Clinics in Surgery

Research Article
Published: 30 Mar, 2017

Quantification of Serum Levels of Two Potential Biomarkers and Clinical Features in Osteoarthritis Patients

*Takashi Kuroiwa[1,2], Hiroshi Ageta[2], Daiki Ikeda[1], Mitsuhiro Morita[1], Kunihiro Tsuchida[2] and Harumoto Yamadaz[1]**

[1]Department of Orthopaedic Surgery, Fujita Health University Hospital, Toyoake, Aichi, Japan

[2]Division for Therapies against Intractable Diseases, Institute for Comprehensive Medical Science, Fujita Health University, Toyoake, Aichi, Japan

Abstract

Introduction: Our previous proteomics analysis of human articular cartilage demonstrated that levels of leukocyte cell-derived chemotaxin 2 (LECT2) and peroxiredoxin 6 (PRDX6) in the cartilage differed significantly between osteoarthritis (OA) and control patients. LECT2 and PRDX6 levels were significantly increased and decreased, respectively, in articular cartilages from OA patients. In the present study, we evaluated the clinical significance of these findings by measuring serum levels of LECT2 and PRDX6 in OA patients.

Materials and Methods: OA samples were obtained from 73 patients with severe hip and knee OA. Control samples were obtained from 16 patients with femoral neck fracture (Fr). Serum LECT2 and PRDX6 levels were measured by enzyme-linked immunosorbent assay.

Results: Serum LECT2 and PRDX6 levels between OA and Fr patients showed the same trends with our previous study. Intriguingly, serum PRDX6 level was markedly elevated in OA patients 3 months after surgery as compared to before surgery. In addition, serum LECT2 and blood urea nitrogen (BUN) levels were correlated, while serum PRDX6 level was correlated with BUN, sodium, and potassium levels.

Conclusion: Our results indicate that PRDX6 will not only be a candidate biomarker for diagnosis of OA before operation, but also a biomarker for the evaluation of healing process of OA.

Keywords: Osteoarthritis; LECT2; PRDX6

OPEN ACCESS

Correspondence:
Harumoto Yamadaz, Department of Orthopaedic Surgery, Fujita Health University Hospital, 1-98 Dengakugakubo, Kutsukake, Toyoake, Aichi, 470-1192, Japan, Tel: +81(562)93-2169; E-mail: hayamada@fujita-hu.ac.jp

Received Date: *20 Dec 2016*
Accepted Date: *21 Mar 2017*
Published Date: *30 Mar 2017*

Citation:
Kuroiwa T, Ageta H, Ikeda D, Morita M, Tsuchida K, Yamadaz H. Quantification of Serum Levels of Two Potential Biomarkers and Clinical Features in Osteoarthritis Patients. Clin Surg. 2017; 2: 1378.

Introduction

Osteoarthritis (OA) is a common disease with a high prevalence among the middle-aged and elderly that is characterized by degeneration and destruction of articular cartilage, subchondral changes, and bone proliferation. The 2005 Research on Osteoarthritis Against Disability study carried out in Japan reported OA in 61.9% of individuals aged 60 years and older (n = 2,282); the prevalence of knee pain and radiographic knee OA with pain is 32.8% and 26.1%, respectively, in the general population [1], and the worldwide prevalence of OA was 250/100,000 in 2010 [2]. As the global population ages, the increase in the number of OA patients will have a major effect on medical costs.

Risk factors for OA include advanced age, obesity, gender, and genetic predisposition as well as local biomechanical factors such as joint instability and trauma and excessive mechanical stress [3]. However, the pathological mechanism of OA is not well understood. It is thought to first develop in cartilage, but is associated with secondary synovitis, bone proliferation, and cartilaginous degeneration. Fibrillation of the cartilage surface occurs in the initial lesion [4] accompanied by biochemical changes such as degeneration of the cartilage matrix, which generates low-molecular weight substances. Proteases such as matrix metalloproteinase and aggrecanase produced by chondrocytes are regulated by cytokines such as interleukin 1 (IL-1), IL-6, and tumor necrosis factor α as well as growth factors such as transforming growth factor β and basic fibroblast growth factor that are also produced by chondrocytes [5]. Following the cartilage destruction caused by these molecules, secondary synovitisas well as the healing of bone and cartilages induced simultaneously.

OA is typically diagnosed by imaging, including simple radiography and magnetic resonance imaging (MRI) [6]. The quantitative evaluation of cartilage has recently become possible with MRI;

Table 1: Clinical data of study subjects. p<0.05, OA vs. Fr group (Student's t and Mann-Whitney U tests). SE: Standard Error; Alb: Albumin; ALT: Alanine Transaminase; AST: Aspartate Transaminase; BMI: Body Mass Index; BUN: Blood Urea Nitrogen; Ca: Calcium; Cl: Chloride; CPK: Creatine Phosphokinase; Cr: Creatinine; CRP: C-reactive Protein; eGFR: Estimated Glomerular Filtration Rate; γ-GTP: γ-glutamyltranspeptidase; Hb: Hemoglobin; HbA1c: Hemoglobin A1c; HDL: High Density Lipoprotein-cholesterol; K: Potassium; Na: Sodium; Plt: Platelet; TChol: Total Cholesterol; TG: Triglyceride; TP: Total Protein; WBC: White Blood Cells.

Variable		Total			OA group			Fr group		p.value
	n	Means	SE	n	Mean	SE	n	Mean	SE	
Gender(Male/Female)	11178			7/66			4/12			
Age (years)	89	71.2	1.2	73	70.2	1.0	16	7620	3.0	0.05
Height (cm)	89	152.4	0.7	73	151.9	0.76	16	155.0	2.0	0.13
Weight (kg)	89	54.3	1.2	73	55.9	1.0	16	47.0	3.0	0.002
WI (kg/rns)	89	23.4	0.5	73	242.0	0.49	16	19.0	0.93	40.0
KL grade 3 (ms/it/ferrate)				0/4						
4 (nwerlisnale)				752						
past medical history										
Hypertension	34			26			8			
Diabetes mellitus	13			10			3			
Variable		Total			OA group			Fr group		p-value
	n	Median	SE	n	Median	SE	n	Median	SE	
Blood samples										
WBC (10Na)	89	6.3	0.6	73	5.9	0.66	16	8.0	0.51	0.08
Hb (gOL)	89	12.4	0.2	73	12.5	0.16	16	12.0	0.47	0.03
Pft (101/pL)	89	222.0	0.7	73	21.8	0.76	16	26.0	2.0	0.11
Hb As. (Y.)	84	5.7	0.1	€8	5.6	0.08	15	6.0	0.24	0.57
Sera										
TP (gicIL)	89	7.0	0.1	73	7.1	0.07	I6	625	0.19	40.0
Alb (gh1L)	89	4.1	0.1	73	4.2	0.06	16	3.0	0.18	40.0
CPl((WL)	88	74.5	10.4	72	76.0	6.0	16	64	51.0	0.02
CRP (mq/dL)	89	0.0	0.3	73	0.0	0.19	16	2.0	1.0	40.0
Liver function										
AST (WL)	89	19.0	2.5	73	19.0	1.0	16	22.0	13.0	0.009
ALT (WL)	89	15.0	2.0	73	15.0	2.0	16	15.0	9.0	0.12
y-GTP (IWL)	88	21.0	5.9	72	20.5	3.0	16	23.0	30.0	0.008
Kidney tunclion										
BIM (mg/dL)	89	15.4	1.2	73	15.0	0.80	16	20.0	5.0	40.0
Cr (mcialL)	89	0.7	0.2	73	0.6	0.08	16	0.86	0.82	0.004
eGFR (mUrnin/1.73m)	89	68.7	2.7	73	72.1	2.60	16	63.0	9.0	0.14
Dyshpidemia										
TChol (Mg/a)	89	190.0	4.1	73	199.0	4.0	16	152	9.0	40.0
TO (mg!dL)	86	101.0	6.5	70	109.0	7.0	16	78	16.0	0.19
Hot (mg/dL)	80	55.0	2.1	64	56.5	2.0	16	50	5.0	0.48
Electrolytes										
Na ImEg/L)	89	141.0	0.4	73	141.0	0.30	16	140.0	1.0	40.0
K (mErpL)	89	4.1	0.1	73	4.1	0.05	16	4.0	0.17	0.07
Cl (mEtS1.)	89	105.0	0.4	73	106.0	0.31	16	102.0	2.0	<0.001
Ca (nuadl)	87	9.6	0.1	71	9.7	0.05	16	9.0	0.52	40.0

however, the procedure is time-consuming, costly, and is invasive owing to the use of contrast medium. Biochemical changes in the cartilage in OA begin earlier than diagnosis by conventional imaging techniques; as such, a more sensitive evaluation method is needed for early diagnosis [7]. In recent years, biomarkers have been developed and tested for this purpose [8]. Biomarkers have already been applied to the diagnosis of other bone and joint diseases, including osteoporosis and rheumatoid arthritis (RA) [9,10]. Similarly, serum or urine biomarkers can potentially be used to measure joint structure metabolism in the assessment of OA [11].

Table 2: Correlations among clinical data and serum LECT2 and PRDX6 levels in pre-operative OA patients. Correlations among clinical data and serum LECT2 and PRDX6 levels in pre-operative OA patients were analyzed using Spearman's rank correlation for all possible combinations. Alb: Albumin; ALP: Alkaline Phosphatase; ALT: Alanine Transaminase; Amy: Amylase; AST: Aspartate Transaminase; BMI: Body Mass Index; BS: Blood Sugar; BUN: Blood Urea Nitrogen; Ca: Calcium Cl: Chloride; CPK: Creatine Phosphokinase; Cr: Creatinine; CRP: C-Reactive Protein; dBP: Diastolic Blood Pressure; eGFR: Estimated Glomerular Filtration Rate; γ-GTP: γ-Glutamyltranspeptidase; Hb: Hemoglobin: HbA1c: Hemoglobin A1c; HDL-cholesterol: High Density Lipoprotein-cholesterol; HR: Heart Rate; K: Potassium; Na: Sodium; P: Phosphorus; Plt: Platelet; TChol: Total Cholesterol; sBP: Systolic Blood Pressure; TBil: Total Bilirubin; TG: Triglyceride; TP: Total Protein; UA: Uric Acid; WBC: White Blood Cells.

		FCT2	PRDX6			LECT2	PRDX6			LECT2	PRDX6
age	r	-76	0.	TIN	r	..109	0.	UA	f	0.	0.
	0	1.	0.		0	0.	0.		/2	1.	0.
	n	73	49		n	73	49		n	69	47
height	r	-139	0.	AST	r	0.	.080	eGFR	f	0.	•59
	P	241	1.		0	1.	1.		P	0.	.690
	n	73	49		n	73	49		n	73	49
weight	r	0.	0.	ALT	r	124	0.	Na	f	-152	-.359 *
	P	870	0.		0	294	0.		P	0.	0.
	9	73	49		n	73	49		n	73	49
BMI	r	0.	0.	ALP	r	0.	0.	K	1	0.	.364 *
	P	1.	0.		p	1.	.510		p	1.	.010
	n	73	49		n	73	49		n	73	49
HR	r	0.	0.	v0TP	r	0.		Cl	r	0.	0.
	0	1.	1.		0	1.			Co	0.	0.
	0	73	49		1.	72			n	73	49
sEIP	r	0.	0.	Any	r	1.	0.	Ca	1	0.	0.
	P	0.	.630		0	0.	1.		P	1.	.700
	n	73	49		n	73	49		n	71	49
06P	r	--.070	0.	CPK	r	0.	56	P	1	0.	104
	P	1.	0.		0	0.	1.		P	1.	.490
	n	73	49		n	72	49		n	66	46
Vil3C	r	0.	0.	TO	r			HbA1c	r	0.	140
	P	0.	.870		0				p	0.	0.
	n	73	49		n				n	69	46
ile	r	0.	0.	Teem	r	0.	0.	BS	r	0.	0.
	0	0.	487		0	1.	0.		P	0.	.810
	n	73	49		n	73	49		11	70	48
rl	r	0.	0.	MX	r	--.110	0.	CRP	r	0.	0.
	P	1.	0.		0	0.	0.		p	0.	1.
	n	73	49		n	64	44		n	73	49
TP	r	on		BUN	1	.310*	.316 *	LECT2	r		0.
	0	1.			0	0.	0.		p		0.
	11	73			n	73	49		n		49
At	I	0.	--.170	Cr	r	0.	0.				
	0	1.	0.		0	0.	1.				
	n	73	49		n	73	49				

In 2006, Burden of disease, Investigative, Prognostic, Efficacy of intervention, and Diagnostic (BIPED) biomarker classification was proposed for OA with the aim of identifying appropriate markers for specific purposes [12]. To data there are no OA biomarkers that are used in general clinical practice, although various studies have addressed this issue using proteomics and protein arrays to probe blood, urine, and synovial fluid samples [13]. Collagen degradation products and complement are useful OA biomarkers; abnormal increases in the expression and activity of complement and the major cartilage components collagen type II and agree can have been detected in the synovium and synovial fluid in OA patients [14,15].

In our previous study, we carried out a comprehensive proteomics analysis of OA patient cartilage using isobaric tags for relative and absolute quantization (iTRAQ), and identified 76 proteins that were differentially expressed between OA patients and control

subjects. Of these, leukocyte cell-derived chemotaxin 2 (LECT2) and peroxiredoxin6 (PRDX6) had not been previously reported as OA biomarkers, and western blot analysis revealed that LECT2 and PRDX6 levels were significantly increased and decreased, respectively, in articular cartilages from OA patients [16].

LECT2 is a secretory protein produced in the liver [17] that is associated with various diseases, including obesity and skeletal muscle insulin resistance [18], atherosclerotic inflammatory reaction [19], hepatocellular carcinoma [20], and amyloidosis [21]. PRDX6 is an enzyme with peroxidase and phospholipase A2 activities that has antioxidant properties [22] and is expressed in various tissues, including the lungs. PRDX6 has been implicated in the repair of cell membranes in pulmonary microvascular endothelial cells following oxidative stress [23].

To identify additional tools for the pathological evaluation of OA, in the present study we quantified the serum levels of LECT2 and PRDX6 by enzyme-linked immunosorbent assay (ELISA) and examined the relationship between patients' physical and blood test data.

Materials and Methods

This study was approved by the steering committee and was carried out under the guidelines for clinical studies of Fujita Health University. Written, informed consent was obtained from all participants.

Background

In our previous study, we performed comprehensive proteomics analysis in human articular cartilage from OA or femoral neck fracture patients 16. To evaluate the clinical significance of these findings, we collected blood samples from patients with OA or femoral neck fracture for the control group. The study population included 89 Japanese subjects (11 male and 78 female; aged 31-91 years) (Table 1) who visited the department of orthopaedic surgery at Fujita Health University (Aichi, Japan) from April 2015 to December 2015 for surgery. OA samples were obtained from 73 patients with severe hip or knee OA who underwent total hip or knee replacement. Patients with osteonecrosis of the femoral head, revision surgery, and RA cases were excluded. Control samples were obtained from 16 patients with femoral neck fracture (Fr) who underwent bipolar femoral head replacement surgery or open reduction internal fixation surgery. Patient characteristics including gender, age, height, weight, body mass index (BMI), heart rate, systolic blood pressure, diastolic blood pressure, and past medical history (hypertension (HT) and diabetes mellitus (DM))were recorded during hospitalization.

Blood sampling

Blood samples were obtained during hospitalization and immediately centrifuged at 3300 rpm at 4°C for 10 min and stored at−80°C. The following biochemical parameters were recorded for each sample: white blood cell count; levels of hemoglobin (Hb), platelet, total protein (TP), albumin (Alb), total bilirubin, aspartate transaminase (AST), alanine transaminase, alkaline phosphatase, γ-glutamyltranspeptidase (γ-GTP), amylase(Amy), creatine phosphokinase (CPK), triglyceride, total cholesterol (TChol), high-density lipoprotein cholesterol, blood urea nitrogen (BUN), creatinine (Cr), uric acid, sodium (Na), potassium (K), chloride (Cl), calcium (Ca), phosphorus, hemoglobin A_{1c}, blood sugar, and C-reactive protein (CRP); and estimated glomerular filtration rate. During the perioperative period, we obtained and examined the blood samples among hospitalization (pre-operation), before discharge (2 weeks after surgery) and the first consultation (3 months after surgery).

Radiographic finding

Anterior-posterior X-rays of both hips, both knees, and lumbar spine were obtained for all patients in the standing position. The severity of OA was radiographically determined according to the Kellgren–Lawrence (KL) grading system [24].

ELISA

LECT2 and PRDX6 concentrations in serum were determined using CUS LECT2 (CSB–EL012855HU; Cusabio) and PRDX6 (CSB–EL018659HU; Cusabio) ELISA kits according to the manufacturer's protocol. The lower detection limits of each kit were defined as 0, and upper limits were defined as values above the measurement range. Inter-assay precision (precision between assays, 8 samples were tested for three assays to assess) was demonstrated by coefficient of variation expressed as a percentage (CV%). Inter-assay precisions of LECT2 and PRDX6 analysis were 15% and 10%, respectively.

Statistical analysis

Data were analyzed using SPSS v.23 (IBM, Tokyo, Japan). Anthropometric and biochemical data are presented as median ± standard error. Non-parametric variables were compared between groups with the Wilcoxon signed-rank, Mann-Whitney U, and Kruskal-Wallis tests. Correlations between variables were assessed with Spearman's rank correlation test. $|r| > 0.3$ and $p<0.05$ were considered statistically significant.

Results

We previously carried out a proteomics analysis of human articular cartilage using the iTRAQ method and found that LECT2 and PRDX6 are differently expressed between OA and Fr patients. In the current study, we measured the levels of LECT2 and PRDX6 inhuman serum by ELISA and examined these parameters with respect to clinical data.

The OA group was composed of 73 patients (seven male and 66 female), and the Fr group of 16 patients (four male and 12 female). Patients' clinical characteristics are shown in Table 1. Mean ages were similar between the OA and Fr groups (70.2 ± 1.17 vs. 76.2 ± 3.48, p=0.05). However, there were statistically significant differences in weight (55.9 ± 1.21 vs. 46.9 ± 2.87, p=0.002) and BMI (24.2 ± 0.49 vs. 19.4 ± 0.93, $p<0.001$; Table 1). Four females were KL grade3, and all other patients were KL grade 4 in the OA group. T he two groups differed in terms of Hb, TP, Alb, CPK, CRP, AST, γ-GTP, BUN, Cr, TChol, Na, Cl, and Ca levels ($p<0.05$).

Prior to operation, LECT2 and PRDX6 levels tended to increase and decrease, respectively, in serum from OA patients. However, their levels did not show statistically significant difference between the OA and Fr groups (LECT2: 0.71 ± 0.58 vs. 0 ± 0.36, p=0.44, PRDX6:119.1 ± 23.8 vs. 195.4 ± 32.4, p=0.10, Figure 1). To determine whether the operative procedure affected these parameters, we compared the serum levels of LECT2 and PRDX6 in the OA group pre-operation (Pre), 2 weeks after surgery (2W) and 3 months after surgery (3M). Serum LECT2 levels were similar at Pre, 2W, and 3M. In contrast, serum PRDX6 level was higher at 3M than at Pre (p=0.02, Figure 2). The same trends were observed in individual patients (p=0.002, Figure 3(a)). In PRDX6 analysis on individual patient, the mean value of the increasing rate (3M/Pre) and CV% between Pre and 3M were

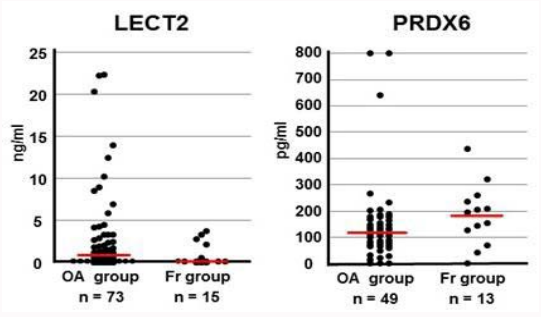

Figure 1: Comparison of serum LECT2 and PRDX6 levels between pre-operative OA and Fr patients. Serum LECT2 and PRDX6 levels were measured by ELISA. Horizontal red bars indicate median values for each group. LECT2: OA, n= 73 and Fr, n= 15 (p=0.44, Mann-Whitney U test). PRDX6: OA, n=49 and Fr, n=13 (p=0.10; Mann-Whitney U test).

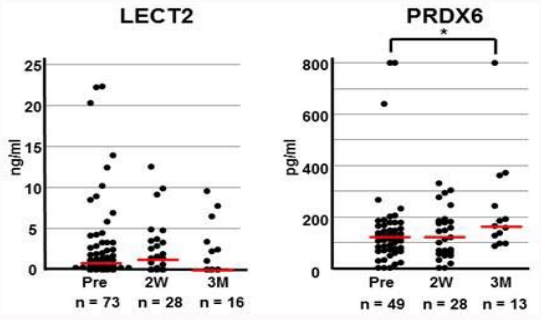

Figure 2: Changes in serum LECT2 and PRDX6 levels in OA patients after operation. Serum LECT2 and PRDX6 levels were measured pre-operation (Pre), 2 weeks after surgery (2W), and 3 months after surgery (3M). Differences between the three groups were evaluated with the Kruskal-Wallis test. Serum PRDX6 level was elevated in the 3M as compared to the Pre group (p=0.02; Mann-Whitney U test). Horizontal red bars indicate median values for each group.

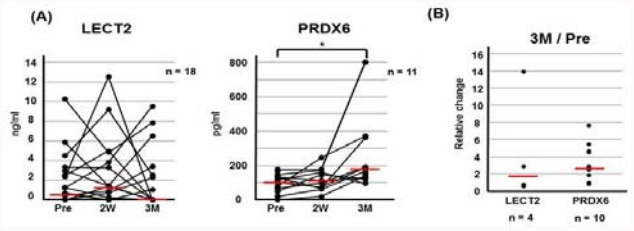

Figure 3: Changes in serum LECT2 and PRDX6 levels in individual patients. (A) Each line represents each patient; horizontal red bars show median value for each group. Pre, pre-operation; 2W, 2 weeks after operation; 3M, 3 months after operation. SerumLECT2 and PRDX6 levels were compared with the Kruskal-Wallis test (LECT2, p=0.44 and PRDX6, p=0.07). PRDX6 but not LECT level differed significantly between Pre and 3M groups with the Wilcoxon signed-rank test (p=0.002 and p=0.53, respectively). (B) The rate of change between Pre and 3M. Zero value at Pre and 3M was not included in this data plot. Each plot represents each patient; horizontal red bars show mean value for each group.

2.7 and 41.7%, respectively. Inter-assay precision of PRDX6 analysis was 10%, indicating that this change was significant (Figure 3(b)).

We investigated whether serum LECT2 and PRDX6 levels were correlated with clinical (anthropometric and biochemical) data in the Pre OA group (Table 2). We found that serum LECT2 and BUN levels were correlated (r=0.310, p=0.008). There were no differences between LECT2 and PRDX levels (r=0.153, p=0.294, Figure 4(a)). On the other hand, the serum level of PRDX6 was correlated with BUN

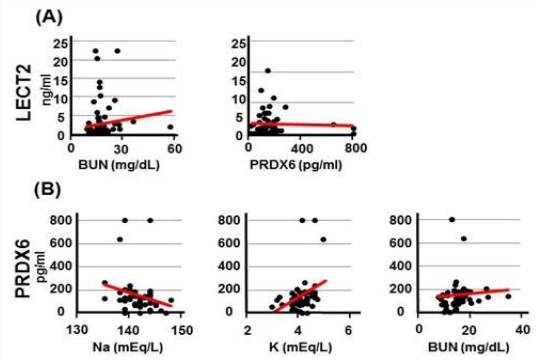

Figure 4: Correlation between clinical data and serum LECT2 (A) and PRDX6 (B) levels in pre-operative OA patients. Correlations between clinical data and serum LECT2 and PRDX6 level were analyzed with Spearman's rank correlation test for all possible combinations. LECT2 was positively correlated with BUN (r=0.310, p=0.008) level. PRDX6 was positively correlated with BUN (r=0.316, p=0.027) and K (r=0.364, p=0.010) level and negatively correlated with Na (r =−0.359, p=0.011).

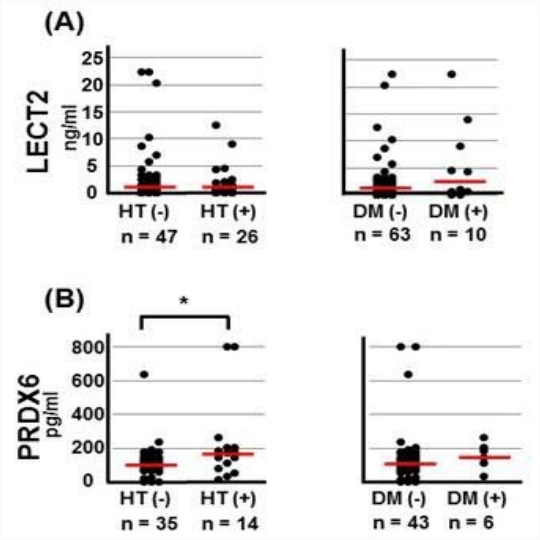

Figure 5: Serum LECT2 (A) and PRDX6 (B) levels in pre-operative OA patients with/without hypertension (HT) and diabetes mellitus (DM). OA patients with and without HT differed significantly with respect to PRDX6 levels (p= 0.02; Mann-Whitney U test).

(r=0.316, p=0.027), Na (r=−0.359, p=0.011), and K (r=0.364, p=0.010, Figure 4(b)) levels.

OA patients with and without HT differed in terms of serum PRDX6 level (p=0.02); on the other hand, there was no difference between OA patients with and without DM. A history of HT or DM had no effects on serum LECT2 level (Figure 5(a)).

Discussion

In the present study, we evaluated the utility of serum LECT2 and PRDX6 levels as candidate biomarkers of OA. This is the first report to quantify the serum concentrations of LECT2 and PRDX6 in OA patients. Although there were no statistically significant difference in serum LECT2 and PRDX6 levels between OA and Fr patients, the same trends with our previous study were observed. Serum biomarkers are modulated or metabolized in various tissues, while changes in cartilage or synovial fluid may not be reflected in serum [25]. It is therefore possible that we were unable to detect significant

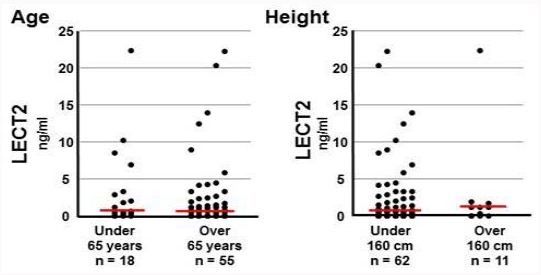

Figure 6: Comparison of serum LECT2 levels by age and height in pre-operative OA patients. Age, LECT2: median ± standard error (<65 years, 0.92 ± 1.34; >65 years, 0.71 ± 0.63); Mann-Whitney U test: p=0.23. Height, LECT2: median ± SE (<160 cm, 0.68 ± 0.59; >160 cm, 1.18 ± 1.98); Mann-Whitney U test: p=0.79. Horizontal red bars show median values for each group.

differences due to the effects of normal joints or other tissues on serum content. Interestingly, we found that serum PRDX6 levels were significantly elevated 3 months after as compared to before surgery. We also found that serum LECT2 level was correlated with BUN, and that serum PRDX6 level was correlated with BUN, Na, and K levels. In addition, serum PRDX6 levels were also higher in OA patients with than in those without HT.

Prognosis analysis of acute liver failure have revealed that serum LECT2 levels are lower in patients who died of acute liver failure than in survivors [26] and are elevated in patients with obesity and fatty liver [27]. Although there are no reports on the relationship between LECT2 and OA, in a mouse RA model, the exacerbation of arthritis resulting from *LECT2* deficiencywas improved by LECT2 over expression [28], suggesting that LECT2 may have an inhibitory effect on inflammation in RA. OA is also characterized by inflammation, although it is less severe than in RA [29]. Our previous finding that in human cartilage, LECT2 expression was higher in OA than in Fr patients suggested that LECT2 levels are upregulated as a biological defense mechanism to inhibit inflammation. Although it was not statistically significant, we observed a similar trend in serum samples from OA patients in the present study. We expect that the difference between OA and Fr patients would be significant using a larger sample size. We also found that serum LECT2 and BUN levels were correlated. Urea nitrogen is a waste product of protein metabolism and reflects renal functioning as serum Cr level [30]. Thus, although serum LECT2 and Cr levels were not correlated, LECT2 may nonetheless be associated with renal function.

Serum PRDX6 level is approximately five times higher in breast cancer patients than in healthy individuals [31]. We previously reported that PRDX6 is down regulated in the cartilage of OA patients. Similarly, in the present study, PRDX6 levels were lower in the serum of OA as compared to Fr patients, although the difference was not statistically significant. PRDX6 is an antioxidant enzyme with both peroxidase and phospholipase A2 activities. Reactive oxygen species (ROS) production is increased in OA [32,33]. Interestingly, PRDX5 levels are upregulated and protects against ROS in OA cartilage [34], and *PRDX5* knockdown stimulates osteoarthritic chondrocyte apoptosis and inhibits the scavenging of endogenous ROS [35]. The PRDX family is classified into three subgroups (2-Cys, atypical 2-Cys, and 1-Cys) according to the number and position of cysteine residues that participate in catalysis. PRDX5 is an atypical 2-Cys whereas PRDX6 is a 1-Cys member [36]. Thus, differences between these two proteins in terms of effects on cartilage may be attributable to structural differences. In the present study, we found that serum level of PRDX6 was markedly elevated 3 months after as compared to before surgery in OA patients; this may be explained by the fact that the ROS-induced reduction in PRDX6 expression was restored by surgery.

In this study, serum level of PRDX6 was correlated to BUN, Na, and K levels. Renal failure is associated with BUN and K concentrations in blood, and the prevalence of OA is higher among cases of renal failure [37]; indeed, approximately, 37% of OA patients have renal impairment as compared to only 27% of the general population [37]. Thus, although we did not find any correlation between serum levels of PRDX6 and Cr, PRDX6 may be associated with renal function. We also found that serum PRDX6 level was higher in OA patients with than in those without concurrent HT. Although the relationship between OA and HT remains unclear, one U.S. study reported that 40% of OA patients have HT [37]. On the contrary, in Korea, OA prevalence was significantly lower in OA patients with HT than in those with OA only after adjusting for patient background, and longer duration of HT attenuated this correlation [38]. Thus, the presence of HT may affect the level of PRDX6, which has a protective effect against OA.

Conclusion

This study is the first to report the measurement of serum LECT2 and PRDX6 by ELISA in OA patients. LECT2 and PRDX6 levels tended to increase and decrease, respectively, in serum from OA patients. Furthermore, we found that serum PRDX6 level was significantly elevated 3 months after as compared to before surgery. In addition, serum PRDX6 level was correlated with BUN, Na, and K levels. Given that serum level of PRDX6 was linked to the healing of OA, PRDX6 may be useful as a candidate biomarker not only for diagnosis of OA but also for monitoring OA improvement.

Acknowledgment

We thank members of the orthopaedic surgery department at Fujita Health University Hospital for providing of clinical data. We also thank Mrs. Yamaguchi and Mrs. Tajima for technical assistance. This work was partly supported by the Program for the Strategic Research Foundation at Private Universities and an Intramural Research Grant (26-8) for Neurological and Psychiatric Disorders of NCNP. All authors declare that there is no conflict of interest.

References

1. Muraki S, Oka H, Akune T, Mabuchi A, En-yo Y, Yoshida M, et al. Prevalence of radiographic knee osteoarthritis and its association with knee pain in the elderly of Japanese population-based cohorts: the ROAD study. Osteoarthritis Cartilage. 2009;17:113743.

2. Murray CJ, Vos T, Lozano R, Naghavi M, Flaxman AD, Michaud C, et al. Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet. 2012;380:2197-2223.

3. Felson DT, Lawrence RC, Dieppe PA, Hirsch R, Helmick CG, Jordan JM, et al. Osteoarthritis: new insights. Part 1: the disease and its risk factors. Ann Intern Med. 2000;133(8):635-46.

4. Panula HE, Hyttinen MM, Arokoski JP, Langsjo TK, Pelttari A, Kiviranta I, et al. Articular cartilage superficial zone collagen birefringence reduced and cartilage thickness increased before surface fibrillation in experimental osteoarthritis. Ann Rheum Dis. 1998;57:237-45.

5. Loeser RF. Molecular mechanisms of cartilage destruction: mechanics,

inflammatory mediators, and aging collide. Arthritis Rheum. 2006;54:1357-60.

6. Hayashi D, Roemer FW, Guermazi A. Imaging for osteoarthritis. Ann Phys Rehabil Med. 2016;59(3):161-9.

7. Ishijima M, Watari T, Naito K, Kaneko H, Futami I, Yoshimura-Ishida K, et al. Relationships between biomarkers of cartilage, bone, synovial metabolism and knee pain provide insights into the origins of pain in early knee osteoarthritis. Arthritis Res Ther. 2011;13:22.

8. Bay-Jensen AC, Reker D, Kjelgaard-Petersen CF, Mobasheri A, Karsdal MA, Ladel C, et al. Osteoarthritis year in review 2015: soluble biomarkers and the BIPED criteria. Osteoarthritis Cartilage. 2016;24:9-20.

9. Diem SJ, Joffe H, Larson JC, Tsai JN, Guthrie KA, LaCroix AZ, et al. Effects of escitalopram on markers of bone turnover: a randomized clinical trial. J Clin Endocrinol Metab. 2014;99(9):E1732-7.

10. Taylor P, Gartemann J, Hsieh J, Creeden J. A systematic review of serum biomarkers anti-cyclic citrullinated Peptide and rheumatoid factor as tests for rheumatoid arthritis. Autoimmune Dis. 2011;2011: 815038.

11. Garnero P, Piperno M, Gineyts E, Christgau S, Delmas PD, Vignon E. Cross sectional evaluation of biochemical markers of bone, cartilage, and synovial tissue metabolism in patients with knee osteoarthritis: relations with disease activity and joint damage. Ann Rheum Dis. 2001;60:619-26.

12. Bauer DC, Hunter DJ, Abramson SB, Attur M, Corr M, Felson D, et al. Classification of osteoarthritis biomarkers: a proposed approach. Osteoarthritis Cartilage. 2006;14(8):723-7.

13. Blanco FJ. Osteoarthritis year in review 2014: we need more biochemical biomarkers in qualification phase. Osteoarthritis Cartilage. 2014;22:2025-32.

14. Elsaid KA, Chichester CO. Review: Collagen markers in early arthritic diseases. Clin Chim Acta. 2006;365(1-2):68-77.

15. Wang Q, Rozelle AL, Lepus CM, Scanzello CR, Song JJ, Larsen DM, et al. Identification of a central role for complement in osteoarthritis. Nat Med. 2011;17(12):1674-9.

16. Ikeda D, Ageta H, Tsuchida K, Yamada H. iTRAQ-based proteomics reveals novel biomarkers of osteoarthritis. Biomarkers. 2013;18(7):565-72.

17. Yamagoe S, Yamakawa Y, Matsuo Y, Minowada J, Mizuno S, Suzuki K. Purification and primary amino acid sequence of a novel neutrophil chemotactic factor LECT2. Immunol Lett. 1996;52:9-13.

18. Lan F, Misu H, Chikamoto K, Takayama H, Kikuchi A, Mohri K, et al. LECT2 functions as a hepatokine that links obesity to skeletal muscle insulin resistance. Diabetes. 2014;63(5):1649-64.

19. Hwang HJ, Jung TW, Hong HC, Seo JA, Kim SG, Kim NH, et al. LECT2 induces atherosclerotic inflammatory reaction via CD209 receptor-mediated JNK phosphorylation in human endothelial cells. Metabolism. 2015;64:1175-82.

20. Okabe H, Delgado E, Lee JM, Yang J, Kinoshita H, Hayashi H, et al. Role of leukocyte cell-derived chemotaxin 2 as a biomarker in hepatocellular carcinoma. PLoS One. 2014;9(6):e98817.

21. Khalighi MA, Yue A, Hwang MT, Wallace WD. Leukocyte chemotactic factor 2 (LECT2) amyloidosis presenting as pulmonary-renal syndrome: a case report and review of the literature. Clin Kidney J. 2013;6: 618-21.

22. Kim TS, Sundaresh CS, Feinstein SI, Dodia C, Skach WR, Jain MK, et al. Identification of a human cDNA clone for lysosomal type Ca2+-independent phospholipase A2 and properties of the expressed protein. J Biol Chem. 1997;272(4):2542-50.

23. Li H, Benipal B, Zhou S, Dodia C, Chatterjee S, Tao JQ, et al. Critical role of peroxiredoxin 6 in the repair of peroxidized cell membranes following oxidative stress. Free Radic Biol Med. 2015;87:356-65.

24. Kellgren JH, Lawrence JS. Radiological assessment of osteo-arthrosis. Ann Rheum Dis. 1957;16(4):494-502.

25. Li L, Jiang BE. Serum and synovial fluid chemokine ligand 2/monocyte chemoattractant protein 1 concentrations correlates with symptomatic severity in patients with knee osteoarthritis. Ann Clin Biochem. 2015;52:276-282.

26. Sato Y, Watanabe H, Kameyama H, Kobayashi T, Yamamoto S, Takeishi T, et al. Serum LECT2 level as a prognostic indicator in acute liver failure. Transplant Proc. 2004;36(8):2359-61.

27. Okumura A, Unoki-Kubota H, Matsushita Y, Shiga T, Moriyoshi Y, Yamagoe S, et al. Increased serum leukocyte cell-derived chemotaxin 2 (LECT2) levels in obesity and fatty liver. Biosci Trends. 2013;7(6):276-83.

28. Okumura A, Saito T, Otani I, Kojima K, Yamada Y, Ishida-Okawara A, et al. Suppressive role of leukocyte cell-derived chemotaxin 2 in mouse anti-type II collagen antibody-induced arthritis. Arthritis Rheum. 2008;58:413-21.

29. Bonnet CS, Walsh DA. Osteoarthritis, angiogenesis and inflammation. Rheumatology (Oxford). 2005;44(1):7-16.

30. Lyman JL. Blood urea nitrogen and creatinine. Emerg Med Clin North Am. 1986;4(2):223-33.

31. Liu FJ, Wang XB, Cao AG. Screening and functional analysis of a differential protein profile of human breast cancer. Oncol Lett. 2014;7(6):1851-1856.

32. Regan E, Flannelly J, Bowler R, Tran K, Nicks M, Carbone BD, et al. Extracellular superoxide dismutase and oxidant damage in osteoarthritis. Arthritis Rheum. 2005;52(11):3479-91.

33. Collins JA, Moots RJ, Winstanley R, Clegg PD, Milner PI. Oxygen and pH-sensitivity of human osteoarthritic chondrocytes in 3-D alginate bead culture system. Osteoarthritis Cartilage. 2013;21:1790-98.

34. Wang MX, Wei A, Yuan J, Trickett A, Knoops B, Murrell GA. Expression and regulation of peroxiredoxin 5 in human osteoarthritis. FEBS Lett. 2002;531(2):359-62.

35. Ma Y, Li R, Zhang Y, Zhou L, Dai Y. Knockdown of peroxiredoxin 5 inhibits the growth of osteoarthritic chondrocytes via upregulating Wnt/Î²-catenin signaling. Free Radic Biol Med. 2014;76:251-60.

36. Kang SW, Baines IC, Rhee SG. Characterization of a mammalian peroxiredoxin that contains one conserved cysteine. J Biol Chem. 1998;273(11):6303-11.

37. Singh G, Miller JD, Lee FH, Pettitt D, Russell MW. Prevalence of cardiovascular disease risk factors among US adults with self-reported osteoarthritis: data from the Third National Health and Nutrition Examination Survey. Am J Manag Care. 2002;8:383-91.

38. Bae YH, Shin JS, Lee J, Kim MR, Park KB, Cho JH, et al. Association between Hypertension and the Prevalence of Low Back Pain and Osteoarthritis in Koreans: A Cross-Sectional Study. PLoS One. 2015;10(9):e0138790.