Biomarkers of Ossification of the Spinal Ligament

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Abstract

Study Design: Literature review.

Objectives: To review biomarkers in patients with ossification of the spinal ligament (OSL), including ossification of the posterior longitudinal ligament and ossification of the ligamentum flavum and to raise the present issues.

Methods: A literature search was performed using PubMed and MEDLINE databases. The biomarkers were classified according to category. The number of the subjects and reproducibility were assessed.

Results: Eleven articles were included in this review. There were 9 articles from Japan, 1 article from Taiwan, and 1 article from China. The biomarkers were classified into calcium-phosphate metabolism markers, bone turnover markers, sclerostin, dickkopf-1, secreted frizzled-related protein-1, fibroblast growth factor-23, fibronectin, menatetrenone, leptin, pentosidine, and hypersensitive C-reactive protein. However, there were several limitations in the research studies, such as small research field, small number of subjects, and a lack of reproducibility.

Conclusions: Although there have been several studies that have analyzed biomarkers for OSL, there are no definitive conclusions to date. Numerous issues will need to be resolved in the future. It is imperative to continue this research because the results might prove beneficial to elucidate the pathology of OSL and the measures to prevent the initiation and progression of the disease.

Keywords

literature review, biomarkers, ossification of the spinal ligament, ossification of the posterior longitudinal ligament, ossification of the ligament flavum

Introduction

Ossification of the spinal ligament (OSL) is a disease that causes narrowing of the spinal canal. Ossification of the posterior longitudinal ligament (OPLL) and ossification of the ligamentum flavum (OLF) are included in the category of this pathology. Patients with OPLL and/or OLF typically develop neurological symptoms, which vary from simple discomfort to severe myelopathy. Patients with severe myelopathy frequently have difficulty walking and present a disturbance in activities of daily living.

OPLL and OLF develop in patients who are generally over 40 years old. There are ethnic differences in the incidence of OPLL. It has been reported that the incidence of OPLL is 3% (1.8% to 4.1%) in Japan, 0.2% to 1.8% in China, 0.95% in Korea, 0.12% in America, and 0.1% in Germany. The incidence of OLF is yet unclarified, but it is less than that in OPLL. OPLL mainly affects the cervical spine, whereas OLF is found in the thoracic spine and lumbar spine. A recent study using whole spine CT revealed that more than half of the patients with cervical OPLL had ossification at the thoracic and/or lumbar levels. Furthermore, more than half of the patients with cervical OPLL had OLF in some region of the spine.

As the cause of these diseases is still unknown, OPLL and OLF are designated as intractable diseases by the Ministry of Health Labour and Welfare in Japan. OPLL and OLF are thought to be multifactorial diseases. In recent studies, a genetic background has been revealed in the pathogenesis of OPLL. A whole genome study clarified 6 causative genes for OPLL.
OPLL. Numerous candidate genes have been identified, to date, and a review article was published in 2017. There are other approaches to determine the pathogenesis of OPLL and OLF using biomarkers. Several biomarkers have been identified for OPLL and OLF, although they have not been confirmed yet. This review article focuses on the biomarkers of OSL. However, there are numerous issues that should be addressed in the future. Here, we review the biomarkers of OSL and discuss the present issues in biomarker research.

**Literature Search Regarding Biomarkers in OPLL and OLF**

Literature was retrieved via a search of the following key words: “ossification of the posterior longitudinal ligament,” “OPLL,” “ossification of the ligamentum flavum,” “OLF,” “ossification of the spinal ligament,” “OYL,” “ossification of the yellow ligament,” and “biomarker.” The search was restricted to English language publications only. The search sources were PubMed and Medline from 1980 to 2017. The articles included were only those that involved human studies. No restriction was made regarding the sample size. Case-control studies were also included. We also added related articles from the reference lists in the selected articles. The search retrieved 44 articles, and the contents were checked by the author. Five articles were added from among references of the selected articles. Finally, 11 articles were included in this review. There were 9 articles from Japan, 1 article from Taiwan, and 1 article from China. No articles from North and South America, European countries, or African countries were found in the search. The selected articles are listed according to the published year in Tables 1 and 2.

**Classification Category in the Biomarkers of OSL**

**Calcium-Phosphate Metabolism Markers**

Serum calcium is stable in the human body. There were no articles retrieved that showed a difference in serum calcium level between patients with OSL and control. In contrast, 2 articles showed that the inorganic phosphate (Pi) level in patients with OSL was lower than that in controls. They et al showed that the tubular reabsorptive capacity for Pi (TmP/GFR) was decreased in patients with OSL, compared with controls. They stated that patients with OSL demonstrated a tendency for low serum Pi with a reduced TmP/GFR. Kawaguchi et al also reported the decrease of Pi in patients with OPLL. However, 2 articles did not show a difference in Pi level between patients with OSL and control.

**Bone Turnover Markers**

It has been well known that patients with OPLL have an increased level of bone mineral density. This increase suggests that an increase of bone formation activity is correlated with the occurrence of this disease. Bone turnover markers were analyzed in 6 articles. The target markers were C-terminal extension peptide of type I procollagen (PICP), carboxyterminal telopeptide of type I collagen (ICTP), osteocalcin, intact osteocalcin, glu-osteocalcin, N-terminal propeptide of type I procollagen (PINP), tartarate-resistant acid phosphate 5b (TRAP5b), and osteopontin in serum, and also pyridinoline (Pyr) and deoxypyridinoline (Dpyr) in urine. Among the selected reports, several showed the difference in PICP, intact osteocalcin, glu-osteocalcin, PINP, and TRAP5b between patients with OSL and control.

| Year | First Author | Country | Journal                         |
|------|--------------|---------|---------------------------------|
| 1985 | Takuwa Y     | Japan   | Acta Endocrinologica             |
| 1993 | Miyamoto S   | Japan   | Spine                           |
| 1996 | Matsui H     | Japan   | Calcified Tissue International   |
| 2000 | Ishihara C   | Japan   | Spinal Cord                      |
| 2003 | Yamada K     | Japan   | Spine                           |
| 2011 | Ikeda Y      | Japan   | European Spine Journal          |
| 2014 | Yoshihara N  | Japan   | Osteoporosis International       |
| 2016 | Kashii M     | Japan   | Journal of Bone and Mineral Metabolism |
| 2017 | Kawaguchi Y  | Japan   | PLoS One                        |
| 2017 | Niu CC       | Taiwan  | BMC Musculoskeletal Disorders   |
| 2017 | Cai GD       | China   | Growth Factors                  |

Sclerostin, dickkopf-1 (DKK-1), and Secreted Frizzled-Related Protein-1 (SFRP-1)

Canonical Wnt/β-catenin signaling is one of the new topics in the research of biomarkers for OSL. Sclerostin, DKK-1, and
| Year | First Author | Materials | Biomarkers | Case (Number) | Control (Number) | Data in Case | Data in Control | P Value | Results |
|------|--------------|-----------|------------|--------------|-----------------|-------------|----------------|---------|---------|
| 1985 | Takuwa Y     | Serum Pi  | 28 PVLO    | 11           | 0.97 mmol/L     | 1.07 mmol/L | .07 Decrease   |         |         |
|      |              | TmP/GFR   | 28 PVLO    | 11           | 0.97 mmol/L     | 1.03 mmol/L | <.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|         |         |
| 1993 | Miyamoto S   | Plasma Fibronectin | 30 OPLL or OLF | 20 | 43.4 ± 1.2 mg/dL | 34.6 ± 1.5 mg/dL | <.0001 Increase |         |         |
| 1996 | Matsui H     | Serum PICP | 40 OPLL    | 36           | 980 ± 350 ng/mL | 360 ± 130 ng/mL | <.05 Increase |         |         |
|      |              | Serum Intact osteocarcin | 40 OPLL | 36           | 38 ± 12 ng/mL | 17 ± 8 ng/mL | <.05 Increase |         |         |
| 2000 | Ishiharu C   | Serum PICP | 22 male OPLL | 20 | 90.4 ± 39.5 mg/dL | 109.8 ± 34.8 mg/dL | NS No difference |         |         |
|      |              | Serum Osteocarcin | 22 male OPLL | 20 | 4.9 ± 2.9 mg/dL | 4.4 ± 2.9 mg/dL | NS No difference |         |         |
|      |              | Serum ICTP | 22 male OPLL | 20 | 3.8 ± 2.3 mg/dL | 3.2 ± 1.1 mg/dL | NS No difference |         |         |
|      |              | Urine Pyr | 22 male OPLL | 20 | 34.1 ± 19.9 mmol/mmol creat. | 32.2 ± 12.6 mmol/mmol creat. | NS No difference |         |         |
|      |              | Urine Dpyr | 22 male OPLL | 20 | 6.7 ± 4.4 mmol/mmol creat. | 4.8 ± 2.0 mmol/mmol creat. | NS No difference |         |         |
| 2003 | Yamada K     | Serum Intact osteocarcin | 8 female OPLL | 8 female | 7.17 ± 0.76 ng/mL | 6.17 ± 0.75 ng/mL | <.05 Increase |         |         |
|      |              | Serum glu-osteocarcin | 8 female OPLL | 8 female | 5.21 ± 1.63 mg/dL | 4.96 ± 1.81 mg/dL | <.05 Increase |         |         |
|      |              | Serum Ca  | 8 female OPLL | 8 female | 3.37 ± 0.42 mg/dL | 3.53 ± 0.61 mg/dL | NS No difference |         |         |
| 2011 | Ikeda Y      | Serum Leptin | 57 female OPLL | 27 female | 9.67 ± 5.1 mg/dL | 6.55 ± 3.67 mg/dL | <.01 Increase |         |         |
|      |              | Serum leptin | 68 male OPLL | 35 male | 3.85 ± 2.2 mg/dL | 3.20 ± 1.4 mg/dL | NS No difference |         |         |
| 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 |         |         |
|      |              | Plasma Pentosidine | 30 OPLL | 1532 none-OPLL | 0.085 ± 0.140 μg/mL | 0.058 ± 0.037 μg/mL | <.0005 Increase |         |         |
| 2016 | Kashii M     | Serum Glycated hemoglobin | 49 male OPLL | 22 male control | 5.7 ± 0.2% | 5.3 ± 0.6% | .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 | .01 Decrease |         |         |
|      |              | Serum Osteocarcin | 49 male OPLL | 22 male control | 3.3 ± 1.6 mg/dL | 3.3 ± 1.5 mg/dL | NS No difference |         |         |
|      |              | Serum TRAP5b | 49 male OPLL | 22 male control | 32.2 ± 128 μU/dL | 42.7 ± 173 μU/dL | .01 Decrease |         |         |
|      |              | Serum Parathyroid hormone | 49 male OPLL | 22 male control | 49.5 ± 14.3 pg/dL | 41.5 ± 11.1 pg/dL | .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 |         |         |

(continued)
| Year  | First Author | Materials | Biomarkers       | Case (Number) | Control (Number) | Data in Case  | Data in Control | P Value | Results |
|-------|--------------|-----------|------------------|---------------|------------------|---------------|-----------------|---------|---------|
|       |              |           | Serum Sclerostin | 49 male OPLL  | 22 male control  | 75.7 ± 42.9 pmol/L | 45.3 ± 16.0 pmol/L | .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% | .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 |
| 2017  | Kawaguchi Y  | Serum       | Serum hs-CRP     | 103 OPLL      | 95               | 0.122 ± 0.141 mg/dL | 0.086 ± 0.114 mg/dL | .047    | Increase |
|       |              |           | Serum Pi         | 103 OPLL      | 95               | 3.19 ± 0.55 mg/dL | 3.36 ± 0.47 mg/dL | .02     | Decrease |
|       |              |           | Serum Ca         | 103 OPLL      | 95               | 9.11 ± 0.35 mg/dL | 9.20 ± 0.44 mg/dL | NS | No difference |
| 2017  | Niu CC       | Serum       | Serum Osteocarcin| 8 OPLL       | 9               | 7.95 ± 3.91 ng/mL | 2.28 ± 1.37 ng/mL | <.01    | Increase |
|       |              |           | Serum DKK-1      | 8 OPLL       | 9               | 395.8 ± 260.1 pg/mL | 792.5 ± 308.6 ng/mL | <.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 ± 101.4 ng/mL | 261.1 ± 111.4 ng/mL | <.01    | Increase |
|       |              |           | Serum Osteoprotegrin | 8 OPLL   | 9               | 316.1 ± 11.2 pg/mL | 792.5 ± 308.6 ng/mL | <.05    | Decrease |
|       |              |           | Serum Osteocarcin | 3 OYL       | 9               | 17.2 ± 8.2 ng/mL | 26.1 ± 15.3 ng/mL | NS | No difference |
|       |              |           | Serum DKK-1      | 3 OYL       | 9               | 368.9 ± 91.4 pg/mL | 261.1 ± 111.4 ng/mL | NS | No difference |
|       |              |           | Serum SFRPs      | 3 OYL       | 9               | 36.1 ± 0.49 ng/mL | 26.1 ± 10.8 ng/mL | NS | No difference |
| 2017  | Cai GD       | Serum       | Serum FGF-23     | 76 male cOPLL | 41 healthy male | 35.11 ± 2.59 pg/mL | 27.05 ± 2.52 pg/mL | .046    | Increase |
|       |              |           | Serum Osteopontin | 76 male cOPLL | 41 healthy male | 17 880 ± 1326 pg/mL | 13 300 ± 1713 pg/mL | .04     | Decrease |
|       |              |           | Serum DKK-1      | 76 male cOPLL | 41 healthy male | 372.4 ± 28.92 pg/mL | 448.7 ± 28.89 pg/mL | .046    | Decrease |
|       |              |           | Serum DKK-1      | 45 female cOPLL | 19 healthy male | 359.1 ± 38.20 pg/mL | 480.4 ± 59.89 pg/mL | .049    | Decrease |

Abbreviations: Pi, inorganic phosphate; PVLO, paravertebral ligament ossification; NS, not significant; TmP/GFR, 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 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 type I procollagen; P-CTX, P-isomerized C-terminal cross-linking telopeptide of type 1 collagen; BAP, bone specific alkaline phosphatase; TRAP5b, tartrate-resistant acid phosphate 5b; DKK-1, dickkopf-1; hs-CRP, hypersensitive C-reactive protein; SFRP, frizzled related protein; FGF-23, fibroblast growth factor-23.
SFRP-1, which are antagonists for canonical Wnt/β-catenin signaling, regulate bone mass by competitive binding to low-density lipoprotein receptor-related protein. Canonical Wnt/β-catenin signaling plays an important role in bone formation, and activation of this signaling pathway results in the propagation of osteoprogenitor cells, as well as reduced apoptosis of osteoblasts, leading to anabolic effects on bone.

Sclerostin was found to be increased in patients with OPLL, compared with controls, in 2 studies. There were also 2 articles that reported a positive decrease in DKK-1 in patients with OSL, but 1 article described a result without any differences. Niu et al reported that there was no difference in SFRP-1 between patients with OSL and controls. SFRP1 is a member of the SFRP family, which act as soluble modulators of Wnt signaling.

Fibroblast Growth Factor-23 (FGF-23)

FGF-23 has been reported to be an interesting protein related to bone metabolism. FGF-23 is secreted by osteophytes/osteoblasts in bone and has a role in regulating the phosphate concentration in plasma.

Fibronectin (FN)

FN is a glycoprotein involved in a wide variety of cellular activities, including the development of bone tissues. FN is one of the essential factors in endochondral ossification. Miyamoto et al found that plasma FN concentrations were significantly elevated in patients with OPLL or OLF compared with control subjects.

Menatetrenone (MK-4)

MK-4 is a vitamin K compound used as a hemostatic agent, and also as an adjunctive therapy for osteoporosis. MK-4 enhances osteoblast function and also inhibits osteoclast function.

Leptin

The Zucker fatty (fa/fa) rat is known to be an animal model of OPLL. The rat reveals hereditary obesity and exhibits hyperglycemia, hyperinsulinemia, hyperlipidemia, and heterotopic ossification of the spine. Furthermore, obesity is a risk factor for OPLL. Hyperleptinemia is a common feature of obese people and leptin is believed to be an important factor in the pathogenesis of OPLL. Ikeda et al focused on leptin as a biomarker for OPLL and found that serum leptin and insulin concentrations were significantly increased in OPLL females compared with non-OPLL female controls. In addition, in females, serum leptin levels were significantly higher in patients in whom OPLL extended to the thoracic and/or lumbar spine than in patients in whom OPLL was limited to the cervical spine. However, no difference was found in males.

Pentosidine

Pentosidine, a biomarker for advanced glycation end-products, is known to correlate with the presence and severity of diabetic complications. OPLL is known to be associated with diabetes mellitus. Yoshimura et al performed a large cohort study in Wakayama, Japan. They detected 30 subjects (17 men, 13 women; 1.9%) who had radiographic OPLL out of 1562 individuals who underwent X-ray examination of the cervical spine. They also found that the plasma pentosidine levels were still significantly related to the presence of OPLL. Based on the results, they speculated that the levels of pentosidine might be associated with ectopic ossification, such as vascular calcification in patients with renal dysfunction, or the presence of OPLL, directly or indirectly.

Hypersensitive CRP (hs-CRP)

Kawaguchi et al hypothesized that OPLL is associated with local inflammation in the spinal ligament. They compared hs-CRP between patients with OPLL and controls and found a higher level of hs-CRP in patients with OPLL, compared with that in controls.

Biomarkers Related to the Extent and Progression of OSL

There were several articles on biomarkers of OSL in which a case-control study was not conducted. However, these studies are also valuable in that they showed the relationships between certain biomarkers and the specific characteristics of OPLL. Seichi et al investigated an oral calcium tolerance test followed by cervical spinal radiography to evaluate OPLL progression. They divided the patients into 2 groups according to their responsiveness to an oral calcium load: a group of 14 patients with decreased response and another group of 25 with normal response. The incidence of the development of cervical OPLL was significantly higher in the decreased response group than that in normal calciuric response group. Akune et al reported that there was positive relationship between insulinogenic index and OPLL extent in the whole spine using 100 patients with OPLL. Sugimori et al found a positive correlation between intact osteocalcin, osteocalcin, and PICP in patients with combinations of cervical, thoracic, or lumbar OPLL using 43 patients with OPLL. In a recent article by Kawaguchi et al, a negative correlation was found between the Pi and OPLL extent in the whole spine. They also found that the mean hs-CRP in the progression group was higher than that in the nonprogression group and there was a positive correlation between the average length of the OPLL progression per year and the hs-CRP. As previously stated, Ikeda et al reported that...
serum leptin levels were higher in female patients with OPLL, which extended to the thoracic and/or lumbar spine than in female patients with OPLL limited to the cervical spine.11 These results might offer important clinical insight to determine the preventive measures for OPLL progression.

Number of Cases and Controls, and Diseases

The number of cases and controls was too small in all of these studies. Only 2 articles exceeded 100 patients with OPLL14,16, however, even in these articles, the number of control subjects was not more than 100. Less than 30 subjects as controls were included in 4 articles.6,9,10,15 Four articles were available either in males or females.9,10,11,13 Among the diseases of OSL, OPLL was the main pathology of focus in most of the articles. Nine studies performed a case-control study using patients with OPLL and controls.7-14,16 In contrast, only 1 article described cases of paravertebral ligament ossification.6 Two articles included OLF and OYL as the same disease category. One article used 20 patients with ankylosing spondylitis, DISH, OPLL, and OYL.15

Reproducibility

There was very little reproducibility for the biomarkers in OSL. As for Pi, there were 2 articles in which serum level of Pi decreased in the patients with OSL6,14; however, 2 articles did not show any difference between patients and controls.10,13 Three articles described the increase in osteocalcin in cases,15 but 2 articles did not show any difference.9,13 Three articles described DKK-1 as biomarkers13,15,16 and DKK-1 decreased in 2 of the studies,15,16 but no difference was shown in the other study.13

Issues in the Research for Biomarkers of OSL

There are numerous issues that need to be resolved in the future.

1. The targets in the research field are very small. There are only 9 categories of biomarkers included in this article. Recent studies have attempted to identify putative biomarkers for OPLL using proteomic profiling. This study allows elucidation of a large range of proteins that might be related to the OSL. Eun et al performed a comparative analysis of serum proteomes to examine biomarkers for OPLL. As a result, there were 9 spots, including PRO2675, human serum albumin in a complex with myristic acid and tri-iodobenzoic acid, an unknown protein, chain B of the crystal structure of deoxy-human hemoglobin beta6, 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), alpha-l-microglobulin/bikunin precursor, that were differentially expressed in the sera of OPLL patients compared with controls.37 Zhang et al conducted a study using proteomics.38 They demonstrated that NAD(P)-dependent steroid dehydrogenase-like, alpha-l collagen VI and nebulin-related anchoring protein were validated by reverse transcriptase-polymerase chain reaction. Based on the results, they concluded that these differentially expressed proteins could play a role in the onset and progression of OPLL. Thus, a variety of biomarkers must be studied.

2. The number of subjects was not sufficient to obtain definitive results. The incidence of OSL has been reported to be 3% in Japan, and many of the clinical articles were published in Asian countries. Many of the candidates might be recruited as cases and controls in Asian countries. However, the number of the subjects was too small. Some studies included only less than 30 patients and controls.6,7,9,10,15 The sample size in these studies was too low. The diagnostic criteria of OSL are easily identified. Thus, it might be easy to collect many samples for the researchers. The numbers of cases and controls should be more than several hundreds.

3. There were very few reproducible results regarding the biomarkers. As the number of the studies on biomarkers of OSL was limited, it was very difficult to obtain reproducible results. In this situation, no reliable results can be obtained. Thus, it was not suitable for clinical application.

Future Perspective

There are numerous issues that need to be resolved in the future.

1. Many of the candidates of biomarkers should be examined. There has been numerous data using proteomics for the biomarkers of OSL. Appropriate candidates should be chosen and examined for their suitability as biomarkers.

2. Many samples should be collected. It might be impossible to collect sufficient samples in a single institute; thus, multicenter studies for sample collection are necessary.

3. Target for the cases should be identified. OSL includes a variety of pathologies, such as OPLL, OLF, AS, and DISH. Among them, OPLL might be considered as the first priority for study because most of the studies for OSL use OPLL. OPLL is a primary disease that can be diagnosed easily by radiographs, using X-ray and computed tomography. OPLL should be the first main focus.

4. Meta-analysis should be performed. One of the advantages of a systematic review is the opportunity to combine research data. However, the targeted biomarkers were diverse and the results were inconsistent. Furthermore, the unit of the markers were different among the studies. Thus, it was very difficult to perform meta-analysis using this data.
Conclusion

Although there have been several studies that have analyzed biomarkers for OSL, there are no definitive and quantitative conclusions to date. Numerous issues will need to be resolved in the future. It is necessary to continue this research because the results might be beneficial to elucidate the pathology of OSL and the measures to prevent initiation and progression of the disease.

Declaration of Conflicting Interests

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