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

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.
2. Summary of the Literature on Biomarkers for OSL

The search for OSL biomarkers started in 1985. Takuwa et al. 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.

A review published in 2021 noted that non-coding RNAs involved in OPLL was published in 2019 (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, menatetitone, 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 levels of DKK1 was published in 2020. 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. 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. (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. 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. 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. A series of their studies found that miRNA-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 miRNA-10a in the regulation of OPLL development. 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. They described how XIST gene inhibition plays an important role in the occurrence of cervical OPLL through the regulation of the miR-17-5p/AHNK/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. 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. 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    | Takuwa Y     | Serum     | Pi         | 28 PVLO       | 11              | 0.97 mmol/L   | 1.07 mmol/L    | 0.07    | Decrease |
|      |              | Serum     | 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 mmol/L   | 46.0 mmol/L    | NS      | No difference |
|      |              | Serum     | 1,25OHD    | 22 PVLO       | 11              | 88.8 pmol/L   | 94.7 pmol/L    | NS      | No difference |
| 2    | 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    | Matsui H     | Serum     | PICP       | 40 OPLL       | 36              | 980±350 ng/mL | 360±130 ng/mL  | <0.05   | Increase |
|      |              | Serum     | PICP       | 40 OPLL       | 36              | 38±12 ng/mL   | 17±8 ng/mL     | <0.05   | Increase |
|      |              | Serum     | Osteocarcin| 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         | 341.9±19.9 mmol cre. | 32.2±12.6 mmol cre. | NS      | No difference |
|      |              | Urine     | Dpyr       | 22 male OPLL  | 20 male         | 6.7±4.4 mmol cre. | 4.8±2.0 mmol cre. | NS      | No difference |
| 4    | Ishiharu C   | 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     | Ca         | 8 female OPLL | 8 female         | 3.37±0.42 ng/mL | 3.53±0.61 ng/mL | NS      | No difference |
|      |              | Serum     | MK-4       | 8 female OPLL | 8 female         | 9.55±0.46 mg/dL | 9.46±0.22 mg/dL | NS      | No difference |
|      |              | Serum     | MK-7       | 8 female OPLL | 8 female         | NS            | NS             | 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         | 4.20±0.52 ng/mL | 4.73±0.50 ng/mL | <0.05   | Increase |
|      |              | Serum     | MK-7       | 16 male OPLL  | 16 male         | NS            | NS             | NS      | No difference |
| 5    | 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     | Ca         | 8 female OPLL | 8 female         | 3.37±0.42 ng/mL | 3.53±0.61 ng/mL | NS      | No difference |
|      |              | Serum     | MK-4       | 8 female OPLL | 8 female         | NS            | NS             | NS      | No difference |
|      |              | Serum     | MK-7       | 8 female OPLL | 8 female         | NS            | NS             | NS      | No difference |
| 6    | 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    | 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±9.9 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 |
| Year | First author | Materials | Biomarkers | Case (number) | Control (number) | Data in case | Data in control | p-value | Results |
|------|--------------|-----------|------------|--------------|-----------------|--------------|----------------|---------|---------|
| 8    | 2016 Kashii M | Serum     | Pentosidine | 30 OPLL      | 1532 none-OPLL  | 0.085±0.140 μg/mL | 0.058±0.037 μg/mL | <0.0005 | Increase |
|      |              |           | OPLL       |              |                  | 5.7%±0.2%     | 5.3%±0.6%     |         |         |
|      |              |           | Plasma     |              |                  |              |               |         |         |
| 8    |              | Serum     | Glycated hemoglobin Ca | 49 male OPLL | 22 male control  | 9.1±0.3 mg/dL | 8.9±0.3 mg/dL | NS       | No difference |
| 8    |              | Serum     | Glycated hemoglobin Pi | 49 male OPLL | 22 male control  | 3.1±0.5 mg/dL | 3.3±0.5 mg/dL | NS       | No difference |
| 8    |              | Serum     | BAP        | 49 male OPLL  | 22 male control  | 14.7±7.8 μg/L | 12.8±3.9 μg/L | NS       | No difference |
| 8    |              | Serum     | PINP       | 49 male OPLL  | 22 male control  | 35.2±16.4 μg/L | 47.7±22.3 μg/L | 0.01     | Decrease |
| 8    |              | Serum     | Osteocarcin | 49 male OPLL | 22 male control  | 3.6±1.6 ng/mL | 3.3±1.5 ng/mL | NS       | No difference |
| 8    |              | Serum     | TRAP5b     | 49 male OPLL  | 22 male control  | 332±128 μU/dL | 427±173 μU/dL | 0.01     | Decrease |
| 8    |              | Serum     | Parathyroid hormone | 49 male OPLL | 22 male control  | 49.5±14.3 pg/dL | 41.5±11.1 pg/dL | 0.01     | Increase |
| 8    |              | Serum     | 1,25-hydroxyvitamin D Sclerostin | 49 male OPLL | 22 male control  | 58.0±18.5 μg/dL | 62.3±25.9 μg/dL | NS       | No difference |
| 8    |              | Serum     | Dickkopf-1 | 49 male OPLL  | 22 male control  | 2069±785 pg/dL | 2355±1076 pg/dL | 0.002   | Increase |
| 8    |              | Serum     | Glycated hemoglobin Ca | 29 female OPLL | 17 female control | 5.8±1.0%     | 5.3±0.5%     | 0.04     | Increase |
| 8    |              | Serum     | Glycated hemoglobin Pi | 29 female OPLL | 17 female control | 9.3±0.5 mg/dL | 9.0±0.2 mg/dL | NS       | No difference |
| 8    |              | Serum     | BAP        | 29 female OPLL | 17 female control | 15.7±6.1 μg/L | 13.1±4.7 μg/L | NS       | No difference |
| 8    |              | Serum     | PINP       | 29 female OPLL | 17 female control | 42.7±14.9 μg/L | 49.2±24.2 μg/L | NS       | No difference |
| 8    |              | Serum     | Osteocarcin | 29 female OPLL | 17 female control | 4.7±1.7 ng/mL | 3.8±1.8 ng/mL | NS       | No difference |
| 8    |              | Serum     | TRAP5b     | 29 female OPLL | 17 female control | 417±161 μU/dL | 397±179 μU/dL | NS       | No difference |
| 8    |              | Serum     | Parathyroid hormone | 29 female OPLL | 17 female control | 58.6±23.3 pg/dL | 46.6±13.7 pg/dL | NS       | No difference |
| 8    |              | Serum     | 1,25-hydroxyvitamin D Sclerostin | 29 female OPLL | 17 female control | 55.6±18.0 μg/dL | 60.9±21.0 μg/dL | NS       | No difference |
| 8    |              | 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 |
| 9    |              | Serum     | Pi         | 103 OPLL     | 95               | 3.19±0.55 mg/dL | 3.09±0.47 mg/dL | 0.02    | Decrease |
| 9    |              | Serum     | Ca         | 103 OPLL     | 95               | 9.1±0.35 mg/dL | 9.2±0.35 mg/dL | NS       | No difference |
| 10   | 2017 Niu CC  | Serum     | Osteocarcin DKK-1 | 8 OPLL      | 9               | 7.95±3.91 ng/mL | 2.28±1.37 ng/mL | <0.01   | Increase |
| 10   |              | Serum     | SFRPs      | 8 OPLL       | 9               | 395.8±260.1 ng/mL | 792.5±308.6 ng/mL | <0.05   | Decrease |
| 10   |              | Serum     | Sclerostin | 8 OPLL       | 9               | 3.82±1.17 ng/mL | 2.61±1.08 ng/mL | NS       | No difference |
| 10   |              | Serum     | Osteoprotegrin | 8 OPLL       | 9               | 499.4±104.1 ng/mL | 261.1±111.4 ng/mL | <0.01   | Increase |
| 10   |              | 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 |
|------|--------------|-----------|------------|---------------|-----------------|--------------|----------------|---------|---------|
| 2017 | Cai GD       | 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         | 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  
TnPiGFR: tubular reabsorptive 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)2D: 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 I collagen  
cOPLL: cervical ossification of the posterior longitudinal ligament  
Pyr: pyridinoline  
Dpyr: deoxypyridinoline  
MK: menatetrenone  
iPTH: intact parathyroid hormone  
PINP: N-terminal propeptide of type I procollagen  
β-CTX: β-isomerised C-terminal cross-linking telopeptide of type I collagen  
BAP: bone specific alkaline phosphatase  
TRAP5b: tartate-resistant acid phosphatase 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\textsuperscript{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\textsuperscript{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\textsuperscript{17}. A Chinese group analyzed diagnostic biomarkers in blood samples of thoracic OLF patients using metabolomics and transcriptomics\textsuperscript{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    | Xu C         | plasma or serum     | 10 miRNAs  | 68 OPLL       | 45 disc herniation, 53 none myelopathy | Increase     | Increase         | Increase | Increase          |
|      |              |                     | 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    | Ohshima Y    | blood               | HbA1C>6.5%-no. (%) | 120 OPLL | 1669 none OPLL | 24 (20%) | 18.5 (11%) | 0.003 | higher incidence |
|      |              |                     | TG>150mg/dL-no. (%) |         |                  | 35 (29%) | 348 (21%) | 0.03  | higher incidence |
|      |              |                     | UA>7.0mg/dL-no. (%) |         |                  | 25 (21%) | 278 (17%) | 0.239 | NS               |
| 3    | Wang P       | plasma               | IL 17RC, rs199772854C/A | 72 T-OPPL |                  |              |                  | 0.001 | IL17RC was higher in A than C polymorphism |
| 4    | Tsuru M      | serum                | chemokine (C-X-C motif) ligand 7 (CXCL7) | 13 OPLL | 7 healthy control |              |                  | <0.05 | Decrease         |
| 5    | 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-apoipoprotein |               |                  |              |                  | 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        |
### Table 2. continued.

| Year | First author | Materials | Biomarkers | Case (number) | Control (number) | Data in case | Data in control | p-value | Results |
|------|--------------|------------|------------|---------------|-----------------|-------------|-----------------|---------|---------|
| 6    | 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    | Oh YM        | PLL tissue | 25 proteins, Upregulated | 12 OPLL | 12 none OPLL | Upregulated |
|      |              |            | Chain A, Thioredoxin peroxidase B |             |                 | Upregulated |
|      |              |            | Immunoglobulin kappa right chainVLJ region |             |                 | Upregulated |
|      |              |            | Ig kappa chain NKG26 Precursor |             |                 | Upregulated |
|      |              |            | Drug-protein interaction: structure of sulfonamide drug complexed with human carbonic anhydrase I | | | Upregulated |
|      |              |            | Hypothetical protein |             |                 | Upregulated |
|      |              |            | 4 proteins, Downregulated |             |                 | Downregulated |
|      |              |            | Apolipoprotein A |             |                 | Downregulated |
|      |              |            | Proapolipoprotein |             |                 | Downregulated |
| 8    | Zhang Y      | PLL tissue (proteomic profiling+mRNA expression) | 3 proteins, up-regulated by proteomic profiling and 1 marker confirmed by mRNA expression | 4 OPLL | 4 none OPLL | Upregulated |
|      |              |            | N-RAP |             |                 | Downregulated |
|      |              |            | 18 proteins, down regulated by proteomic profiling and 2 markers confirmed by mRNA expression |             |                 | 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  
VIαⅠ: collagen VI alpha-1
Ligament tissue samples from patients with OSL and control subjects were used in two studies for proteome analyses to understand the pathobiology 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 patients on two-dimensional electrophoresis gels between the ossified proteins that were significantly and consistently different to understand the pathophysiology of OSL. One study found controls, used in two studies for proteome analyses, difficult to use data from ligament cells as biomarkers.

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

| Calcium phosphate metabolism marker       | inorganic phosphate (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)                                            |
|                                          | Osteoprotegerin                                                                       |
|                                          | Osteopontin                                                                           |
|                                          | Sclerostin                                                                            |
|                                          | Dickkopf-1 (DKK-1)                                                                     |
| Glycoprotein of the extracellular matrix  | Fibronectin                                                                            |
|                                          | Glycated hemoglobin                                                                    |
| 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)                                               |

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”. 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. Cirulating 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. Very recent our paper showed that the serum level of periostin reflected the progression of OPLL. 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.

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