Decreased Serum Levels of High Mobility Group Box 1 (HMGB-1) After Graft Replacement or Stenting of Abdominal Aortic Aneurysm

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Brief statement: High mobility group box 1 (HMGB-1) has been associated with inflammation and atherosclerosis, which results in elevated HMGB-1 levels in patients with abdominal aortic aneurysms (AAA) because aneurysms contain a large amount of atherosclerotic tissue. There are no data regarding changes in HMGB-1 levels following surgical interventions for AAA. We investigated the serum HMGB-1 levels before and after either endovascular aortic repair (EVAR) or open aortic repair (OAR). The serum HMGB-1 was higher in patients with AAA than in controls. However, the elevated HMGB-1

levels normalized after graft replacement or stent placement for AAA. This is the first report describing changes in serum HMGB-1 after surgical treatment of AAA.

Abstract

Objectives: High-mobility group box 1 (HMGB-1) is a key substance mediating inflammation and development of atherosclerotic lesions (AL), including abdominal aortic aneurysms (AAA). Serum levels of HMGB-1 are increased in patients with AAA than in normal controls because the ALs in AAAs secrete HMGB-1. We therefore postulate that the serum HMGB-1 level should decrease after endovascular aortic repair (EVAR) or open aortic repair (OAR). However, there is no evidence of this in the literature. The purpose of this study was to investigate the changes in HMGB-1 levels after surgical intervention for AAA. We also aimed to determine if the HMGB-1 levels varied between the two procedures.

Design: Prospective study.

Materials and methods: Serum HMGB-1 levels were determined in 24 patients with AAA and 25 healthy controls. Twelve of the 24 AAA patients underwent EVAR while the other half underwent OAR. The relationship between HMGB-1 levels and presence of AAA or influence of operative methods on the serum HMGB-1 level were prospectively investigated.

Results: Serum HMGB-1 levels in AAA patients were significantly higher than in healthy controls (9.4 ± 5.7 vs. 4.1 ± 2.0 ng/mL, $P < 0.01$). The serum HMGB-1 levels in both the EVAR group and the OAR group were significantly decreased from baseline at both 3 months and 1 year after surgery.

Conclusions: Removal or isolation of AL via surgical intervention significantly decreases serum HMGB-1 levels. The significant post-operative reduction in HMGB-1 levels suggests that important endocrinological changes occur after surgical treatment of AAA.

Key Words: Abdominal aortic aneurysm, Atherosclerosis, Covered stenting, High-mobility group box 1

Introduction

High-mobility group box 1 (HMGB-1) is a nonhistone DNA-binding protein consisting of 215 amino acid residues organized into 3 domains that include 2 tandem HMGB domains (A box and B box) arranged in an L-shape configuration and a 30 amino acid long C-terminal tail.[1, 2] HMGB-1 functions as an intracellular regulator of gene transcription and promotes secretion of several inflammatory cytokines including interleukin (IL), tumor necrosis factor (TNF)–α, γ-interferon, and macrophage inflammatory proteins-1α and -1β.[3,4,5,6] HMGB-1 is therefore regarded as a key mediator of inflammation-related responses, including inflammation, tissue regeneration, cancer, infections, and development of atherosclerotic lesions (AL), including abdominal aortic aneurysms (AAA).[5]

Elevated HMGB-1 expression has been detected in ALs in endothelial cells, vascular smooth muscle cells, and macrophages.[7] Increased HMGB-1 expression leads to progression of ALs and may result in development of AAAs. The ALs in AAAs then secrete more HMGB-1, further accelerating growth of the AAAs.[8,9] There are two surgical treatments for AAA: open aortic repair (OAR) and endovascular aortic repair (EVAR). In patients with AAA, serum HMGB-1 is increased compared to that in normal controls,[8] probably because ALs in AAAs secrete HMGB-1. Therefore, theoretically, the serum HMGB-1 level will decrease after OAR or EVAR because these procedures result in a large amount of the AL in the AAA being removed or isolated from the patient's blood circulation. However, there is no published evidence supporting this. The purpose of this study was to investigate the changes in HMGB-1 secretion after surgical intervention for AAA.

Materials and Methods

Patients

Figure 1 shows the study design. We enrolled 49 subjects, consisting of 24 AAA patients and 25 healthy volunteers. All 24 AAA patients underwent surgical interventions—12 underwent an OAR using a Dacron graft (the AL was removed from the blood circulation) and the other 12 underwent EVAR (the AL was isolated from the blood circulation). There was no endoleak after EVAR. The serum HMGB-1 levels were measured at baseline in all participants and were not repeated in the control group. In the AAA group, serum HMGB-1 levels were repeated at 3 months and 1 year after surgery. All patients and volunteers gave informed consent, and the study was approved by the Institutional Review Board at Okayama University Hospital (Okayama, Japan).

Measurements

Blood samples were collected in the conventional manner and centrifuged (3000 rpm, 10 min) to obtain serum, which was stored at –80ºC. The concentration of HMGB-1 in serum samples was determined using an enzyme-linked immunosorbent assay kit according to the manufacturer's protocol (Shino-Test, Sagamihara, Japan). We analyzed cell counts and biochemistry using standard methods established by the Department of the Central Clinical Laboratory, Okayama University Hospital.

Statistical Analysis

The characteristics of the AAA group and the control group were compared and the difference in serum HMGB-1 levels at baseline between the two groups was analyzed. Baseline serum HMGB-1 levels were compared against levels obtained at 3 months and l year post

surgery. Comparisons were also made between the EVAR and OAR groups to investigate the effect of the different surgical procedures on the serum levels of HMGB-1. All data were expressed as mean ± SEM or SD. The Mann-Whitney U test was used to analyze differences between the quantitative data in the AAA and control groups. The chi-square test was used for analysis of the categorical variables. Time-course variation of serum HMGB-1 levels from their preoperative values to the values 1 year post intervention between the OAR and EVAR groups were tested using 2-way ANOVA. A probability value of < 0.05 was considered to be statistically significant. Statistical analyses were performed with IBM SPSS software, version 19.0.0 (SPSS Inc., Chicago, Illinois).

Results

Baseline characteristics of all patients

The baseline demographic and clinical characteristics of the AAA patients and controls are shown in **Table I**. The AAA group had significantly higher pulse wave velocity than the control group. Smoking, diabetes, and hyperlipidemia were more prevalent in the AAA group than in the control group.

Table II shows the laboratory data for the entire patient cohort. The AAA group had significantly higher levels of fibrinogen degradation products and D-dimer, and lower levels of hemoglobin, activated partial thromboplastin time, albumin, and high-density lipoprotein (HDL) cholesterol than the control group. Serum HMGB-1 levels were significantly higher in the AAA group than in the control group (**Figure 2**).

Time-course variations in HMGB-1 after vascular surgery

All patients in the AAA group survived surgery and were well 1-year post intervention with no major complications. Serum HMGB-1 levels were significantly decreased after surgical intervention (**Figure 3**). Both OAR and EVAR significantly decreased serum HMGB-1 levels at 3 months and 1 year after the surgery (**Figure 4-A,B**). In addition, 2-way ANOVA analysis showed that there were no significant differences in the degree of reduction in serum HMGB-1 levels between the OAR group and the EVAR group 1 year after intervention (**Figure 4-C**).

Discussion

This study showed significantly increased serum HMGB-1 levels in AAA patients than in controls. These results are consistent with previous reports that HMGB-1 expression is enhanced in all layers of the aortic wall, including atheromatous lesions in AAA patients,[3] and that plasma HMGB-1 levels are increased in AAA patients.[8] The results of this study confirmed the relationship between increased serum HMGB-1 levels and the presence of AAA.

This study is the first to demonstrate a significant reduction in serum HMGB-1 levels in AAA patients after surgical intervention with similar reductions seen after either OAR or EVAR. The postulated reason for this phenomenon is that a large amount of HMGB-1 secreting AL was removed or isolated from the blood circulation by graft replacement or covered-stent placement. A previous study showed that HMGB-1 was highly expressed in inflammatory cells in the adventitia, media, and atherosclerotic plaques. HMGB-1 was also expressed in smooth muscle cells and endothelial cells in AAA tissue.[3] In this study, there is a possibility that the remaining aortic wall may continue to secret HMGB1 after EVAR or after graft replacement and the secreted HMGB1 will enter the circulation via the remaining vasa vasorum. However, the decrease of HMGB1 after AAA surgery was significant. In addition, the only difference between

pre- and post-operative conditions in the patients was removal or isolation of the atherosclerotic plaque. Furthermore, there were no cases of endoleak after EVAR in this study. These results suggest that the main cause of HMGB1 increase in patients with AAA was secretion from the atherosclerotic plaque in AAA, not from the aortic wall itself.

The long-term outcome of decreased HMGB-1 could not be determined from this study. Our observations indicate that the eventual post-operative reduction in serum HMGB-1 is not related to adverse outcomes in terms of immediate survival or incidence of major post-surgical complications. The decreased HMGB-1 levels following surgical treatment of AAA clearly demonstrate that these interventions trigger an important endocrinological change, which suggests surgical intervention is likely to have a significant impact in terms of long-term outcomes. In a study using a transgenic mouse model, reduction of inflammatory cytokines, including HMGB-1, was shown to reduce the development of atherosclerotic changes.[10] Therefore, decreased HMGB-1 levels after surgery for AAA may have beneficial effects for long-term vascular outcomes. Further study is necessary to determine if this is truly the case.

Study Limitations

This study was limited by the relatively small number of patients.

Conclusion

The baseline serum HMGB-1 levels were significantly increased in the AAA group compared to those in the control group. The HMGB-1 levels in AAA patients significantly decreased after OAR or EVAR. Removal or isolation of large AL may suppress progression of atherosclerotic disease due to the decreased secretion of HMBG-1 after intervention. Further studies are required

to determine whether decreased HMBG-1 levels truly improve outcomes of atherosclerotic vascular diseases.

Conflict of Interest Statement

The authors report no conflicts of interest. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sector.

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Table I. Baseline demographic and clinical characteristics of all patients

	Control (n = 25)	AAA (n = 24)	p value
Male % (n)	56.0 (14)	73.6 (18)	0.28
Age (years)	69.8 ± 5.3	71.8 ± 6.7	0.2
BMI	24.1 ± 3.8	23.2 ± 4.0	0.15
Systolic blood pressure (mmHg)	122.4 ± 16.7	130.1 ± 19.7	0.13
Diastolic blood pressure (mmHg)	75.5 ± 9.1	73.7 ± 11.6	0.87
ABI (right)	1.18 ± 0.11	1.08 ± 0.13	0.67
ABI (left)	1.15 ± 0.13	1.05 ± 0.18	0.62
PWV (right) (cm/s)	1547 ± 366	2089 ± 481	< 0.01
PWV (left) (cm/s)	1540 ± 370	2022 ± 488	0.02
Smoker % (n)	8.0 (2)	24.0 (10)	0.01
Medication			
Hypertension % (n)	48.0 (12)	58.5 (14)	0.18
Diabetes % (n)	0	29.2 (7)[j]	0.01
Hyperlipidemia % (n)	24.0 (6)	62.5 (15)	< 0.01

Data are shown as mean ± SD (or percentage and number). AAA; abdominal aortic aneurysm, ABI; ankle branch index, BMI; body mass index, PWV; pulse wave velocity.

Table II. Baseline laboratory based parameters of all subjects

Laboratory values	control (n = 25)	AAA (n = 24)	p value
WBC × 10^4 (/μL)	6.2 ± 2.7	5.9 ± 1.6	0.70
Hb (mg/dl)	13.7 ± 2.0	12.6 ± 1.7	0.03
Hct (%)	40.0 ± 5.1	37.8 ± 4.6	0.08
Plt × 10^4 (/μL)	212 ± 46	224 ± 86	0.45
APTT (sec)	113 ± 18	102 ± 26	0.048
Fibrinogen (mg/dl)	396 ± 127	487 ± 204	0.16
FDP (μg/ml)	4.8 ± 1.8	16.7 ± 14.3	0.03
D-dimer (ng/ml)	1.5 ± 1.0	8.3 ± 7.0	0.03
TP (g/dL)	7.1 ± 0.5	7.1 ± 0.5	0.87
Albumin (g/dL)	4.2 ± 0.3	3.9 ± 0.5	0.01
Creatinine (mg/dL)	1.3 ± 1.2	1.0 ± 1.2	0.94
T-chol (mg/dL)	194 ± 27	192 ± 38	0.78
HDL-chol (mg/dL)	67.8 ± 15.1	49.5 ± 10.8	< 0.01
HbA1c (%)	5.8 ± 0.4	5.9 ± 0.5	0.47
BNP (pg/mL)	31.6 ± 57.5	54.5 ± 62.8	0.29
CRP (mg/dL)	0.5 ± 0.9	2.2 ± 4.8	0.14
HMGB-1 (ng/ml)	4.1 ± 2.0	9.4 ± 5.7	< 0.01

Data are shown as mean ± SD. AAA; abdominal aortic aneurysm, APTT; activated partial thromboplastin time, BNP; brain natriuretic peptide, CRP; C-reactive protein, FDP; fibrinogen degradation products, Hb; hemoglobin, Hct; hematocrit, HDL-chol; high-density lipoprotein-cholesterol, HMGB-1, high-mobility group box 1, Plt; platelet, T-chol; total cholesterol, TP; total protein, WBC; white blood cell.

Figure legends

Figure 1. Tree diagram of patients enrolled in this study

AAA; abdominal aortic aneurysm, EVAR; endovascular aortic repair, HMGB-1; high-mobility group box 1, OAR; open aortic repair

Figure 2. Serum HMGB-1 levels before intervention

The serum HMGB-1 level was higher at baseline in the AAA group than in the control group. All data are expressed as mean ± SEM. *P < 0.01, AAA vs. control group.

Figure 3. Time course variance of changes in HMGB-1 levels after surgical intervention for AAA

Following intervention, the AAA patients showed decreased serum HMGB-1 levels on follow up at 3 months and 1 year compared to baseline. All data are expressed as mean ± SEM. *P < 0.01, 3 months or 1 year vs. baseline.

Figure 4. Changes in serum HMGB-1 levels after EVAR or OAR

The serum HMGB-1 levels decreased after surgery in both EVAR and OAR groups at 3 months and 1 year follow-up compared with baseline (A, B). No significant differences in post-surgical HMGB-1 levels were observed between the two groups (C). *P < 0.05, baseline vs. 3 months, **P < 0.01, baseline vs. 1 year. EVAR; endovascular aortic repair, OAR; open aortic repair.

Figure 1. Tree diagram of patients enrolled in this study

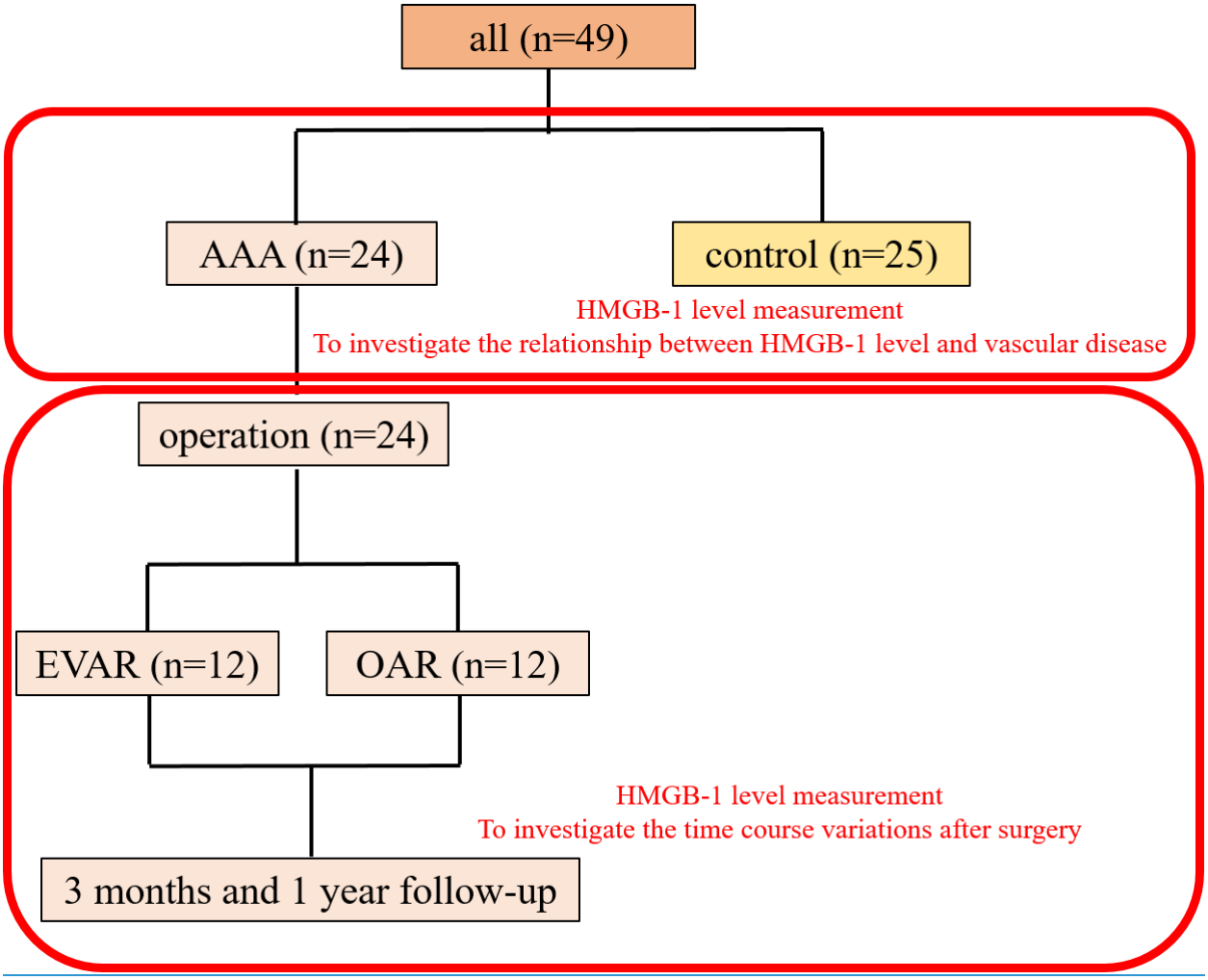
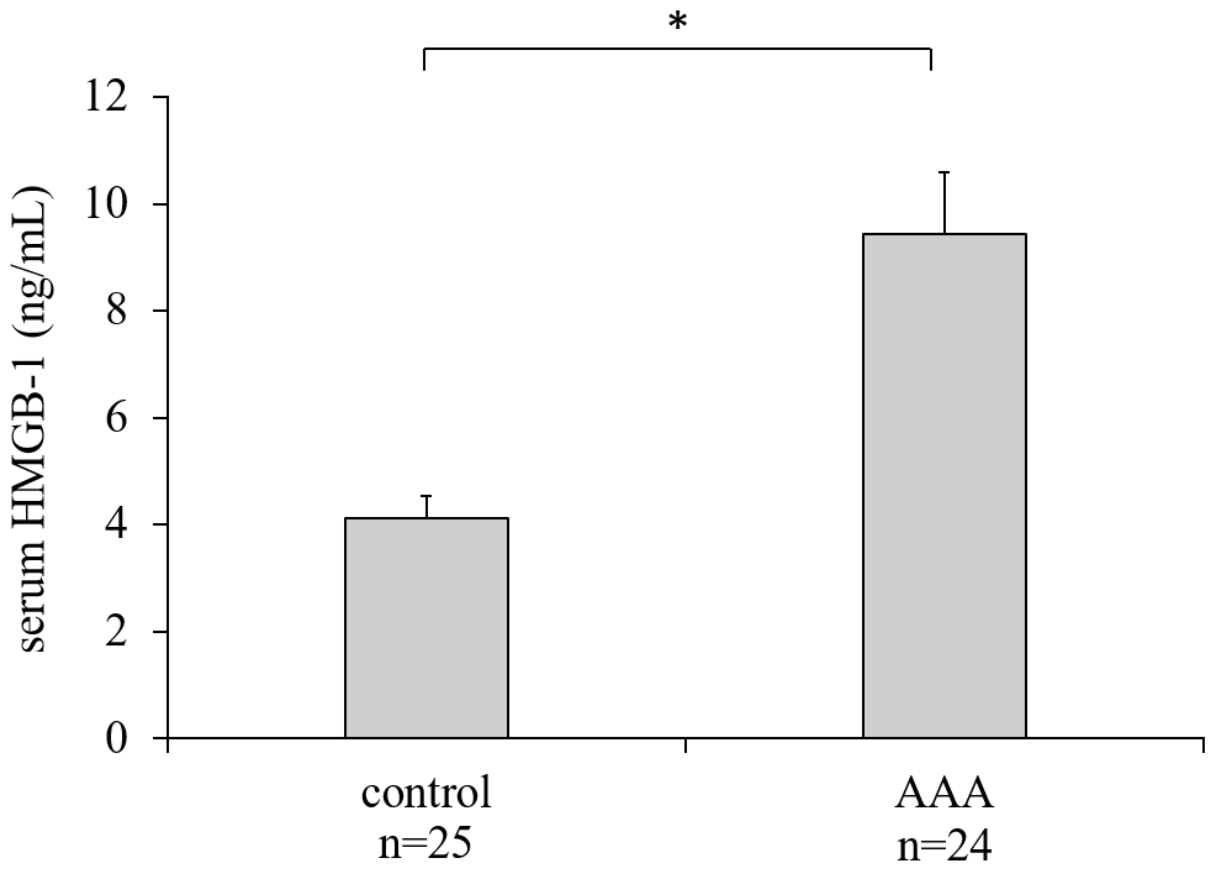


Figure 2. Serum HMGB-1 levels before intervention



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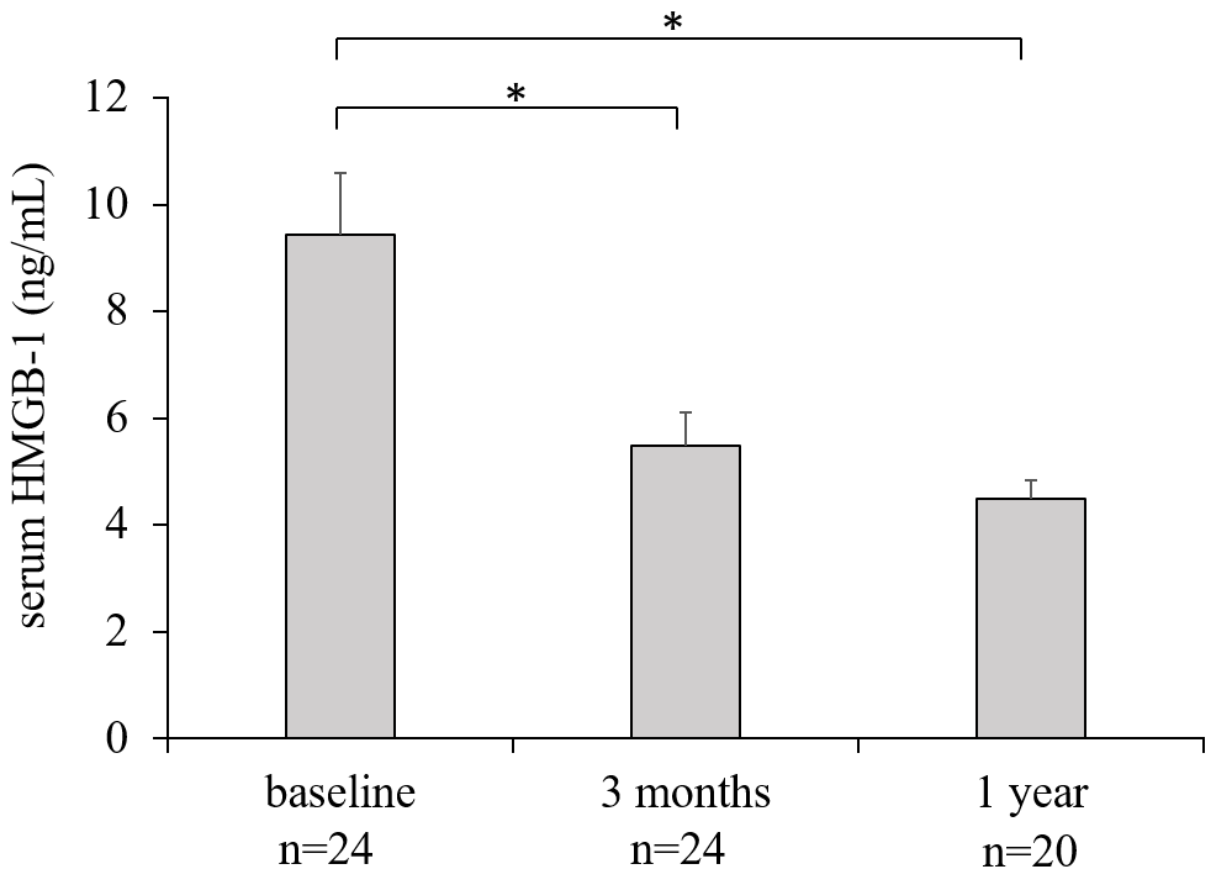

Figure 4. Changes in serum HMGB-1 levels after EVAR or OAR