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Utility of soluble lectin-like oxidized low-density lipoprotein receptor-1 (sLOX-1) in the postmortem diagnosis of ischemic heart disease

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ABSTRACT

Purpose: Ischemic heart disease (IHD) is a major cause of death in developed countries. Postmortem IHD diagnosis using biochemical markers is difficult because of the postmortem changes. In the present study, we investigated the utility of soluble lectin-like low-density lipoprotein receptor-1 (sLOX-1) in body fluids obtained from forensic autopsy cases.

Methods: We measured pericardial fluid, urine, and serum sLOX-1 levels; these samples were obtained from medicolegal autopsy cases (n = 149, postmortem interval < 72 h), and the utility of these biomarkers postmortem acute IHD diagnosis was evaluated.

Results: The pericardial fluid and urine of patients with acute IHD had higher sLOX-1 levels ($p < .05$) compared to the controls. No significant differences were found between the sLOX-1 level and the degree of coronary atherosclerosis, body mass index, and postmortem interval.

Conclusion: sLOX-1 levels in pericardial fluid and urine samples obtained postmortem are useful markers of acute IHD.

1. Introduction

Ischemic heart disease (IHD) is a major cause of sudden death in developed countries. Biochemical markers are central to the clinical diagnosis of IHD. However, the usefulness of biochemical markers for the postmortem diagnosis of IHD in forensic autopsy cases is uncertain because of postmortem changes. In general, the autopsy diagnosis of IHD is achieved through both macroscopic and microscopic findings based on anamnesis, death-related details, or both. Because early-stage pathological consequences of IHD can only be observed 6 h after onset, the diagnosis of IHD in sudden death cases is challenging.[1]

Several biochemical markers, such as N-terminal pro-brain natriuretic peptide (NT-proBNP), heart-type fatty acid-binding protein (hFABP), creatine kinase MB (CKMB), myoglobin, cardiac troponin (cTn) T, and cardiac myosin light chain I, are known to increase in patients with IHD.[2–16] Previous studies have measured the levels of these biochemical markers in forensic autopsy cases. Although some studies revealed a significant difference in serum cTnT, NT-proBNP, and hFABP levels and pericardial fluid myoglobin, CKMB, and cTnT levels, postmortem elevation of these markers was a major concern.[17–29]

Atherosclerosis plays a major role in the pathogenesis of IHD. Oxidized low-density lipoprotein (LDL) is associated with atherogenesis, and lectin-like oxidized LDL receptor-1 (LOX-1) is the endothelial receptor of oxidized LDL.[30,31] LOX-1 is a type II membrane protein that belongs to the C-type lectin family and is overexpressed in the endothelial cells, macrophages, and smooth muscle cells of atherosclerotic lesions.[30,31] LOX-1 overexpression in the atherosclerotic lesions in blood vessels releases, by a cleavage process, the extracellular domain of this receptor into the blood as soluble LOX-1 (sLOX-1).[32,33] Tumor necrosis factor alpha-converting enzyme (TACE)/ADAM17, a disintegrin metalloproteinase domain-containing protein 10, and interleukin-18 are involved in this cleavage process,[34–36] and sLOX-1 may be associated with plaque vulnerability. Recent studies have reported increased serum sLOX-1 levels in patients with acute coronary syndrome (ACS), suggesting that measurement of sLOX-1 may be useful in the clinical diagnosis of acute-phase IHD.[37–44] Other studies have revealed serum sLOX-1 elevation in patients with obesity, type II diabetes, atherosclerotic conditions, and aortic dissection.[45–47]

To the best of our knowledge, no studies have investigated the use of sLOX-1 in forensic autopsy cases. In the present study, we measured the level of sLOX-1 not only in serum, but also in pericardial fluid and urine because serum biomarkers are likely to be unstable owing to postmortem changes, and we validated their use in the postmortem diagnosis of IHD.

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2. Methods

2.1. Case selection

The present study included forensic autopsies performed at the Jikei University School of Medicine, between January 2015 and March 2017. We examined 70 cases of acute IHD (mean age: 61.9 years; range: 35–87 years) and 79 control cases (mean age: 62.2 years; range: 22–89 years). The diagnosis of acute IHD was based on both macroscopic findings such as coronary stenosis and signs of abrupt death (blood fluidity, organ congestion), and microscopic findings such as coronary embolism/plaque rupture and myocardial necrosis. In the absence of microscopic findings, a diagnosis by exclusion of other possible causes of death was made. For all cases, the postmortem interval (PMI) was 72 h or less. The enforcement of cardiopulmonary resuscitation was determined by the medical records presented by the police.

The Ethics Committee of the Jikei University School of Medicine for Biochemical Research approved this study. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

2.2. Sample collection and laboratory assays

The cardiac blood serum, pericardial fluid, and urine samples obtained during autopsies were stored at −80 °C as quickly as possible and were kept frozen until biomarker measurement. sLOX-1 levels were measured by a sandwich chemiluminescence enzyme immunoassay (NK Medico Co., Ltd.) using two kinds of monoclonal antibody against the extracellular domain of LOX-1, that is, B017M and a chicken monoclonal anti human LOX-1 antibody HUC3-48.

Table 1 shows the number of samples. Among the 149 cases in the study, pericardial fluid was obtained in 103 cases, and urine was obtained in 82 cases. We measured serum sLOX-1 in 70 acute IHD cases and 79 control cases (23 asphyxia, 11 drowning, 9 congestive heart failure 10 drug intoxications, 9 hypothermia, 8 carbon monoxide intoxication, 4 head traumas, 2 cervical cord traumas, 2 hyperthermia, and 1 thoracic injury). Pericardial fluid sLOX-1 level was measured in 48 acute IHD cases and 55 control cases (15 asphyxia, 11 drowning, 9 congestive heart failure, 1 drug intoxication, 11 hypothermia, 1 carbon monoxide intoxication, 2 head traumas, 1 cervical cord trauma, 3 hyperthermia, and 1 thoracic injury), and urine sLOX-1 level was measured in 35 acute IHD cases and 47 control cases (10 asphyxia, 4 drowning, 4 congestive heart failure, 8 drug intoxications, 10 hypothermia, 5 carbon monoxide intoxication, 2 head traumas, 2 cervical cord traumas, 1 hyperthermia, and 1 thoracic injury).

2.3. Pathological findings in acute IHD cases

Hematoxylin–Eosin staining of heart tissues was performed for the pathological diagnosis of coronary thrombosis/plaque rupture and for pathological acute myocardial necrosis findings such as eosinophilic changes, contraction band necrosis, and neutrophilic infiltration.

2.4. Evaluation of coronary atherosclerosis

We evaluated coronary atherosclerosis according to the degree of coronary atherosclerotic stenosis, and divided cases into three groups. The classification of the groups were non-coronary atherosclerosis (no observation of coronary stenosis), mild to moderate (maximum coronary stenosis between 10% and 75%), and severe (maximum coronary stenosis over 75%). Since all cases in the acute IHD group had a maximum coronary atherosclerosis over 75%, they were classified as single-vessel disease, double-vessel disease, and triple-vessel disease cases.

2.5. Statistical analyses

Statistical analyses were performed using STATA 13.0 (Stata Corp). Student's t-test was used to evaluate the differences in baseline characteristics between the groups. The Kruskal–Wallis equality-of-population rank test was used to compare the measured values between the groups. A receiver operating characteristic (ROC) curve was generated for values that differed significantly between the groups. The cutoff value was determined by using the Youden Index. Spearman's rank correlation coefficient was used to evaluate the correlation between the values.

3. Results

3.1. Baseline characteristics

Table 2 shows the baseline characteristics of the acute IHD and control cases. No significant differences in age were found between the groups. Mean body mass index (BMI) and heart weight were significantly higher in acute IHD group compared to the control cases.

3.2. sLOX-1 levels between acute IHD and control cases

As shown in Table 3, we observed a significant increase in sLOX-1 levels in the pericardial fluid and urine of acute IHD cases compared to the control cases. The median levels of serum sLOX-1 in acute IHD and control cases were 18875.8 pg/ml (range: 181.3–49845.0 pg/ml) and 14213.3 pg/ml (range: 335.9–96697.6 pg/ml), respectively. The median levels of pericardial fluid sLOX-1 in acute IHD and control cases were 1085.5 pg/ml (range: 222.3–19274.6 pg/ml) and 487.1 pg/ml (range: 0–3720.7 pg/ml), respectively. The median values of urine sLOX-1 in acute IHD and control cases were 6415.7 pg/ml (range: 53.0–35397.6 pg/ml) and 3346.7 pg/ml (range: 323.7–62953.0 pg/ml), respectively. Fig. 1, Fig. 2, and Fig. 3 show the box charts generated for sLOX-1 level in the serum, pericardial fluid, and urine, respectively. Although significant differences were observed in pericardial fluid and urine sLOX-1 levels, there was no correlation between the values for both the samples in the 54 cases from whom both the specimens were obtained ($p = 0.35$).

Fig. 4 shows the ROC curve for pericardial fluid sLOX-1 levels for the postmortem diagnosis of acute IHD. The area under the curve was 0.70 (95% CI = 0.59–0.80). The pericardial fluid sLOX-1 cutoff value for diagnosing acute IHD was 551.7 pg/ml (sensitivity 91.7%, specificity 52.7%).

Fig. 5 shows the ROC curve for urine sLOX-1 levels in the postmortem diagnosis of acute IHD. Area under the curve was 0.66 (95% CI = 0.54–0.78). The urine sLOX-1 cutoff value for diagnosing acute IHD was 4128.8 pg/ml (sensitivity 77.1%, specificity 55.3%).

Table 1
Number of samples.

	IHD	Control
Serum	70	79
Pericardial fluid	48	55
Urine	35	47

IHD, ischemic heart disease.

Table 2
Baseline characteristics (mean ± SD) in the IHD and control cases.

Baseline characteristics	IHD (n = 70)	control (n = 79)	p value
Age (y)	61.9 ± 13.2	62.2 ± 18.7	0.9
BMI (kg/m^2)	23.7 ± 5.2	21.8 ± 5.4	< 0.05
Heart weight (g)	457.7 ± 106.9	362.8 ± 94.3	< 0.05

BMI, body mass index; IHD, ischemic heart disease; SD, standard deviation.

Table 3
Comparison of sLOX-1 level (mean ± SD) between the acute IHD and control cases.

	Acute IHD	Control	p value
Serum (pg/ml)	20369.9 ± 11622.8	19423.4 ± 17367.1	0.07
Pericardial fluid (pg/ml)	1933.8 ± 2979.4	968.5 ± 1013.1	< 0.05
Urine (pg/ml)	11171.7 ± 10419.2	6904.2 ± 10285.7	< 0.05

sLOX-1, soluble lectin-like oxidized low-density lipoprotein receptor-1; SD, standard deviation.

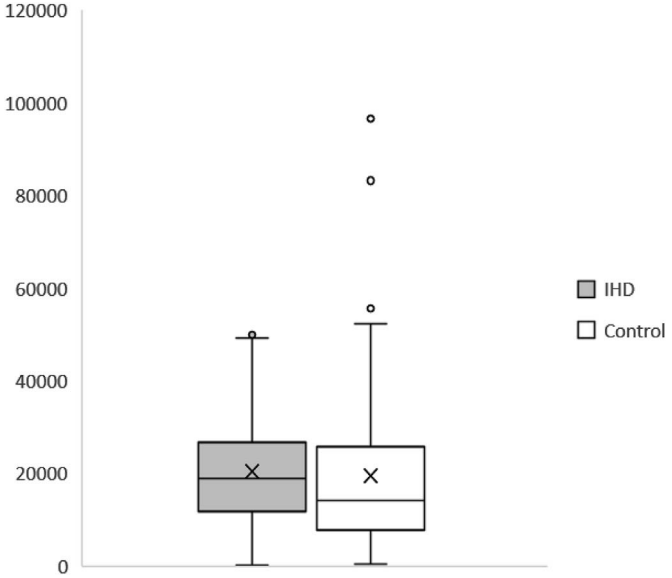

Fig. 1. Box chart of soluble lectin-like oxidized low-density lipoprotein receptor-1 (pg/ml) in the serum of patients from the acute ischemic heart disease and control cases. × represents the mean value for each specimen.

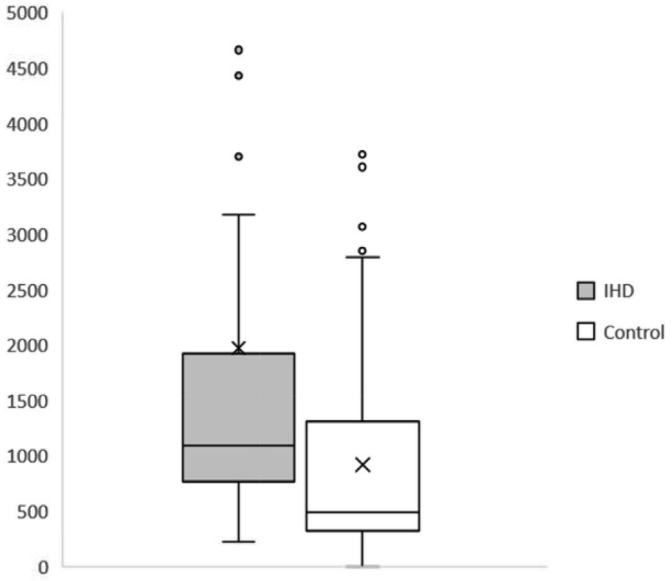

Fig. 2. Box chart of soluble lectin-like oxidized low-density lipoprotein receptor-1 (pg/ml) in the pericardial fluid of patients from the acute ischemic heart disease and control cases. × represents the mean value for each specimen.

3.3. Evaluation of sLOX-1 level and pathological findings in the acute IHD cases

Among the acute IHD cases, coronary thrombosis/plaque rupture was observed in 23 cases (23 serum, 15 pericardial fluid, and 15 urine

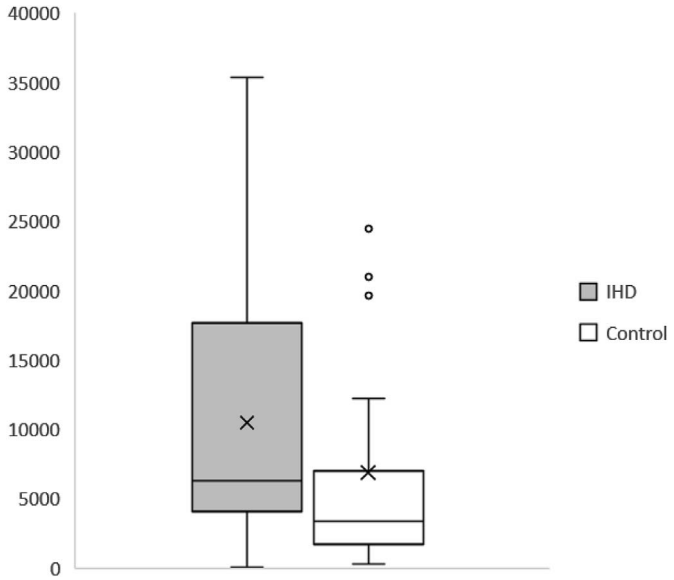

Fig. 3. Box chart of soluble lectin-like oxidized low-density lipoprotein receptor-1 (pg/ml) in the urine of patients from the acute ischemic heart disease and control cases. × represents the mean value for each specimen.

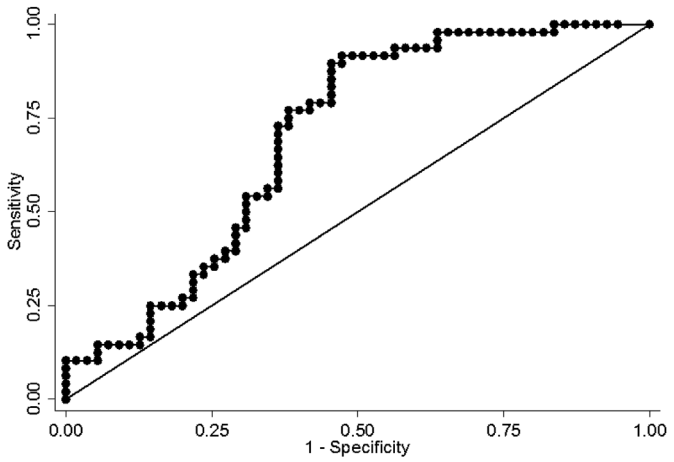

Fig. 4. ROC curve of pericardial fluid sLOX-1 for the postmortem diagnosis of acute IHD.

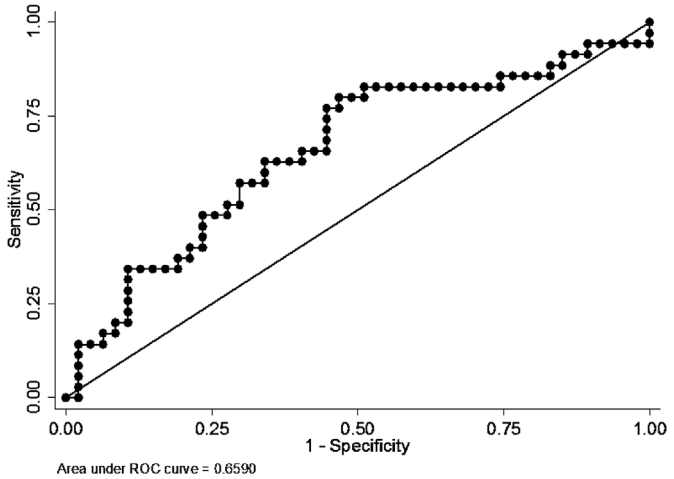

Fig. 5. ROC curve of urine sLOX-1 for the postmortem diagnosis of acute IHD.

Table 4
Comparison of pericardial fluid and urine sLOX-1 levels (mean ± SD) between IHD cases with and without coronary thrombosis/plaque rupture.

	With thrombosis/plaque rupture	Without thrombosis/ plaque rupture	p value
Serum (pg/ml)	22243.4 ± 11707.3	18816.9 ± 10863.3	0.23
Pericardial fluid (pg/ml)	1977.1 ± 1828.9	1914.2 ± 3401.9	0.33
Urine (pg/ml)	14681.4 ± 11528.7	8607.1 ± 8843.4	0.13

sLOX-1, soluble lectin-like oxidized low-density lipoprotein receptor-1; SD, standard deviation; IHD, ischemic heart disease.

samples). Pathological acute myocardial necrosis was observed in 28 cases (28 serum, 18 pericardial fluid, and 16 urine samples). The sLOX-1 levels in acute IHD patients with and without acute coronary findings (thrombosis/plaque rupture) show no significant differences (Table 4). The median level of serum sLOX-1 in acute IHD cases with and without acute coronary findings were 19901.1 pg/ml (range: 2665.2–49107.9 pg/ml) and 18224.2 pg/ml (range: 181.3–49845.0 pg/ml), respectively. The median level of pericardial fluid sLOX-1 in acute IHD cases with and without acute coronary findings were 1090.9 pg/ml (range: 391.8–6674.4 pg/ml) and 1080.0 pg/ml (range: 222.3–19274.6 pg/ml), respectively. The median level of urine sLOX-1 in acute IHD cases with and without acute coronary findings were 13222.5 pg/ml (range: 728.4–35397.6 pg/ml) and 6095.1 pg/ml (range: 53.0–32405.8 pg/ml), respectively.

Although serum sLOX-1 was significantly higher in cases without pathological acute myocardial necrosis groups, there were no significant differences in pericardial fluid and urine sLOX-1 levels in patients with IHD with and without pathological acute myocardial necrosis (Table 5). The median levels of serum sLOX-1 in acute IHD cases with and without acute myocardial necrosis were 21003.2 pg/ml (range: 10532.4–49107.9 pg/ml) and 16274.6 pg/ml (range: 181.3–49845.0 pg/ml), respectively. The median levels of pericardial fluid sLOX-1 in acute IHD cases with and without acute myocardial necrosis were 1104.3 pg/ml (range: 363.6–4430.8 pg/ml) and 1026.1 pg/ml (range: 222.3–19274.6 pg/ml), respectively. The median levels of urine sLOX-1 in acute IHD cases with and without acute myocardial necrosis were 7581.5 pg/ml (range: 728.4–34920.1 pg/ml) and 6135.4 pg/ml (range: 53.0–35397.6 pg/ml), respectively.

Among the acute IHD cases, both acute coronary findings and myocardial necrosis was absent in 33 cases.

3.4. Evaluation of sLOX-1 level and the degree of BMI in the control cases

Pericardial fluid and urine sLOX-1 levels were compared between the control cases with BMI above and below 25 (Table 6). No significant difference in sLOX-1 level was observed. The median levels of pericardial fluid sLOX-1 in BMI < 25 and 25 < BMI cases were 484.9 pg/ml (range: 0–3699.4 pg/ml) and 727.2 pg/ml (range: 147.6–3720.7 pg/ml), respectively. The median levels of urine sLOX-1 in BMI < 25 and 25 < BMI cases were 3182 pg/ml (range: 567.9–62953.0 pg/ml) and 3617.2 pg/ml (range: 323.7–21004.2 pg/ml), respectively.

Table 5
Comparison of sLOX-1 (mean ± SD) between IHD cases with and without pathological acute myocardial necrosis.

	With pathological acute myocardial necrosis	Without pathological acute myocardial necrosis	p value
Serum (pg/ml)	23152.1 ± 9764.9	17778.4 ± 11673.6	< 0.05
Pericardial fluid (pg/ml)	1444.6 ± 996.5	2227.4 ± 3683.3	0.65
Urine (pg/ml)	11640.5 ± 9633.2	10848.2 ± 11218.7	0.56

sLOX-1, soluble lectin-like oxidized low-density lipoprotein receptor-1; SD, standard deviation; IHD, ischemic heart disease.

Table 6
Comparison of sLOX-1 levels (mean ± SD) between BMI in control cases.

	BMI < 25	25 < BMI	p value
Pericardial fluid (pg/ml)	888.1 ± 925.7 (n = 40)	1182.7 ± 1226.6 (n = 15)	0.50
Urine (pg/ml)	7378.8 ± 11460.2 (n = 35)	5520.2 ± 5801.9 (n = 12)	0.77

sLOX-1, soluble lectin-like oxidized low-density lipoprotein receptor-1; SD, standard deviation; BMI, body mass index.

3.5. Evaluation of sLOX-1 level and the degree of coronary atherosclerosis

In the control cases, the number of cases within non-coronary atherosclerosis, mild to moderate, and severe coronary atherosclerosis groups was 46 (46 serum, 26 pericardial fluid, and 31 urine samples), 29 (29 serum, 25 pericardial fluid, and 12 urine samples), and 4 (4 serum, 4 pericardial fluid, and 4 urine samples) cases respectively. The median level of serum sLOX-1 in each degree was 16115.0 pg/ml (range: 335.9–83214.0 pg/ml), 13359.8 pg/ml (range: 1985.6–96697.6 pg/ml), and 28022.65 pg/ml (range: 7951.8–30186.4 pg/ml), respectively. The median level of pericardial fluid sLOX-1 in each degree was 421.1 pg/ml (range: 0–3720.7 pg/ml), 727.2 pg/ml (range: 0–3605.8 pg/ml), and 898.1 pg/ml (range: 257.6–1102.6 pg/ml), respectively. The median level of urine sLOX-1 in each degree was 3049.0 pg/ml (range: 323.7–24674.9 pg/ml), 5730.6 pg/ml (range: 737.0–62953.0 pg/ml), and 3489.1 pg/ml (range: 2765.0–21004.2 pg/ml), respectively.

In the acute IHD cases, the number of single-vessel, double-vessel, and triple-vessel disease cases was 34 (34 serum, 19 pericardial fluid, and 18 urine samples), 23 (23 serum, 18 pericardial fluid, and 11 urine samples), and 13 (13 serum, 11 pericardial fluid, 6 urine samples) cases, respectively. The median level of serum sLOX-1 in each degree was 20530.5 pg/ml (range: 2665.2–48720.4 pg/ml), 16274.6 pg/ml (range: 181.3–49107.9 pg/ml), and 20996.7 pg/ml (range: 12279.8–49845.0 pg/ml), respectively. The median level of pericardial fluid sLOX-1 in each degree was 928.3 pg/ml (range: 222.3–1969.8 pg/ml), 1081.5 pg/ml (range: 362.6–7919.6 pg/ml), and 1522.6 pg/ml (range: 369.1–19274.6 pg/ml), respectively. The median level of urine sLOX-1 in each degree was 7262.0 pg/ml (range: 53.0–29052.0 pg/ml), 6135.4 pg/ml (range: 3861.2–35397.6 pg/ml), and 11069.0 pg/ml (range: 728.4–34920.1 pg/ml), respectively.

No significant difference was observed in the sLOX-1 level between the varying degrees of coronary atherosclerosis (Tables 7 and 8).

3.6. Evaluation of sLOX-1 level and the degree of PMI in the control cases

The cases with PMI under 24 h, 24–48 h, and 48–72 h were compared in the control cases (Table 9). The median levels of serum sLOX-1 in cases with PMI under 24 h, 24–48 h, and 48–72 h were 12981.5 pg/ml (range: 5083.5–52241.4 pg/ml), 16314.2 pg/ml (range: 3012.1–96697.6 pg/ml), and 10983.1 pg/ml (range: 335.9–41506.1 pg/ml), respectively. The median levels of pericardial fluid sLOX-1 in cases with PMI under 24 h, 24–48 h, and 48–72 h were 322.5 pg/ml (range: 47.4–2620.3 pg/ml), 726.8 pg/ml (range: 0–3720.7 pg/ml), and 975.2 pg/ml (range: 0–3699.4 pg/ml), respectively. The median levels of urine sLOX-1 in cases with PMI under 24 h, 24–48 h, and 48–72 h were 5694.3 pg/ml (range: 567.9–12223.3 pg/ml), 2934.1 pg/ml (range: 323.7–62953.0 pg/ml), and 4134.9 pg/ml (range: 652.6–24674.9 pg/ml), respectively.

Table 7
Comparison of sLOX-1 (mean ± SD) between the degrees of coronary atherosclerosis in control cases.

	Non	Mild-moderate	Severe	p value
Serum (pg/ml)	19518.5 ± 16778.6	18704.0 ± 19308.9	23545.9 ± 10565.4	0.48
Pericardial fluid (pg/ml)	906.9 ± 1074.3	1061.3 ± 1034.4	789.1 ± 388.6	0.81
Urine (pg/ml)	5173.4 ± 5995.8	11114.7 ± 17169.9	7686.8 ± 8890.8	0.33

sLOX-1, soluble lectin-like oxidized low-density lipoprotein receptor-1; SD, standard deviation.

Although there was a time-depended tendency for elevation in pericardial fluid sLOX-1 level, we found no significant differences between the groups.

3.7. Evaluation of sLOX-1 and the enforcement of cardiopulmonary resuscitation in control cases

There were 33 cases who underwent cardiopulmonary resuscitation in the control group. No significant difference in sLOX-1 level in all specimens were observed compared to the patient without cardiopulmonary resuscitation.

4. Discussion

sLOX-1 is suggested to be associated with plaque vulnerability.[38,39] Recent studies show serum sLOX-1 elevation in ACS.[37–44] Hayashida et al.[37] discovered earlier peak value of serum sLOX-1 compared to TnT in ACS patients. Furthermore, the peak value of serum sLOX-1 in ACS patients were observed at the time of hospital arrival, suggesting that serum sLOX-1 levels may begin to rise before the onset of ACS.[37] Kume et al.[40] suggested that sLOX-1 elevation has a different etiology from myocardial injury.

In the present study, serum sLOX-1 levels were higher than clinical references in all cases. Postmortem increase in serum biochemical marker levels of myoglobin, CKMB, and cTnT have been previously reported.[17–20] Postmortem increases in NT-proBNP, hFABP, CKMB, and cTnT serum levels may be attributable to myocardium autolysis, whereas, the increase in serum myoglobin and cardiac myosin light chain I levels may be explained by myocardium and skeletal muscle autolysis.[4–6,10,15,16,48–55] Although earlier studies have reported significant increases in serum sLOX-1 level in patients with ACS, no significant difference was observed between the acute IHD and control groups in the present study. Furthermore, the levels found in this study were remarkably higher than those found in living patients with ACS.[37,38] LOX-1 is reportedly expressed *in vivo* in the aortic intima and vascular-rich organs such as the placenta, lungs, brain, and liver.[30,31] High serum sLOX-1 levels can be explained by the autolysis of these organs and postmortem redistribution. These results suggest that serum sLOX-1 levels cannot be used as a diagnostic marker in postmortem acute IHD diagnosis.

Pericardial fluid is an ultrafiltrate of blood plasma, and therefore, biomarker levels in the pericardial fluid are reflective of their levels in the plasma of living patients.[56] Few studies have shown significant differences in the levels of brain natriuretic peptide, myoglobin, CKMB, and cTnT, between pericardial fluid samples obtained from normal patients and the deceased.[17,20,24–29] In the present study, the pericardial fluid level of sLOX-1 was significantly higher in the acute IHD group compared to the control group (Table 3). Furthermore, measured values in the present study were comparable to the serum cutoff value (1000 pg/ml) in living patients as reported by Hayashida et al.[37] for diagnosing acute myocardial infarction (AMI).

Biochemical markers that exist in the myocardium may permeate through the epicardium because of postmortem myocardium autolysis. Compared to some of the other biochemical markers that are used to diagnose IHD, such as NT-proBNP, hFABP, CKMB, myoglobin, cTnT, and cardiac myosin light chain I, sLOX-1 is absent in the myocardium. Therefore, the pericardial fluid sLOX-1 level might be less affected by postmortem myocardium autolysis and is more likely to reflect the antemortem serum levels. However, the diffusion of sLOX-1 from the coronary arteries immediately beneath the epicardium must be considered. LOX-1 is expressed on the atherosclerotic lesion of the arterial intima; thus, LOX-1 on the intima and sLOX-1 in the blood must permeate through the intima, tunica media, tunica adventitia, and epicardium to diffuse in to the pericardial fluid. The pericardial fluid sLOX-1 level in this study was comparable to the serum level in living individuals, and no significant time-dependent elevation was discovered (Tables 3 and 9). Therefore, we presumed the diffusion of sLOX-1 from the coronary artery to be negligible in cases with PMI shorter than 72 h.

Kobayashi et al.[38] compared serum sLOX-1 levels among patients with ACS having plaque rupture, patients with ACS without plaque rupture, and patients with stable angina pectoris. The sLOX-1 level was significantly higher in patients with ACS compared to patients with stable angina pectoris. Furthermore, sLOX-1 level was significantly higher in ACS patients with plaque rupture compared to those without plaque rupture. Therefore, sLOX-1 was suggested to be associated with plaque vulnerability.[38,39] Although significant differences in pericardial fluid sLOX-1 levels were observed between the acute IHD and control groups in the present study, we were unable to detect coronary thrombosis/plaque rupture (Table 4). Kume et al.[40] measured sLOX-1, hFABP, and cTnT levels in patients with ACS and showed that sLOX-1 had the highest sensitivity and specificity in diagnosing ACS. Furthermore, no correlation was found between cTnT and sLOX-1 levels, suggesting that sLOX-1 elevation has a different etiology from myocardial injury. Consistent with the observations in previous studies,[40] pericardial fluid sLOX-1 level was not affected by the degree of pathological acute myocardial necrosis in this study (Table 5).

In the present study, BMI and degree of coronary atherosclerosis were higher in the acute IHD group than in the control group, and might have contributed to the significant difference in sLOX-1 level. However, because sLOX-1 level was unaffected by BMI and coronary atherosclerosis (Tables 6–8), it could be said that postmortem pericardial fluid sLOX-1 level does not strongly increase as a function of BMI and coronary atherosclerosis alone. Although we were unable to

Table 8
Comparison of sLOX-1 (mean ± SD) between the degrees of coronary atherosclerosis in acute IHD cases.

	Single-vessel	Double-vessel	Triple-vessel	p value
Serum (pg/ml)	21230.4 ± 11012.6	16925.2 ± 10612.6	25904.0 ± 13324.5	0.18
Pericardial fluid (pg/ml)	1088.1 ± 529.3	1963.9 ± 2188.5	3419.1 ± 5401.2	0.24
Urine (pg/ml)	10193.8 ± 9314.5	11440.6 ± 11394.5	13752.5 ± 13741.8	0.93

sLOX-1, soluble lectin-like oxidized low-density lipoprotein receptor-1; SD, standard deviation; IHD, ischemic heart disease.

Table 9
Comparison of sLOX-1 levels (mean ± SD) between PMI in control cases.

	< 24 h	24–48 h	48–72 h	p value
Serum (pg/ml)	16551.7 ± 13483.4 (n = 14)	21560.7 ± 19718.4 (n = 49)	15390.7 ± 11254.9 (n = 16)	0.61
Pericardial fluid (pg/ml)	514.6 ± 746.6 (n = 10)	989.4 ± 952.4 (n = 32)	1266.2 ± 1260.5 (n = 13)	0.09
Urine (pg/ml)	5790.1 ± 4083.0 (n = 9)	8115.4 ± 13549.5 (n = 24)	5544.2 ± 5806.9 (n = 14)	0.66

sLOX-1, soluble lectin-like oxidized low-density lipoprotein receptor-1; SD, standard deviation; PMI, postmortem interval.

detect acute coronary thrombosis/plaque rupture in the acute IHD group, the usefulness of pericardial fluid sLOX-1 levels in postmortem acute IHD diagnosis in cases wherein PMI is 72 h or shorter is worth considering.

Urine levels of sLOX-1 were significantly higher in the acute IHD group than in the control group (Table 3). Several previous studies in living patients demonstrated an increase in urine hFABP in AMI patients.[7,8] Nayashida et al.[57] demonstrated the importance of the renal clearance of hFABP compared to that of CKMB and TnT. Bjurman et al.[58] measured serum cTnT, cTnI, NT-proBNP, hFABP, and copeptin levels and showed that NT-proBNP, hFABP, and copeptin levels are elevated in patients with low glomerular filtration rate. The authors suggested that compared to cTnT (37 kDa) and cTnI (24 kDa), low-molecular-weight molecules including NT-proBNP (8.5 kDa), hFABP (15 kDa), and copeptin (5 kDa) can easily permeate through the glomerular filtration membrane, and, the kidneys play a key role in their excretion.

sLOX-1 has a molecular weight of 35 kDa.[34] Therefore, we believe that sLOX-1 is not excreted in the urine of living patients. In the present study, urine sLOX-1 levels were relatively high, and no significant correlation was found between its urine and pericardial fluid levels, possibly owing to postmortem autolysis and LOX-1 expression in the bladder. Compared to the coronary artery directly beneath the epicardium, the number of vessels of the bladder wall is greater and the vessels are thinner. Therefore, the diffusion of sLOX-1 to the urine could have occurred earlier and more massively compared to pericardial fluid.

The limitations of the present study include the small sample size and lack of information regarding baseline characteristics. Levels of sLOX-1 are also known to increase in conditions such as diabetes, aortic dissection, and cerebral infarction. Information regarding antemortem diabetic conditions is often unknown in forensic autopsy cases. Furthermore, we used preserved specimens in this study; thus, HbA1c could not be measured because of hemolysis. Fatal aortic dissection cases were often accompanied by hemopericardium. In such cases, pericardial fluid could not be obtained; thus, aortic dissection cases were excluded from this study. Cerebral infarction cases that fit the criteria of this study were not found during this study period.

In the present study, we measured sLOX-1 level in body fluids collected from postmortem specimens. Although further large-scale confirmatory studies are required, the results suggest that pericardial fluid and urine sLOX-1 levels are useful in postmortem acute IHD diagnosis, in cases with a postmortem sampling interval of 72 h or shorter. In the field of forensic medicine, postmortem changes in biochemical markers are a major concern because of autolysis. sLOX-1 is a biochemical marker that is absent in the myocardium. Therefore, pericardial fluid sLOX-1 level was comparable to the serum cutoff level in living patients.

Coronary atherosclerosis without any demonstration of macroscopic or microscopic changes of myocardial necrosis is often the only finding in acute IHD cases with a survival time under 6 h after onset. In such cases, the diagnosis is made by exclusion of other possible causes of death. In the present study, significant elevation of sLOX-1 level was observed not only in acute IHD cases with histopathological findings, but also in acute IHD cases without specific histopathological findings. Therefore, measuring sLOX-1 in pericardial fluid can be a useful additional diagnostic tool for diagnosing acute IHD in cases where no histopathological findings are observed.

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