three pathological categories: cervical ossification of the posterior longitudinal ligament (cervical OPLL); thoracic ossification of the posterior longitudinal ligament (thoracic OPLL); and thoracic ossification of the ligamentum flavum (thoracic OLF). According to the guidelines, the incidence of cervical OPLL is approximately 3% (1.9%-4.3%) in Japanese patients. Rates in East Asian countries are approximately equal to that in Japan, including rates of 2.8%-3.0% among Taiwanese, 0.95%-3.6% among Korean people, and 1.1%-1.7% among Chinese. However, the incidence of cervical OPLL is lower in Caucasian populations than in Asian populations. Cervical OPLL is predominant in male patients, whereas thoracic OPLL is predominant in female patients. Surgical treatment for thoracic OPLL can be very difficult. OLF is often associated with OPLL and is frequently seen in the upper (T3-T5) and lower thoracic spine (T10-12). OSL, including cervical OPLL, thoracic OPLL, and thoracic OLF, is characterized by ectopic new bone formations in the spinal ligament. However, the etiology of OSL has not yet been fully elucidated. It is very important to clarify the pathogenesis of OSL. There are two possible approaches for the research of OSL pathology as follows: a genetic and a biomarker approach. To date, numerous candidate genes have been identified, which were reviewed in an article published in 2017[2]. Additionally, several biomarkers for OPLL and OLF have been identified, but have not yet been confirmed. One review article on potential biomarkers for OSL was published in 2019[3]. This review paper summarizes the contents of the previous reviews, introduces recent advances in the study of OSL and discusses future perspectives.

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Received: November 25, 2021, Accepted: December 31, 2021, Advance Publication: April 12, 2022

2. Summary of the Literature on Biomarkers for OSL

The search for OSL biomarkers started in 1985. Takuwa et al.[4] were the first to determine that inorganic phosphate levels were lower in OSL patients than in controls. They also showed that the tubular resorptive capacity for phosphate to glomerular infiltration rate (TmP/GFR) was decreased in patients with OSL compared with controls, and stated that patients with OSL demonstrated a tendency for low serum inorganic phosphate with a reduced TmP/GFR. These results were related to the high incidence of OPLL in patients with calcium and phosphate metabolism disorders, vitamin D-resistant rickets, and hypoparathyroidism and hyperparathyroidism[1].

A review of the literature that investigated the biomarkers involved in OPLL was published in 2019[3] (Table 1).The data were extracted from articles published from 1985 to 2017. There were nine articles from Japan, one article from Taiwan, and one article from China. The literature search found no articles from North or South America, European countries, or African countries. This is because OSL is more common in Asian countries than in Western countries. The review cited 11 reports in which a calcium phosphate metabolism marker, bone turnover markers, sclerostin, dickkopf-1 (DKK1), secreted frizzled-related protein-1, fibroblast growth factor-23 (FGF-23), fibronectin, menatetrenone, leptin, pentosidine, and hypersensitive C-reactive protein were examined as markers. However, the numbers of cases and controls were too small in all these studies; only two articles included more than 100 patients with OPLL, and four included fewer than 30 subjects as controls. The small number of subjects makes definitive conclusions difficult. In addition, limited data were available to reproduce studies that employed the possible candidate biomarkers. A study that could reproduce these data in terms of the serum level of DKK1 was published in 2020[5]. The level of DKK1 decreased in patients with OPLL in comparison with those without OPLL. This finding was similar to that in a previous study[6]. Most importantly, no studies functionally demonstrated how the candidate biomarkers brought about ectopic ossification in the spinal ligament. Therefore, no definite conclusion has been reached regarding biomarkers for OSL. Table 1 summarizes the biomarkers for OSL in a case-control study published in the Global Spine Journal (GSJ) in 2019[3]. (The table is inserted in this paper with the permission of GSJ.)

3. Recent Advances Regarding Biomarkers for OSL (Table 2)

A review published in 2021 noted that non-coding RNAs (ncRNAs) might be useful biomarkers for OSL[7]. Non-coding RNAs include microRNAs (miRNAs), long non-coding RNAs, and circular RNAs. Recent studies have revealed that ncRNAs are involved in many physiological and pathological processes, such as cancer, inflammation, and degenerative diseases. A Chinese group found significant differences in miR-10a-3p, miR-10a-5p, miR-563, miR-210-3p, and miR-218-3p when comparing blood samples from OPLL and non-OPLL patients[8]. They used high-throughput miRNA sequencing data from OPLL and non-ossified posterior longitudinal ligament cells and selected the 10 most differentially expressed miRNAs. Then, they analyzed the levels of miRNA in the blood samples of patients and performed a case-control study. The authors stated that blood tests for these markers may be useful in a clinical setting for early detection of OPLL. This study was based on previous results using ligament cells from OPLL and non-OPLL patients by the same Chinese research group; they found an OPLL-specific miRNA and described its regulatory network[9]. A series of their studies found that microRNA-10a actively modulates the ossification of posterior ligament cells in vitro. By modulating the ID3/RUNX2 axis using OPLL model mice, the authors identified a critical role for the highly increased levels of microRNA-10a in the regulation of OPLL development[10]. They also found that the long non-coding RNA X-inactive-specific transcript (XIST) has four binding sites for miR-17-5p and that miR-17-5p was also significantly decreased in OPLL ligament fibroblast compared with non-OPLL ligament fibroblast cells[11]. They described how XIST gene inhibition plays an important role in the occurrence of cervical OPLL through the regulation of the miR-17-5P/AHNAK/BMP2 signaling pathway. Their recent study using ligament tissues from OPLL and non-OPLL patients indicated that miR-181a-5p also plays an important role in the development of OPLL and that PBX1 is responsible for the osteogenic phenotype of miR-181a-5p[12]. Therefore, the methods that use ncRNAs to analyze the pathomechanisms of OSL have been a hot topic in recent years.

One Japanese study published in 2020 used routine medical checkup data, in the form of blood samples and whole-body computed tomography, to determine the characteristics of cervical OPLL in 120 OPLL subjects out of 1789 asymptomatic subjects[13]. In comparing data between subjects with and without OPLL, they found that OPLL patients were older, were more likely to be men, had higher body mass indexes, had a higher incidence of hypertension, and had higher levels of HbA1c, triglycerides, and uric acid (UA). Furthermore, carotid artery ultrasounds showed higher maximum intima-media thickness and a higher incidence of plaques in subjects with OPLL. This study had the advantage of using data from a large cohort. These results indicate that triglycerides and UA serum levels might be biomarkers for OPLL.

Recent research on biomarkers for OSL revealed that specific markers are altered in both the blood and ligament tissue of patients with OSL. A study found elevated interleukin-17 receptor C (IL17RC) levels in the plasma of patients with thoracic OPLL with rs199772854A compared with thoracic OPLL patients with rs199772854C, indicating

Table 1. Comparison of the Results of Biomarkers between Cases and Controls.

	Year	First author	Materials	Biomarkers	Case (number)	Control (number)	Data in case	Data in control	p-value	Results
1	1985	Takuwa Y	Serum	Pi	28 PVLO	11	0.97 mmol/L	1.07 mmol/L	0.07	Decrease
				TmP/GFR	28 PVLO	11	0.97 mmol/L	1.03 mmol/L	<0.05	Decrease
			Serum	Ca	28 PVLO	11	2.20 mmol/L	2.25 mmol/L	NS	No difference
			Serum	25OHD	24 PVLO	11	85.9 nmol/L	46.0 nmol/L	NS	No difference
			Serum	1,25OHD	22 PVLO	11	88.8 pmol/L	94.7 pmol/L	NS	No difference
2	1993	Miyamoto S	Plasma	Fibronectin	30 OPLL or OLF	20	43.4±1.2 mg/dL	34.6±1.5 mg/dL	<0.0001	Increase
3	1996	Matsui H	Serum	PICP	40 OPLL	36	980±350 ng/mL	360±130 ng/mL	<0.05	Increase
			Serum	Intact osteocarcin	40 OPLL	36	38±12 ng/mL	17±8 ng/mL	<0.05	Increase
4	2000	Ishiharu C	Serum	PICP	22 male OPLL	20 male	90.4±39.5 ng/mL	109.8±34.8 ng/mL	NS	No difference
			Serum	Osteocarcin	22 male OPLL	20 male	4.9±2.9 ng/mL	4.4±2.9 ng/mL	NS	No difference
			Serum	ICTP	22 male OPLL	20 male	3.8±2.3 ng/mL	3.2±1.1 ng/mL	NS	No difference
			Urine	Pyr	22 male OPLL	20 male	34.1±19.9 nmol/mmol creat.	32.2±12.6 nmol/mmol creat.	NS	No difference
			Urine	Dpyr	22 male OPLL	20 male	6.7±4.4 nmol/mmol creat.	4.8±2.0 nmol/mmol creat.	NS	No difference
5	2003	Yamada K	Serum	Intact osteocarcin	8 female OPLL	8 female	7.17±0.76 ng/mL	6.17±0.75 ng/mL	<0.05	Increase
			Serum	Glu-osteocarcin	8 female OPLL	8 female	5.21±1.63 ng/mL	4.96±1.81 ng/mL	<0.05	Increase
			Serum	Pi	8 female OPLL	8 female	3.37±0.42 mg/dL	3.53±0.61 mg/dL	NS	No difference
			Serum	Ca	8 female OPLL	8 female	9.55±0.46 mg/dL	9.46±0.22 mg/dL	NS	No difference
			Serum	MK-4	8 female OPLL	8 female			NS	No difference
			Serum	MK-7	8 female OPLL	8 female			NS	No difference
			Serum	Intact osteocarcin	16 male OPLL	16 male	4.20±0.52 ng/mL	4.73±0.50 ng/mL	NS	No difference
			Serum	Glu-osteocarcin	16 male OPLL	16 male	2.10±0.37 ng/mL	2.07±0.40 ng/mL	NS	No difference
			Serum	Pi	16 male OPLL	16 male	3.05±0.35 mg/dL	3.29±0.66 mg/dL	NS	No difference
			Serum	Ca	16 male OPLL	16 male	9.42±0.29 mg/dL	9.28±0.42 mg/dL	NS	No difference
			Serum	MK-4	16 male OPLL	16 male			<0.05	Increase
			Serum	MK-7	16 male OPLL	16 male			NS	No difference
6	2011	Ikeda Y	Serum	Leptin	57 female OPLL	27 female	9.67±5.1 ng/mL	6.55±3.67 ng/mL	<0.01	Increase
			Serum	Leptin	68 male OPLL	35 male	3.85±2.2 ng/mL	3.20±1.4 ng/mL	NS	No difference
7	2014	Yoshimura N	Serum	Total cholesterol	30 OPLL	1532 none-OPLL	209.6±36.2 mg/dL	208.8±34.5 mg/dL	NS	No difference
			Serum	Uric acid	30 OPLL	1532 none-OPLL	5.24±1.21 mg/dL	4.84±1.30 mg/dL	NS	No difference
			Serum	HbA1c	30 OPLL	1532 none-OPLL	5.38%±0.79%	5.17%±0.70%	NS	No difference
			Serum	iPTH	30 OPLL	1532 none-OPLL	41.2±14.2 pg/mL	41.2±34.4 pg/mL	NS	No difference
			Serum	PINP	30 OPLL	1532 none-OPLL	52.6±29.9 μg/L	57.9±27.0 μg/L	NS	No difference
			Urine	β-CTX	30 OPLL	1532 none-OPLL	150.4±79.1 μg/mmol Cr	187.2±121.3 μg/mmol Cr	NS	No difference

Table 1. continued.

	Year	First author	Materials	Biomarkers	Case (number)	Control (number)	Data in case	Data in control	p-value	Results
			Plasma	Pentosidine	30 OPLL	1532 none-OPLL	0.085±0.140 μg/mL	0.058±0.037 μg/mL	<0.0005	Increase
8	2016	Kashii M	Serum	Glycated hemogrobin	49 male OPLL	22 male control	5.7%±0.2%	5.3%±0.6%	0.02	Increase
			Serum	Ca	49 male OPLL	22 male control	9.1±0.3 mg/dL	8.9±0.3 mg/dL	NS	No difference
			Serum	Pi	49 male OPLL	22 male control	3.1±0.5 mg/dL	3.3±0.5 mg/dL	NS	No difference
			Serum	BAP	49 male OPLL	22 male control	14.7±7.8 μg/L	12.8±3.9 μg/L	NS	No difference
			Serum	PINP	49 male OPLL	22 male control	35.2±16.4 μg/L	47.7±22.3 μg/L	0.01	Decrease
			Serum	Osteocarcin	49 male OPLL	22 male control	3.6±1.6 ng/mL	3.3±1.5 ng/mL	NS	No difference
			Serum	TRAP5b	49 male OPLL	22 male control	332±128 mU/dL	427±173 mU/dL	0.01	Decrease
			Serum	Parathyroid hormone	49 male OPLL	22 male control	49.5±14.3 pg/dL	41.5±11.1 pg/dL	0.01	Increase
			Serum	1,25-hydroxyvitamin D	49 male OPLL	22 male control	58.0±18.5 pg/dL	62.3±25.9 pg/dL	NS	No difference
			Serum	Sclerostin	49 male OPLL	22 male control	75.7±42.9 pmol/L	45.3±16.0 pmol/L	0.002	Increase
			Serum	Dickkopf-1	49 male OPLL	22 male control	2069±785 pg/dL	2355±1076 pg/dL	NS	No difference
			Serum	Glycated hemoglobin	29 female OPLL	17 female control	5.8%±1.0%	5.3%±0.5%	0.04	Increase
			Serum	Ca	29 female OPLL	17 female control	9.3±0.5 mg/dL	9.0±0.2 mg/dL	NS	No difference
			Serum	Pi	29 female OPLL	17 female control	3.5±0.5 mg/dL	3.5±0.3 mg/dL	NS	No difference
			Serum	BAP	29 female OPLL	17 female control	15.7±6.1 μg/L	13.1±4.7 μg/L	NS	No difference
			Serum	PINP	29 female OPLL	17 female control	42.7±14.9 μg/L	49.2±24.2 μg/L	NS	No difference
			Serum	Osteocarcin	29 female OPLL	17 female control	4.7±1.7 ng/mL	3.8±1.8 ng/mL	NS	No difference
			Serum	TRAP5b	29 female OPLL	17 female control	417±161 mU/dL	397±179 mU/dL	NS	No difference
			Serum	Parathyroid hormone	29 female OPLL	17 female control	58.6±23.3 pg/dL	46.6±13.7 pg/dL	NS	No difference
			Serum	1,25-hydroxyvitamin D	29 female OPLL	17 female control	55.6±18.0 pg/dL	60.9±21.0 pg/dL	NS	No difference
			Serum	Sclerostin	29 female OPLL	17 female control	44.4±21.3 pmol/L	44.5±20.2 pmol/L	NS	No difference
			Serum	Dickkopf-1	29 female OPLL	17 female control	1928±924 pg/dL	2443±812 pg/dL	NS	No difference
9	2017	Kawaguchi Y	Serum	hs-CRP	103 OPLL	95	0.122±0.141 mg/dL	0.086±0.114 mg/dL	0.047	Increase
			Serum	Pi	103 OPLL	95	3.19±0.55 mg/dL	3.36±0.47 mg/dL	0.02	Decrease
			Serum	Ca	103 OPLL	95	9.11±0.35 mg/dL	9.20±0.44 mg/dL	NS	No difference
10	2017	Niu CC	Serum	Osteocarcin	8 OPLL	9	7.95±3.91 ng/mL	2.28±1.37 ng/mL	<0.01	Increase
			Serum	DKK-1	8 OPLL	9	395.8±260.1 pg/mL	792.5±308.6 ng/mL	<0.05	Decrease
			Serum	SFRPs	8 OPLL	9	3.82±1.17 ng/mL	2.61±1.08 ng/mL	NS	No difference
			Serum	Sclerostin	8 OPLL	9	499.4±104.1 pg/mL	261.1±111.4 ng/mL	<0.01	Increase
			Serum	Osteoprotegrin	8 OPLL	9	17.2±8.2 ng/mL	26.1±15.3 ng/mL	NS	No difference
			Serum	Osteocarcin	3 OYL	9	5.62±1.78 ng/mL	2.28±1.37 ng/mL	<0.05	Increase

Table 1. continued.

	Year	First author	Materials	Biomarkers	Case (number)	Control (number)	Data in case	Data in control	p-value	Results
			Serum	DKK-1	3 OYL	9	316.1±112.1 pg/mL	792.5±308.6 ng/mL	<0.01	Decrease
			Serum	SFRPs	3 OYL	9	3.61±0.49 ng/mL	2.61±1.08 ng/mL	NS	No difference
			Serum	Sclerostin	3 OYL	9	368.9±91.4 pg/mL	261.1±111.4 ng/mL	NS	No difference
			Serum	Osteoprotegrin	3 OYL	9	18.7±3.79 ng/mL	26.1±15.3 ng/mL	NS	No difference
11	2017	Cai GD	Serum	FGF-23	76 male cOPLL	41 healthy male	35.11±2.599 pg/mL	27.05±2.526 pg/mL	0.046	Increase
			Serum	Osteopontin	76 male cOPLL	41 healthy male	17880±1326 pg/mL	13300±1713 pg/mL	0.04	Increase
			Serum	DKK-1	76 male cOPLL	41 healthy male	372.4±28.92 pg/mL	448.7±28.89 pg/mL	0.046	Decrease
			Serum	DKK-1	45 female cOPLL	19 healthy male	359.1±38.20 pg/mL	480.4±59.89 pg/mL	0.049	Decrease

Pi: inorganic phosphate PVLO: paravertebral ligament ossification NS: not significant
TmP/GFR: tubular reabsroptive capacity for Pi OPLL: ossification of the posterior longitudinal ligament
Ca: calcium OLF: ossification of the ligamentum flavum
25OHD: 25-hydroxyvitamin D AS: ankylosing spondylitis
1,25 (OH) $_2$D: 1,25-dihydroxyvitamin D DISH: diffuse idiopathic spinal hyperostosis
PICP: C-terminal extension peptide of type I procollagen OYL: ossification of the yellow ligament
ICTP: carboxyterminal telopeptide of type 1 collagen cOPLL: cervical ossification of the posterior longitudinal ligament
Pyr: pyridinoline
Dpyr: deoxypyridinoline
MK: menatetrenone
iPTH: intact parathyroid hormone
PINP: N-terminal propeptide of typeI procollagen
β-CTX: β-isomerised C-terminal cross-linkingtelopeptide of type I collagen
BAP: bone specific alkaline phosphatase
TRAP5b: tartate-resistant acid phosphate 5b
DKK-1: dickkopf-1
hs-CRP: hypersensitive C reactive protein
SFRPs: frizzled-related proteins
FGF-23: fibroblast growth factor-23

that the gene polymorphism is a susceptibility gene for OSL, and IL17RC staining in the ligament tissue of these patients was positive[14,15]. A Japanese group performed a serum proteomic analysis in both patients with OPLL and healthy subjects to identify factors potentially involved in the development of OPLL, and found reduced levels of chemokine (C-X-C motif) ligand 7 (CXCL7) in patients with OPLL[16]. They generated a CXCL7 knockout mouse model to study the molecular mechanisms underlying OPLL and found that CXCL7-null mice presented with an OPLL phenotype. These results indicated that CXCL7 may be a useful serum marker for OPLL progression.

Other approaches to discover biomarkers for OSL include proteome and transcriptome analyses. A Korean group compared the two-dimensional electrophoresis patterns of sera from OPLL patients and healthy subjects. They identified nine spots that were differentially expressed in the sera of OPLL patients as follows: PRO2675; human serum albumin in a complex with myristic acid and triiodobenzoic acid; an unknown protein; chain B of the crystal structure of deoxy human hemoglobin beta 6; pro-apolipoprotein; ALB protein; retinol-binding protein; and chain A of human serum albumin mutant R218h complexed with thyroxine (3,3',5,5'; tetraiodo-L-thyronine) were upregulated, whereas the 1-microglobulin/bikunin precursor was downregulated[17]. A Chinese group analyzed diagnostic biomarkers in blood samples of thoracic OLF patients using metabolomics and transcriptomics[18]. The authors included 25 patients with OLF and recruited 23 healthy volunteers for the control group. Using liquid chromatography-mass spectrometry, they identified 37 metabolites in OLF samples, including UA and hypoxanthine. Transcriptomic data revealed a substantial change in the purine metabolism in OLF patients, with xanthine dehydrogenase as the key regulatory factor. Based on the results, the authors concluded that UA is a potential biomarker for OLF and could play an important role within the pathway; xanthine dehydrogenase could affect the purine metabolism by suppressing the expression of hypoxanthine and xanthine, leading to low serum UA levels in OLF patients.

Table 2. Comparison of the Results of Biomarkers between Cases and Controls in Recent Studies.

	Year	First author	Materials	Biomarkers	Case (number)	Control (number)	Data in case	Data in control	p-value	Results
1	2019	Xu C	plasma or serum	10 miRNAs	68 OPLL	45 disc herniation, 53 none myelopathy				
				miR-10a-3p						Increase
				miR-10a-5p						Increase
				miR-563						Increase
				miR-210-3p						Increase
				miR218-3p						Increase
				miR-196b-5p						Decrease
				miR-129-3p						Decrease
				miR-199b-5p						Decrease
				miR212-3p						Decrease
				miR-218-3p						Decrease
2	2020	Ohshima Y	blood	HbA1C>6.5%-no. (%)	120 OPLL	1669 none OPLL	24 (20%)	185 (11%)	0.003	higher incodence
				TG>150mg/dL-no. (%)			35 (29%)	348 (21%)	0.03	higher incodence
				UA>7.0mg/dL-no. (%)			25 (21%)	278 (17%)	0.239	NS
3	2019	Wang P	plasma	IL 17RC, rs199772854C/A	72 T-OPLL				<0.001	IL17RC was higher in A than C polymorphism
4	2018	Tsuru M	serum	chemokine (C-X-C motif) ligand 7 (CXCL7)	13 OPLL	7 healthy control			<0.05	Decrease
5	2007	Eun JP	serum (proteomics)	9 spots	6 OPLL	6 normal subjects			change in ratio	
				PRO2675					2.81±0.40	Increase
				Human serum albumin in a complex with myristic acid and tri-iodobenzoic acid					3.98±0.65	Increase
				Unknown (protein for IMAGE: 3934797)					2.55±0.38	Increase
				Chain B, crystal structure of deoxy-human hemoglobin beta6					9.12±0.95	Increase
				Pro-apolipoprotein					7.66±0.87	Increase
				ALB protein					4.79±0.68	Increase
				Retinol binding protein					3.10±0.56	Increase
				Chain A, human serum albumin mutant R218h complexed with thyroxine (3,3,5,5, tetraiodo-L-thyronine)					2.36±0.33	Increase
				1-microglobulin/bikunin precursor					0.19±0.15	Decrease

Spine Surg Relat Res 2022; 6(3): 224-232

dx.doi.org/10.22603/ssrr.2021-0229

Table 2. continued.

	Year	First author	Materials	Biomarkers	Case (number)	Control (number)	Data in case	Data in control	p-value	Results
6	2020	Li J	serum (metabolomics and transcriptomics)	uric acid	25 T-OLF	23 healthy volunteers				Increase
				triacetin						Increase
				hypoxanthine						Increase
				pyrimidine metabolism						Increase
				purine metabolism						Increase
7	2014	Oh YM	PLL tisuue	25 proteins, Upregulated	12 OPLL	12 none OPLL				
				Chain A, Thiredoxin peroxidase B						Upregulated
				Immunogloblin kappa right chainVLJ region						Upregulated
				Ig kappa chain NIG26 Precursor						Upregulated
				Drug-protein interaction: structure of sulfonamide drug complexed with human carbonic anhydrase I						Upregulated
				Hypothetical protein						Upregulated
				4 proteins, Downregulated						
				Apolipoprotein A						Downregulated
				Proapolipoprotein						Downregulated
8	2015	Zhang Y	PLL tissue (proteomic profiling+mRNA expression)	3 proteins, up-rehulated by proteomic profiling and 1 marker confirmed by mRNA expressuion	4 OPLL	4 none OPLL				
				N-RAP						Upregulated
				18 proteins, down regulated by proteomic profiling and 2 markers confirmed by mRNA expressuion						
				NSDHL						Downregulated
				Viα1						Downregulated

PLL: posterior longitudinal ligament HbA1C: glycated hemoglobin OPLL: ossification of the posterior longitudinal ligament NS: not significant
TG: triglycerides OLF: ossification of the ligamentum flavum
UA: uric acid T-OPLL: thoracic OPLL
IL17RC: interleukin-17 receptor C T-OLF: thoracic OLF
N-RAP: nebulin-related anchoring protein
NSDHL: NAD (P) dependent steroid dehydrogenase-like
VIα1: collagen VI alpha-1

Table 3. The Classification of the Serum Boimarkers Which Might Be Related to Ossification of the Spinal Ligament.

Calcium phospahe metabolism marker
inorganic phpsphate (Pi)
the tubular reabsorptive capacity for Pi
Fibroblast growth factor-23 (FGF-23)
Bone turnover marker
C-terminal extension peptide of type I procollagen (PICP)
intact osteocalcin
Glu-osteocalcin
N-terminal propeptide of type I procollagen (PINP)
Tartate-resistant acid phosphate 5b (TRAP5b)
Osteoprotegrin
Osteopontin
Sclerostin
Dickkopf-1 (DKK-1)
Glycoprotein of the extracellular matrix
Fibronectin
Glycated hemogrobin
Vitamin K2
Matetrenone (MK-4)
Hormone
Leptin
Parathyroid hormone
Advanced glycation end products
Pentosidine
Inflammation
Hypersensitive C-reactive protein (hs-CRP)
Erythrocyte sedimentation rate (ESR)
MicroRNA
miR-10a-3p, miR-10a-5p, miR-563, miR-210-3p, and miR-218-3p
Others
Triglycerides
Uric acid
Interleukin 17 receptor C (IL17RC) gene expression
Chemokine (C-X-C motif) ligand 7 (CXCL7)

Ligament tissue samples from patients with OSL and control subjects were used in two studies for proteome analyses to understand the pathophysiology of OSL. One study found 25 proteins that were significantly and consistently different on two-dimensional electrophoresis gels between the ossified posterior longitudinal ligament tissue samples from patients with OPLL and the non-ossified posterior longitudinal ligament tissue samples from healthy subjects[19]. Among these proteins, 21, including chain A, thioredoxin peroxidase B, and immunoglobulin kappa light chain VLJ region, were upregulated in the patients with OPLL, whereas the remaining 4 were downregulated. The other study identified 21 proteins or peptides that were distinct in OPLL samples, of which carbonic anhydrase I, the NAD(P)-dependent steroid dehydrogenase-like, biliverdin reductase B, and alpha-1 collagen VI were downregulated, whereas osteoglycin and the nebulin-related anchoring protein were upregulated[20]. However, these studies did not show any blood sample data. It is difficult to use data from ligament cells as biomarkers.

4. Future Perspectives Regarding Biomarkers for OSL

There have been numerous reports regarding biomarkers of OSL (Table 3). Information on candidate biomarkers and methodological progress increase every year. However, several issues have been raised in previous studies. First, the research fields focusing on the target markers are few. Second, the number of subjects has not been sufficient to obtain definitive results. Third, very few results regarding biomarkers have been reproducible. Fourth, there are very few functional studies on how biomarkers bring about ectopic ossification in the spinal ligament. Fifth, there are many studies from Asia but very few from other regions, such as North and South America and European countries. These issues were described in the Japanese OSL guideline, which stated, "The limitations include the few types of markers targeted to date, the small sample size, and the fact that these markers were not reproducible. Therefore, biomarkers have yet to be conclusively investigated"[1]. Furthermore, useful biomarkers for clinical practice have several requirements. First and foremost, the samples must be easy to obtain. Although previous studies used ligament tissue from patients and controls, obtaining this tissue requires a surgical procedure. Circulating blood samples would be easier to use. However, if the secretion levels of the candidate biomarkers are very small, detecting them in blood samples might be difficult. However, if the candidate biomarkers are detectable in blood samples, it might be possible to diagnose and evaluate the disease activity of OSL earlier, without employing radiological examination. Our earlier studies on hypersensitive C-reactive protein and FGF-23 might be useful in detecting the progression of OPLL[21,22]. Very recent our paper showed that the serum level of periostin reflected the progression of OPLL[23]. Another benefit of detecting biomarkers for OSL would be clarifying the pathomechanism of the disease. As previously mentioned, the etiology and pathomechanism of OSL have not yet been fully elucidated. Determining the pathomechanism might be very useful in seeking a therapeutic strategy for OSL. Research using both biomarkers and data from ligament tissue is very important in clarifying the pathomechanism. In the near future, this research should be applicable in treating patients with OSL.

5. Conclusions

This paper reviewed the recent progress toward determining biomarkers for OSL, and research seeking these biomarkers is ongoing. There are several issues in this research field. Once these issues are overcome, results from research should be applied to treatment of patients with OSL.

Disclaimer: Prof. Yoshiharu Kawaguchi is one of the Editors of Spine Surgery and Related Research and on the journal's Editorial Committee. He was not involved in the edito-

rial evaluation or decision to accept this article for publication at all.

Conflicts of Interest: The author declares that there are no relevant conflicts of interest.

Sources of Funding: This research received no external funding.

Acknowledgement: The work reported in this article was supported by grants from the Ministry of Health, Labour and Welfare of Japan: Committee for Study of Ossification of Spinal Ligament and Committee for Research and Development of Therapies for Ossification of The Posterior Longitudinal Ligament.

Author Contributions: Y. Kawaguchi wrote and prepared the manuscript.

Ethical Approval: Not applicable

Informed Consent: Not applicable

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