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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.[1] 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.[2] Furthermore, more than half of the patients with cervical OPLL had OLF in some region of the spine.[3]

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

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Table 1. Previous Studies Regarding Biomarkers in the Ossification of the Spinal Ligament.

	Year	First Author	Country	Journal
1	1985	Takuwa Y	Japan	*Acta Endocrinologica*
2	1993	Miyamoto S	Japan	*Spine*
3	1996	Matsui H	Japan	*Calcified Tissue International*
4	2000	Ishihara C	Japan	*Spinal Cord*
5	2003	Yamada K	Japan	*Spine*
6	2011	Ikeda Y	Japan	*European Spine Journal*
7	2014	Yoshimura N	Japan	*Osteoporosis International*
8	2016	Kashii M	Japan	*Journal of Bone and Mineral Metabolism*
9	2017	Kawaguchi Y	Japan	*PLoS One*
10	2017	Niu CC	Taiwan	*BMC Musculoskeletal Disorders*
11	2017	Cai GD	China	*Growth Factors*

OPLL.[4] Numerous candidate genes have been identified, to date, and a review article was published in 2017.[5] 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,[6-14] 1 article from Taiwan,[15] and 1 article from China.[16] 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.[6,14] Takuwa et al showed that the tubular reabsorptive capacity for Pi (TmP/GFR) was decreased in patients with OSL, compared with controls.[6] 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.[14] However, 2 articles did not show a difference in Pi level between patients with OSL and control.[10,13]

Bone Turnover Markers

It has been well known that patients with OPLL have an increased level of bone mineral density.[17] 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.[8-10,13,15,16] 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), tartate-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 TRRAP5b between patients with OSL and control.[8,10,15,16] Matsui et al reported that serum PICP and intact osteocalcin are increased in patients with OPLL, compared with controls.[8] A similar result of increased intact osteocalcin and glu-osteocalcin was found by Yamada et al[10] and Niu et al.[15] However, Ishihara et al reported that there was no difference in serum osteocalcin, PICT, and ICTP as well as urine Pyr and Dpyr between patients and controls.[9] Kashii et al did not find any difference in serum osteocalcin either.[13] Niu et al described that osteoprotegrin is decreased in patients with OSL, including ankylosing spondylitis (AS), diffuse idiopathic spinal hyperostosis (DISH), OPLL, and ossification of the yellow ligament (OYL) that is the same as OLF.[15] Osteoprotegerin (OPG) is a decoy receptor for the receptor activator of nuclear factor kappa B ligand (RANKL). By binding RANKL, OPG prevents RANK-mediated nuclear factor kappa B (NF-κB) activation.[18] OPG can reduce the production of osteoclasts by inhibiting the differentiation of osteoclast precursors.[19] A recent paper by Cai et al reported that osteopontin is increased in patients with cervical OPLL, compared with controls.[16] Osteopontin is biosynthesized by a variety of tissue types including preosteoblasts, osteoblasts, osteocytes, and odontoblasts. Osteopontin has been implicated as an important factor in bone remodeling.[20]

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

Table 2. 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
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 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
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 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
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 ng/mL	4.96 ± 1.81 ng/mL	<.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			<.05	Increase
		Serum	MK-7	16 male OPLL	16 male			NS	No difference
2011	Ikeda Y	Serum	Leptin	57 female OPLL	27 female	9.67 ± 5.1 ng/mL	6.55 ± 3.67 ng/mL	<.01	Increase
		Serum	Leptin	68 male OPLL	35 male	3.85 ± 2.2 ng/mL	3.20 ± 1.4 ng/mL	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 hemogrobin	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	3.6 ± 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	.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)

Table 2. (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 hemogrobin	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
		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
2017	Kawaguchi Y	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	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 ± 104.1 pg/mL	261.1 ± 111.4 ng/mL	<.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	<.05	Increase
		Serum	DKK-1	3 OYL	9	316.1 ± 112.1 pg/mL	792.5 ± 308.6 ng/mL	<.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
2017	Cai GD	Serum	FGF-23	76 male cOPLL	41 healthy male	35.11 ± 2.599 pg/mL	27.05 ± 2.526 pg/mL	.046	Increase
		Serum	Osteopontin	76 male cOPLL	41 healthy male	17880 ± 1326 pg/mL	13300 ± 1713 pg/mL	.04	Increase
		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 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; $1,25(OH)_2D$, 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-isomerized C-terminal cross-linking telopeptide 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; 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 5.[21] 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.[22] Sclerostin was found to be increased in patients with OPLL, compared with controls, in 2 studies.[13,15] There were also 2 articles that reported a positive decrease in DKK-1 in patients with OSL,[15,16] but 1 article described a result without any diffrences.[13] Niu et al reported that there was no difference in SFRP-1 between patients with OSL and controls.[15] SFRP1 is a member of the SFRP family, which act as soluble modulators of Wnt signaling.[23]

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.[24-26] FGF-23 is known to act on the kidney. It decreases reabsorption and increases excretion of phosphate.[27] A recent article by Cai et al found that the serum FGF-23 level in patients with cervical OPLL was increased compared with that in control.[16]

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.[28] Miyamoto et al found that plasma FN concentrations were significantly elevated in patients with OPLL or OLF compared with control subjects.[7]

Menatetrenone (MK-4)

MK-4 is a vitamin K compound used as a hemostatic agent, and also as an adjunctive therapy for osteoporosis.[29] MK-4 enhances osteoblast function and also inhibits osteoclast function.[30] Yamada et al reported that the serum MK-4 level was increased in male patients with OPLL, compared with control male subjects; however, there was no difference in females.[10]

Leptin

The Zucker fatty (fa/fa) rat is known to be an animal model of OPLL.[31] The rat reveals hereditary obesity and exhibits hyperglycemia, hyperinsulinemia, hyperlipidemia, and heterotopic ossification of the spine. Furthermore, obesity is a risk factor for OPLL.[11] Hyperleptinemia is a common feature of obese people and leptin is believed to be an important factor in the pathogenesis of OPLL.[11] 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.[11] 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.[32] OPLL is known to be associated with diabetes mellitus.[33] 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.[12] 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.[14] 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.[34] 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.[35] 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.[36] In a recent article by Kawaguchi et al, a negative correlation was found between the Pi and OPLL extent in the whole spine.[14] 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 OPLL[14,16]; 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 OSL[6,14]; 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 biomarkers[13,15,16] 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 regarding the present research of biomarkers in OSL.

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), alpha1-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-1 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.

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