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Serum Lipid Oxidative Stress Products as Risk Factors Are the Candidate Predictive Biomarkers for Human Abdominal Aortic Aneurysms

Feng Shi, PhD[1], Changcheng Ma, MD[2], Chao Ji, PhD[3], Mu Li, MD[4], Xun Liu, MD[4], and Yanshuo Han, MD[5]

Abstract

This research was designed to determine the association of serum lipid peroxidation products with disease severity in patients with abdominal aortic aneurysm (AAA). In total, 76 pairs of AAA cases as well as matched controls were enrolled in our research using propensity score matching (PSM). And their malondialdehyde (MDA), lipid hydroperoxide (LPO), and glutathione peroxidase (GSH-Px) levels were also detected through enzyme-linked immunosorbent assay (ELISA). Additionally, the relative clinical data of enrolled participants were extracted. The serum biomarker concentrations were measured in 76 patients with AAAs (diameter between 30 and 54 mm, n = 54; diameter ≥55 mm, n = 22) and 76 control patients from observational cohort study. After PSM adjustment for clinical variables, including age, gender, heart ratio, body mass index, smoking, hypertension, diabetes mellitus, coronary heart disease, and stroke, the serum MDA and LPO among AAA cases were remarkably increased compared with those from the normal patients. Inversely, serum GSH-Px was significantly decreased in patients with AAA compared to the control group. Besides, the serum levels of MDA and LPO were independently associated with AAA risk. Typically, there was significantly positive correlation between MDA level and LPO level (R = 0.358) but negative correlation of MDA level with GSH-Px (R = -0.203) level in patients with AAA. Meanwhile, the area under the receiver operating characteristic curve was 0.965 when MDA was used to diagnose AAA, and the optimal threshold value was 0.242 nmol/mL. Moreover, serum MDA level was significantly increased in cases with rupture AAA compared to those in selective AAA cases. Logistic regression analysis suggested that a higher serum MDA level indicated an elevated risk of AAA rupture (odds ratio = 2.536; 95% CI: 1.037-6.203; *P* =0.041). Our present findings suggest that serum peroxidation contents were evidently changed among AAA cases. Serum MDA and LPO concentrations could be used to predict disease severity in patients with AAA. Moreover, serum MDA may serve as the candidate biomarker for diagnosis of AAA and accurate identification of increased risks of AAA rupture.

Keywords

abdominal aortic aneurysm, lipid oxidative stress, malondialdehyde, glutathione peroxidase

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[1] Department of Health Management, Shengjing Hospital of China Medical University, Shenyang, China
[2] Department of Clinical Laboratory, Shengjing Hospital of China Medical University, Shenyang, China
[3] Department of Clinical Epidemiology, Shengjing Hospital of China Medical University, Shenyang, China
[4] Department of General Surgery, Shengjing Hospital of China Medical University, Shenyang, China
[5] School of Life and Pharmaceutical Sciences, Dalian University of Technology, Panjin, China

Corresponding Author:
Yanshuo Han, School of Life and Pharmaceutical Sciences, Dalian University of Technology, No. 2 Dagong Road, Liaodongwan New District, Panjin 124221, China.
Email: yanshuohan@dlut.edu.cn

Introduction

Abdominal aortic aneurysm (AAA) is a common vascular disorder featured by abnormal dilation and degeneration of focal aortaventralis. Abdominal aortic aneurysm is commonly asymptomatic before its cataclysmic presentation when ruptured, including acute abdominal pain and hemorrhagic shock.[1,2] The prevalence of AAA ranges from 4% to 7%, with over 175 000 deaths due to the rupture of AAA worldwide.[3,4] The diameter of aneurysm is a crucial determinant of rupture. Despite the accessible endovascular aneurysm repair as well as open surgical repair, the mortality of rupture AAA (rAAA) is as high as 80%.[5,6] Therefore, the prevention of AAA is of significance due to the extremely high mortality caused by its rupture. Nevertheless, there is no therapeutic agent to prevent the progression and rupture of AAA.[7,8] Moreover, there is an increasing appreciation that growth of AAA is nonlinear, and the risk of rupture varies with time.[9] Therefore, it will be meaningful to detect the reasons for AAA rupture and to subsequently decrease its incidence.

The pathological progression in the aortic wall is associated with the generation and involvement of various biomarkers, with extensive investigations on these biomarkers worldwide.[10,11] Biomarkers, especially those related to pathophysiological processes of inflammation and aortic wall degradation, are attractive potential candidates.[12,13] However, no biomarker has yet been sufficiently validated with additional prognostic value on AAA diameter for clinical practice.[14,15] Inflammation and tissue degeneration play vital roles in the pathogenesis of AAA formation and rupture.[16,17] Elevated production of free radicals could trigger endothelial injury, phenotype from a contractile to an inflammatory phenotype in smooth muscle cells (SMCs), ultimately causing apoptosis. More importantly, free radicals can also mediate lipid peroxidation, causing atherosclerosis, thereby contributing to hemodynamic stress as well as hypertensive pathology, all of which are considered as integral elements of aneurysm development.[18,19] This process is mostly correlated with cellular damage due to oxidative stress, and the formation of various aldehydes and malondialdehyde (MDA) would occur in the case of breakdown of lipid hydroperoxides (LPO) in biological systems. Inversely, glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) are[20] free radical cleaning enzymes, with essential roles in cleaning these radicals against tissue defect.[21,22] To our knowledge, few studies are accessible on detailed comparison of serum lipid oxidative stress product levels in AAA, and it remains unclear of the performance of serum peroxidation products to identify aortic aneurysm.

The present study was designed to examine the performance of personalized biomarkers in AAA diagnostics to correlate the AAA diameter or rAAA and selected biomarkers, followed by multivariate analysis to construct multiparameter model for stratification in patients with AAA. This model could be utilized as a diagnostic tool for the identification of potential risk aneurysms, which would facilitate in the individualized therapeutic approach. Therefore, evidence gathering on risk factors of developing AAA would enhance the cost-effectiveness of screening programs and detection rate of AAA.

Materials and Methods

Abdominal Aortic Aneurysm Population

A hospital-based, cross-sectional, observational, case–control study was carried out in this study, aiming to analyze the serum lipid peroxidation contents among patients with AAA and the matched control patients at the Shengjing Hospital of China Medical University (CMU). Peripheral blood specimens were collected from 116 consecutive AAA cases from Department of Clinical Laboratory (N = 116) at Shengjing Hospital of CMU from January 2016 to July 2019. The clinical data were extracted from patients upon admission from the Department of Health Management (n = 76). Seven and 69 out of these 76 cases were diagnosed with ruptured AAA and selective AAA, respectively, during hospitalization (Figure 1). Patients were diagnosed with AAA on the basis of imaging findings (including echocardiography, magnetic resonance imaging and computed tomography), and the classification of AAA was based on the clinical practice guidelines of the European Society for Vascular Surgery (ESVS).[23] Our study protocol gained approval from the Ethics Committee (No. 2016PS085K) at Shengjing Hospital of CMU. Written informed consent was provided by all patients.

Definitions

In all participants, abdominal aortic diameter was defined as the maximal anteroposterior inner wall to inner wall diameter of the infrarenal aorta.[24] Patients with expanded aortic diameter between 40 mm and 54 mm were categorized as small abdominal aortic aneurysms,[25] and rAAA is indicated by the triad of sudden-onset mid-abdominal or flank pain (possible radiation into the scrotum), shock, and a pulsatile abdominal mass.[26,27] Additionally, hypertension was diagnosed according to the diastolic blood pressure (BP) $\geq$90 mm Hg and/or the systolic BP $\geq$140 mm Hg, followed by administration of antihypertensive agents. Meanwhile, diabetes mellitus was diagnosed when the glycosylated hemoglobin A1c level was $\geq$6.5%, the fasting blood glucose was $\geq$7.0 mmol/L, and insulin or oral hypoglycemic agents were administered. Further, status of current smoker was determined according to the self-report by patients. Additionally, lower and upper serum lipid peroxidation contents were determined according to the threshold based on the receiver operating characteristic (ROC) curve in this study.

Exclusion Standards

In this study, 31 cases were excluded due to the acute, chronic, or traumatic artic dissection (n = 22), Marfan syndrome, intramural aortic hematoma, thoracic aortic aneurysm or the involvement of additional connective tissues, and cases who received steroids or non-steroidal anti-inflammatory drugs (n = 9). A previous history of cardiac surgery, myocardial infarction, valvular heart disease, infection, or other inflammatory

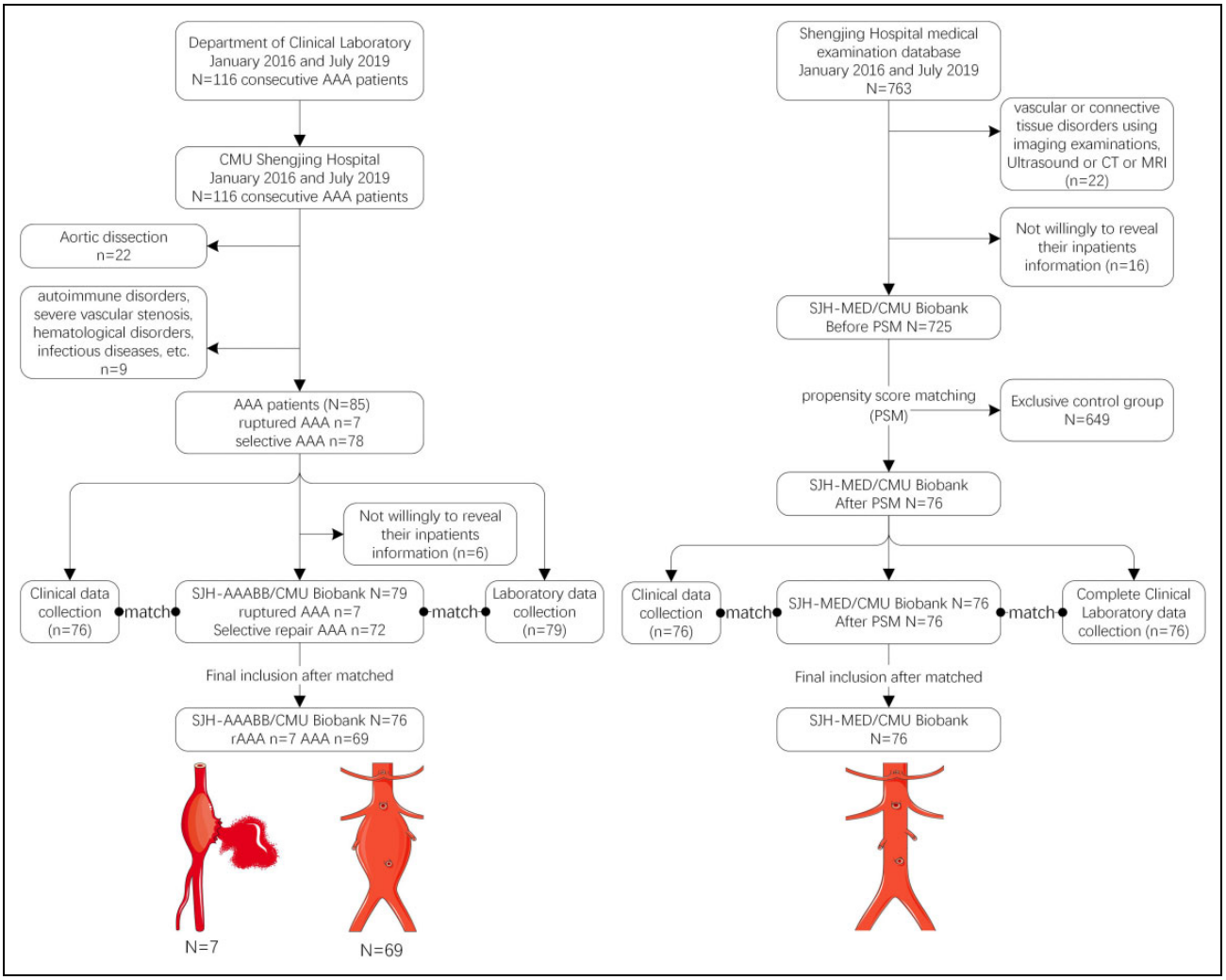

Figure 1. Flowchart of patient selection and blood specimen selection from patients with AAA and healthy control group from the Shengjing Hospital of CMU Abdominal Aortic Aneurysm Blood sample Biobank (SJH-AAABB/CMU) and Shengjing Hospital of CMU Medical Examination Blood sample Biobank (SJH-MED/CMU).

diseases was known to affect the serum lipid peroxidation levels. Thus, patients with these above conditions, who were examined by echocardiography, laboratory tests, imaging examinations, angiography diagnosis, and additional medical examinations based on patients' medical history and clinical presentations, were also excluded from this study. Eventually, 85 patients were potentially identified as AAA and were potentially suitable for inclusion in this study.

Control Group (Ctrl)

Our controls were negative to systemic or topical application of drugs, such as anti-inflammatory drugs, anticonvulsants, steroids, or antifungals, and had no concurrent cutaneous or systemic disorders. The Medical Examination Database of Shengjing Hospital of CMU was utilized to identify normal patients by thoroughly retrieving each control patients between 2016 and 2019. The control population was identified from 725 originally healthy individuals undergoing comprehensive routine health examination at outpatient department. Among them, 76 were confirmed to have normal findings on physical and echocardiographic examinations, with matched age and gender with those in the study group. Moreover, patients diagnosed with connective tissue or vascular diseases upon admission based on imaging examinations were also eliminated from control group. Further, patients with immune-associated diseases, history of drug application, infection, or malignant cancer were also excluded from the control group. This study was carried out in line with the Declaration of Helsinki. The participant selection and inclusion process are summarized in Figure 1.

Aortic Aneurysm and Medical Examination Blood Biobank

According to the exclusion criteria of unqualified patients, 6 cases were further excluded due to their unwillingness to offer the inpatient records for publication, blood samples, or clinical data (Figure 1). Eventually, the blood samples were collected from 76 eligible AAA cases (including 7 with rAAA and 69

with selective AAA) to perform additional analyses for the blood Biobank. Typically, the Biobank was registered as the Shengjing Hospital of CMU Abdominal Aortic Aneurysm Blood sample Biobank (SJH-AAABB/CMU). Meanwhile, blood samples were also collected from 76 healthy controls, and registered as the Shengjing Hospital of CMU Medical Examination Blood sample Biobank (SJH-MED/CMU). Intravenous puncture was performed in all eligible cases upon admission, followed by extraction of blood samples into the anticoagulant ethylenediaminetetraacetic acid plastic tubes (5.0 mL, BD Vacutainer lavender) as well as the silica/gel plastic tubes (5.0 mL, SST BD Vacutainer gold). In addition, the blood samples were subjected to centrifugation to extract serum, which was further preserved at −80 °C prior to test (as long as 1 year). Moreover, peripheral blood mononuclear cells were collected from these eligible AAA cases using the density gradient centrifugation of Ficoll-sodium diatrizoate according to prior description.[28] This Biobank study also gained approval from Ethics Committee of Shengjing Hospital of CMU and was carried out according to the Declaration of Helsinki.

Serum Lipid Peroxidation Contents

The Human malondialdehyde (Human MDA) ELISA Kit (ml062874, mlBio; Shanghai Enzyme-linked Biotechnology Co, Ltd) was purchased to detect the serum MDA levels according to the enzyme-linked immunosorbent assay (ELISA) strictly following manufacturer's protocols. The Human lipid hydroperoxides (Human LPO) ELISA Kit (ml320104, mlBio; Shanghai Enzyme-linked Biotechnology Co, Ltd) was purchased to assess the serum LPO. Human serum GSH-Px levels were also measured by ELISA using GSH-Px ELISA kits (ml065582, mlBio; Shanghai Enzyme-linked Biotechnology Co, Ltd) in line with the manufacturer's protocol. Additionally, those raw standards provided by the kits were utilized to construct standard curves after proper dilution, and the biomarker concentrations within samples were calculated using standard curves. Each sample was tested in duplicate.

Laboratory Examinations

The Department of Clinical Laboratory of Shengjing Hospital of CMU was responsible for each blood test in SJH-MED/CMU Biobank and SJH-AAABB/CMU Biobank. Afterward, the relevant clinical information was extracted, the corresponding blood Biobank was established, and laboratory tests were conducted as previously described.[28] In total, 76 qualified patients were eventually enrolled based on the patient inclusion criteria. The patient screening and inclusion procedures were summarized in Figure 1.

Lipid panel. The selective solubilization approach (Determiner L HDL or LDL-C test Kit, Kyowa Medex) was utilized to directly detect the plasma contents of high-density lipid cholesterol and low-density lipid cholesterol. Besides, enzymatic approaches were used to detect triglyceride and total cholesterol (TC) contents. The lipid profiles were produced using the automatic biochemistry analyzer (ARCHIRECT ci16200, Abbott Laboratories).

Other biochemical tests. The method proposed by the International Federation of Clinical Chemistry (Abbott Laboratories) was used to determine the levels of glutamic oxalacetic transaminase (aspartate aminotransferase [AST]) and alanine aminotransferase. Additionally, the biuret approach (FUJIFILM Wako Pure Chemical industries Ltd) was adopted to determine the total protein (TP) level in plasma. The glucose oxidase and urease glutamate dehydrogenase approaches (DiaSys Diagnostic Systems GmbH) were utilized to determine fasting plasma glucose level.

Statistical Analysis

The propensity score matching (PSM) method was adopted to decrease selection bias between cases and controls, and to reduce the potential clinical confounders using the 1:1 matching protocol in the current observational case–control study. Besides, the Hosmer-Lemeshow degree of fitting test was used to evaluate the model, to carry out logistic regression analysis and the C-statistic test. Before PSM, Student *t* test was adopted to compare clinical characteristics of patients with those in control group (for continuous variables), whereas χ^2 test was adopted to compare categorical variables. After PSM was performed, nonparametric Mann-Whitney test was conducted to detect the differences between both groups. Thereafter, those categorical variables were shown as numbers and percentages, followed by χ^2 test to determine the differences of these 2 groups (for clinical and biochemical factors). Linear regression and Spearman correlation were performed to assess possible correlations. Moreover, partial correlation analyses regarding smoking status, gender, and age were carried out to analyze the correlations across those continuous variables. Multiple logistic regression analysis was performed to assess the predictive significance of serum lipid peroxidation products in AAA or rAAA risk after adjusting for potential confounding factors. The relevant threshold values were determined using the logistic models as well as the ROC curves with corresponding area under curve (AUC) values. Youden index was used to determine the optimal cutoff for lipid peroxidation contents (sensitivity + specificity-1). SPSS version 25.0 software (SPSS Inc) was employed to all statistical analysis. A P value <.05 was considered to be statistically significant.

Results

Baseline Clinical Characteristics for PSM

The clinical characteristics for 76 patients with AAA and 725 control patients are shown in Table 1. Five clinical variables, including age, gender, body mass index, heart ratio, and hypertension, were statistically significant between both groups before PSM, which was used to reduce the selection bias and potential clinical confounders. Using PSM, those 76 matched pairs of AAA cases and control patients were compared through logistic regression analysis. Moreover, the Hosmer–

Table 1. Clinical Characteristics in Patients With AAA and Control Patients Before and After PSM.[a]

Characteristics	Before PSM			After PSM				
	Control N = 725	AAA N = 76	P	Control N = 76	AAA N = 76	P	AAA diameter (30-54 mm) n = 54	AAA diameter ≥55mm n = 22	P
Age, years	54.80 ± 13.34	63.59 ± 11.74	<.001	63.64 ± 13.42	63.59 ± 11.74	.859	61.13 ± 11.69	69.50 ± 9.76	.004
Men, n (%)	371 (51.17%)	65 (8.97%)	<.001	65 (85.53%)	65 (85.53%)	1.000	46 (85.19%)	19 (86.36%)	1.000
BMI	24.66 ± 5.27	23.31 ± 5.22	.034	23.09 ± 5.18	23.31 ± 5.22	.796	23.20 ± 4.62	23.59 ± 6.57	.770
HR, bmp	78.63 ± 14.21	89.42 ± 12.67	<.001	90.15 ± 12.39	89.42 ± 12.67	.723	88.93 ± 10.91	90.64 ± 16.47	.597
Current smoker, n (%)	144 (19.86%)	18 (23.68%)	.453	18 (23.68%)	18 (23.68%)	1.000	11 (20.37%)	7 (31.82%)	.373
Hypertension, n (%)	313 (43.17%)	49 (64.47%)	<.001	47 (61.84%)	49 (64.47%)	.867	32 (59.26%)	17 (77.27%)	.188
Type 2 diabetes mellitus, n (%)	81 (11.17%)	11 (14.47%)	.448	10 (13.16%)	11 (14.47%)	.811	8 (14.81%)	3 (13.64%)	1.000
Prior CHD, n (%)	154 (21.24%)	21 (27.63%)	.242	20 (26.32%)	21 (27.63%)	1.000	14 (25.93%)	7 (31.82%)	.587
Prior stroke, n (%)	55 (7.59%)	5 (6.58%)	1.000	5 (6.58%)	5 (6.58%)	1.000	4 (7.41%)	1 (4.55%)	1.000

Abbreviations: AAA, abdominal aortic aneurysm; BMI, body mass index; CHD, coronary heart disease; HR, heart rate; LPO, lipid hydroperoxides; PSM, propensity score matching.
[a]Values are mean ± SD or n (%).

Figure 2. Analysis of lipid peroxidation levels in different groups. A comparison of malondialdehyde (A), lipid hydroperoxides (B), and glutathione peroxidase (C) levels among controls and AAA and subgroup analysis of lipid peroxidation levels between small AAA and patients with AAA, between ruptured AAA and selective AAA. *$P < .05$, **$P < .01$, ***$P < .001$, ns indicates no significance.

Lemeshow degree of fit test revealed $P = .72$, C-statistic test indicated $P = .85$. Following PSM, the differences in clinical characteristics between the 2 groups were eliminated. Among the 76 cases in AAA group, 54 patients were diagnosed with small AAA (<55 mm), while the remaining 22 were identified as AAA (diameter more than 55 mm) based on guidelines of the ESVS.[23] The clinical characteristics for patients with AAA are elaborated in Table 1.

Serum Levels of Lipid Peroxidation Products Among AAA Cases

Serum MDA levels were significantly increased among AAA cases in comparison to those in control group (3.40 ± 2.37 vs 1.23 ± 0.69 nmol/mL, $P < .001$; Figure 2A). Serum MDA

Table 2. Comparisons of Blood Parameters Between Different Subgroups in AAA Patient Group.

Serum lipid peroxidation products		AAA diameter 30-54 mm	AAA diameter >55 mm		Non-rupture	Rupture	
	Subgroup	n = 54	n = 22	P	n = 7	n = 69	P
	MDA, nmol/mL	3.12 ± 0.95	3.75 ± 2.54	.276	2.94 ± 0.97	5.73 ± 5.12	**.001**[a]
	LPO, μmmol/L	5.33 ± 3.35	5.76 ± 3.08	.643	5.61 ± 3.15	6.16 ± 2.51	.656
	GSH-Px, pg/mL	131.52 ± 87.67	153.88 ± 169	.548	133.9 ± 90.59	193.89 ± 164.41	.132
Basic statistics							
	Age, year	63.89 ± 11.39	69.5 ± 9.76	.072	63.94 ± 10.97	57.33 ± 18.38	.188
	BMI	22.77 ± 4.21	23.59 ± 6.57	.594	23.45 ± 5.33	21.06 ± 3.11	.251
	HR, bmp	88.5 ± 10.3	90.64 ± 16.47	.577	89.01 ± 12.26	93.57 ± 18.19	.375
Routine blood indexes, leukocyte							
	WBC, $\times 10^9$/L	7.92 ± 3.07	6.33 ± 2.12	**.043**[b]	7.62 ± 3.07	8.14 ± 2.97	.719
	BA, $\times 10^9$/L	0.07 ± 0.14	0.08 ± 0.19	.858	0.08 ± 0.16	0.09 ± 0.07	.852
	EO, $\times 10^9$/L	0.34 ± 0.47	0.2 ± 0.16	.197	0.25 ± 0.33	0.33 ± 0.54	.615
	LY, $\times 10^9$/L	1.7 ± 0.68	1.8 ± 0.69	.605	1.63 ± 0.66	1.7 ± 0.61	.825
	MO, $\times 10^9$/L	0.76 ± 0.99	0.8 ± 1.36	.919	0.69 ± 0.89	1.65 ± 2.23	.052
	NE, $\times 10^9$/L	5.45 ± 3.05	3.79 ± 1.69	**.027**[b]	5.2 ± 3.1	5.76 ± 2.76	.700
Routine blood indexes, erythrocyte							
	RBC, $\times 10^{12}$/L	4.34 ± 0.78	4.33 ± 0.55	.943	4.31 ± 0.7	4.19 ± 0.41	.704
	HGB, g/L	132.11 ± 25.76	130.5 ± 20.64	.813	131.55 ± 23.15	123 ± 15.43	.424
	HCT, L/L	0.4 ± 0.07	0.4 ± 0.06	.934	0.4 ± 0.07	0.38 ± 0.04	.596
	MCH, pg	30.31 ± 2.24	30.1 ± 2.11	.740	30.49 ± 2.03	29.36 ± 2.4	.242
	MCHC, g/L	327.82 ± 12.98	325.32 ± 10.65	.468	328.04 ± 11.42	319.6 ± 9.04	.115
	MCV, fL	92.45 ± 5.67	92.48 ± 4.93	.986	92.93 ± 5.13	91.88 ± 6.32	.668
	RDW, %	11.69 ± 1.94	10.92 ± 1.3	.213	11.28 ± 1.55	12.83 ± 2.26	.078
Routine blood indexes, thrombocyte							
	PLT, $\times 10^9$/L	208.45 ± 87.16	225.38 ± 94.33	.594	212.97 ± 89.05	197.25 ± 62.55	.734
	MPV, fL	10.24 ± 1.00	9.83 ± 0.57	.187	10.02 ± 0.78	10.85 ± 1.18	.060
	PCT, L/L	0.22 ± 0.08	0.24 ± 0.10	.558	0.22 ± 0.09	0.23 ± 0.06	.927
	PDW, 10GSD	12.8 ± 1.09	13.3 ± 1.58	.271	12.92 ± 1.28	12.98 ± 0.79	.930
Liver function							
	ALT, U/L	17.32 ± 7.50	18.08 ± 9.20	.792	19.78 ± 12.91	15.5 ± 5.97	.520
	AST, U/L	17.70 ± 3.16	19.15 ± 3.63	.232	18.70 ± 3.80	14.75 ± 2.63	**.047**[b]
	ALB, g/L	38.36 ± 4.67	37.15 ± 5.85	.504	37.78 ± 4.54	37.55 ± 8.28	.929
	GGT, U/L	27.55 ± 19.58	26.62 ± 16.30	.886	31.25 ± 23.51	26.00 ± 18.07	.669
	ALP, U/L	74.32 ± 17.76	69.85 ± 20.16	.498	75.17 ± 21.21	68.5 ± 8.43	.541
	BChE, 1000U/L	7.35 ± 2.10	7.32 ± 2.02	.968	7.21 ± 2.08	7.18 ± 2.99	.980
	PA, mg/dL	21.92 ± 5.92	22.65 ± 7.14	.765	21.79 ± 7.09	21.13 ± 5.62	.878
	TP, g/L	64.63 ± 5.85	65.22 ± 6.71	.793	64.37 ± 5.33	67.48 ± 7.11	.291
	TBIL, μmol/L	11.99 ± 4.85	11.36 ± 5.13	.719	11.71 ± 4.35	17.00 ± 8.70	.045
	DBIL, μmol/L	6.16 ± 1.51	7.5 ± 0.57	.298	7.23 ± 2.17	7.10 ± 0.01	.958
Renal function and serum electrolyte							
	Crea, mmol/L	83.6 ± 15.24	76.5 ± 16.26	.606	90.29 ± 22.21	73.00 ± 21.21	.361
	Urea, mmol/L	6.83 ± 2.47	8.16 ± 3.61	.205	7.32 ± 2.93	5.80 ± 1.78	.318
	FPG, mmol/L	5.58 ± 1.74	5.53 ± 0.74	.920	5.77 ± 1.58	5.38 ± 0.34	.623
	CYSC, mg/L	1.08 ± 0.29	1.54 ± 0.99	.066	1.26 ± 0.65	0.95 ± 0.31	.437
	Ca, mmol/L	2.21 ± 0.13	2.21 ± 0.16	.907	2.20 ± 0.14	2.21 ± 0.08	.883
	K, mmol/L	4.10 ± 0.45	3.86 ± 0.33	.100	4.01 ± 0.44	3.74 ± 0.38	.241
	CL, mmol/L	104.61 ± 3.36	105.48 ± 2.53	.425	104.87 ± 3.1	104.05 ± 2.43	.615
	Na, mmol/L	141.03 ± 3.79	140.94 ± 1.48	.936	140.73 ± 3.18	141.6 ± 1.93	.598
	P, mmol/L	1.12 ± 0.25	1.12 ± 0.19	.997	1.1 ± 0.23	1.27 ± 0.10	.147
	HCO_3, mmol/L	24.69 ± 2.48	23.78 ± 2.00	.268	23.95 ± 2.10	25.2 ± 4.36	.319
Serum lipid profile							
	TC, mmol/L	4.43 ± 0.87	4.86 ± 0.91	.216	4.63 ± 0.90	4.19 ± 0.24	.409
	TG, mmol/L	2.04 ± 2.38	1.48 ± 0.81	.462	1.71 ± 1.88	1.86 ± 0.72	.910
	LDL-C, mmol/L	2.80 ± 0.83	3.26 ± 0.76	.140	2.97 ± 0.82	2.74 ± 0.37	.639
	HDL-C, mmol/L	1.02 ± 0.24	1.16 ± 0.34	.192	1.11 ± 0.34	1.02 ± 0.06	.628

(continued)

Table 2. (continued)

Serum lipid peroxidation products	Subgroup	AAA diameter 30-54 mm n = 54	AAA diameter >55 mm n = 22	P	Non-rupture n = 7	Rupture n = 69	P
Cardiovascular injury-related parameters							
	CK, U/L	74.38 ± 31.20	72.18 ± 35.70	.867	71.54 ± 29.30	78.67 ± 49.94	.709
	LDH, U/L	212.06 ± 46.01	210.73 ± 71.05	.953	204.32 ± 39.3	268.00 ± 122.99	**.044**[b]
Blood coagulation function							
	PT, s	13.55 ± 0.78	13.54 ± 1.52	.970	13.47 ± 1.06	14.06 ± 1.32	.249
	APTT, s	38.49 ± 5.35	38.53 ± 4.35	.981	38.40 ± 5.16	40.60 ± 4.82	.362
	PTA, s	91.68 ± 8.41	92.09 ± 12.83	.892	92.38 ± 9.65	87.60 ± 12.22	.303
	INR	1.06 ± 0.07	1.07 ± 0.14	.732	1.06 ± 0.09	1.10 ± 0.12	.395
	D-Dimer, μg/mL	2.10 ± 2.32	3.89 ± 3.85	.105	3.26 ± 3.80	2.37 ± 2.71	.696

Abbreviations: AAA, abdominal aortic aneurysm; ALT, alanine aminotransferase; ALB, albumin; ALP, alkaline phosphatase; APTT, activated partial thromboplastin time; AST, aspartate aminotransferase; BChE, cholinesterase; BA, basophil; BMI, body mass index; Ca, serum calcium; CK, creatine kinase; CL, serum chlorine; Crea, Creatinine; CYSC, Cystatin C; DBIL, direct bilirubin; EO, eosinophil; FPG, fasting plasma glucose; GSH-Px, glutathione peroxidase; GGT, gamma glutamyl transpeptidase; HCT, hematocrit; HDL-C, high-density lipoprotein-cholesterol; HGB, hemoglobin; HR, heart rate; INR, international normalized ratio; K, serum kalium; LDH, lactate dehydrogenase; LY, lymphocyte; LPO, lipid hydroperoxides; LDL-C, low-density lipoprotein-cholesterol; MO, monocyte; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MDA, malondialdehyde; MPV, mean platelet volume; Na, serum sodium; NE, neutrophil; RBC, red blood cells; RDW, red blood cell volume distribution width; P, serum phosphorus; PCT, platelet hematocrit; PDW, platelet distribution width; PLT, platelet; PA, pre-albumin; PT, prothrombin time; PTA, prothrombin activity; TP, total protein; TBIL, total bilirubin; Urea, Carbamide; TC, total cholesterol; TG, triglycerides; WBC, white blood cells.
[a] $P < .01$.
[b] $P < .05$.

level was not significantly different between small AAA and AAA (3.12 ± 0.95 vs 3.75 ± 2.54 nmol/mL; $P = .276$). Interestingly, a significant difference was observed in the serum MDA level between non-rAAA and rupture patients with AAA (2.94 ± 0.97 vs 5.73 ± 5.12 nmol/mL, $P = .001$; Table 2).

Serum LPO levels were significantly elevated among AAA cases than those in control patients (5.71 ± 3.06 vs 3.29 ± 0.97 μmol/L, $P < .001$; Figure 2B). Serum MDA level was not significantly different between small AAA and AAA (5.33 ± 3.35 vs 5.76 ± 3.08 μmol/L; $P = .643$), or between non-rAAA and rupture patients with AAA (5.61 ± 3.15 vs 6.61 ± 2.51, $P = .656$; Table 2).

As expected, the level of GSH-Px was significantly decreased in patients with AAA than that in control group (148.61 ± 127.72 vs 162.42 ± 56.08 pg/mL, $P < .001$; Figure 2C). Serum GSH-Px level was not significantly different between small AAA and AAA (131.52 ± 87.67 vs 153.88 ± 169.99 pg/mL; $P = .548$), or between non-rAAA and rupture patients with AAA (133.90 ± 90.59 vs 193.89 ± 164.41, $P = .132$; Table 2).

The subgroup analyses for AAA cases are summarized in Table 2. A significant difference was observed in the white blood cell counts (7.92 ± 3.07 vs 6.33 ± 2.12 × 10^9/L, $P = .043$) and neutrophil counts (5.45 ± 3.05 vs 3.79 ± 1.69 × 10^9/L, $P = .027$) between small AAA and AAA (Table 2). Consequently, AST concentrations were significantly decreased and serum lactate dehydrogenase (LDH) levels were markedly increased among rAAA cases in comparison with those in nonrupture patients with AAA (Table 2).

Associations Between Serum Lipid Peroxidation Products and Clinical Characteristics

The possible associations between serum lipid peroxidation content and clinical characteristics were evaluated in patients with AAA. As a result, MDA level was positively correlated with TP (R = .381), LPO level was positively correlated with red cell volume distribution width (RDW, R = 0.353) and prothrombin time (PT, R = 0.323). Additionally, GSH-Px level was negatively associated with serum TC (R = −0.348) in patients with AAA (Table 3). The association of lipid peroxidation products with clinical features is shown in Table 3.

Correlations between individual lipid peroxidation products were evaluated by Spearman rank correlation coefficient (as shown in Table 4). The results demonstrated that LPO level was significantly and positively related to MDA (R = 0.358, $P < .001$), while negatively correlated with GSH-Px level (R = −0.203, $P = .032$).

Predictive and Diagnostic Significance of Serum Lipid Peroxidation Content for AAA

Receiver operating characteristic analysis was used to determine the threshold lipid peroxidation content, in order to evaluate AAA (as shown in Table 5). The AUC for AAA was 0.965, and the optimal threshold was 2.242 nmol/mL, while the sensitivity was 90.8% and the specificity was 91.9% for MDA level (Figure 3A). Furthermore, the values of AUC were 0.780 and 0.741 for LPO and GSH-Px, respectively, and the

Table 3. The Correlations Between Individual Serum Peroxidation Products and Clinical Features in Patients With AAA.

Characterization	Indices	MDA (nmol/mL)	LPO (umol/L)	GSH-Px (pg/mL)
Basic statistics				
	BMI	−0.040	0.037	−0.009
	HR, bmp	−0.132	−0.014	−0.051
	Maximum diameter, mm	0.238	0.050	0.137
Routine blood indexes, leukocyte				
	WBC, $\times 10^9$/L	0.004	−0.034	0.020
	BA, $\times 10^9$/L	−0.001	−0.177	−0.036
	EO, $\times 10^9$/L	0.041	−0.074	−0.040
	LY, $\times 10^9$/L	0.211	−0.037	**0.257**[a]
	MO, $\times 10^9$/L	0.075	−0.074	0.016
	NE, $\times 10^9$/L	−0.053	−0.048	−0.050
Routine blood indexes, erythrocyte				
	RBC, $\times 10^{12}$/L	0.118	−0.244	0.127
	HGB, g/L	0.036	−0.198	0.091
	HCT, L/L	0.082	−0.251	0.115
	MCH, pg	−0.168	0.061	−0.067
	MCHC, g/L	−0.169	0.045	−0.068
	MCV, fL	−0.108	0.037	−0.038
	RDW, %	−0.076	**0.353**[a]	−0.109
Routine blood indexes, thrombocyte				
	PLT, $\times 10^9$/L	0.010	0.005	0.017
	MPV, fL	−0.050	−0.080	−0.142
	PCT, L/L	0.029	−0.185	0.027
	PDW, 10GSD	−0.023	−0.089	−0.142
Liver function				
	ALT, U/L	0.027	−0.153	−0.023
	AST, U/L	0.280	−0.091	0.252
	ALB, g/L	0.209	−0.148	0.145
	GGT, U/L	−0.182	−0.135	−0.127
	ALP, U/L	−0.083	−0.142	−0.089
	BChE, 1000U/L	0.283	-0.082	0.058
	PA, mg/dL	−0.097	−0.256	−0.085
	TP, g/L	**0.381**[a]	−0.066	0.232
	TBIL, μmol/L	0.135	−0.014	0.132
	DBIL, μmol/L	0.707	−0.117	0.284
Renal function and serum electrolyte				
	Crea, mmol/L	−0.027	**−.667**[a]	0.206
	Urea, mmol/L	−0.148	0.035	−0.086
	FPG, mmol/L	0.154	−0.116	0.010
	CYSC, mg/L	−0.230	0.077	−0.205
	Ca, mmol/L	0.176	−0.066	0.063
	K, mmol/L	−0.021	−0.021	0.038
	CL, mmol/L	0.110	0.109	0.049
	Na, mmol/L	0.132	−0.019	0.034
	P, mmol/L	−0.130	0.010	−0.208
	HCO_3, mmol/L	0.325	−0.052	0.271
Serum lipid profile				
	TC, mmol/L	−0.116	0.150	**−.348**[a]
	TG, mmol/L	0.177	−0.159	−0.203
	LDL-C, mmol/L	−0.101	0.105	−0.194
	HDL-C, mmol/L	−0.257	0.203	−0.150
Cardiovascular injury-related parameters				
	CK, U/L	0.063	−0.198	0.121
	LDH, U/L	0.238	−0.181	0.217
Blood coagulation function				
	PT, s	−0.013	**0.323**[a]	−0.003
	APTT, s	0.137	0.230	−0.019
	PTA, s	−0.004	−0.061	−0.025
	INR	−0.021	0.161	−0.001
	D-Dimer, μg/mL	−0.155	0.089	−0.131

Abbreviations: AAA, abdominal aortic aneurysm; ALT, alanine aminotransferase; ALB, albumin; ALP, alkaline phosphatase; APTT, activated partial thromboplastin time; AST, aspartate aminotransferase; BChE, cholinesterase; BA, basophil; BMI, body mass index; Ca, serum calcium; CK, creatine kinase; CL, serum chlorine; Crea, Creatinine; CYSC, Cystatin C; DBIL, direct bilirubin; EO, eosinophil; FPG, fasting plasma glucose; GSH-Px, glutathione peroxidase; GGT, gamma glutamyl transpeptidase; HCT, hematocrit; HDL-C, high-density lipoprotein-cholesterol; HGB, hemoglobin; HR, heart rate; INR, international normalized ratio; K, serum kalium; LDH, lactate dehydrogenase; LY, lymphocyte; LPO, lipid hydroperoxides; LDL-C, low-density lipoprotein-cholesterol; MO, monocyte; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MDA, malondialdehyde; MPV, mean platelet volume; Na, serum sodium; NE, neutrophil; RBC, red blood cells; RDW, red blood cell volume distribution width; P, serum phosphorus; PCT, platelet hematocrit; PDW, platelet distribution width; PLT, platelet; PA, pre-albumin; PT, prothrombin time; PTA, prothrombin activity; TP, total protein; TBIL, total bilirubin; Urea, Carbamide; TC, total cholesterol; TG, triglycerides; WBC, white blood cells.
[a]$P < .05$.

optimal thresholds were 5.185 μmol/L and 129.38 pg/mL, respectively. The sensitivity value in diagnosis was 56.6% for LPO (Figure 3B) and 69.3% for GSH-Px (Figure 3C), respectively, and the values of specificity were 97.3% and 78.4%, respectively.

As shown in Table 6, multiple logistic regression analysis was further carried out to assess the prediction value of serum lipid peroxidation content as a risk factor for AAA under different adjustment models. After adjusting all possible confounding factors, serum MDA level remained significant correlation with AAA risk (odds ratio [OR] = 13.706 per unit increase, 95% CI = 5.888-31.909, $P < .01$) and serum LPO was significantly associated with AAA risk (OR: 1.891 per unit increase, 95% CI = 1.451-2.464, $P < .001$).

Univariate and Multivariate Logistic Regression Analysis for AAA Rupture

The following 4 variables had been previously found to be correlated with AAA rupture, MDA level, AST concentration, TBIL, and serum LDH concentration ($P < .05$). Additional 3 variables, including monocyte counts, red cell volume distribution width, and mean platelet volume, were indicated to be potentially associated with rAAA (all P values were approximately .05 in univariate analysis; Table 2). As a result, the above 7 variables, together with the maximum diameter of AAA, hypertension, smoker, and pulsating sensations in the

Table 4. The Correlations Between Corresponding Serum Peroxidation Products Within AAA Patients' Group.

		MDA (nmol/mL)	LPO (ummol/L)	GSH-Px (pg/mL)
MDA (nmol/mL)	Coefficient	-	0.358[a]	−0.021
	P value	-	<.001	.835
	N	-	76	76
LPO (ummol/L)	Coefficient		-	−0.203
	P value		-	.032
	N		-	76
GSH-Px (pg/mL)	Coefficient			-
	P value			-
	N			-

Abbreviations: AAA, abdominal aortic aneurysm; GSH-Px, glutathione peroxidase; LPO, lipid hydroperoxides; MDA, malondialdehyde.
[a]P<0.05

Table 5. Diagnostic Value of Individual Lipid Peroxidation Products for Patients With AAA.

	AUC	95% CI	P value	Cutoff value	Sensitivity (%)	Specificity (%)
MDA (nmol/mL)	0.965	0.934-0.995	<.001	2.242 nmol/mL	90.8	91.9
LPO (μmmol/L)	0.780	0.701-0.859	<.001	5.185 umol/L	56.6	97.3
GSH-Px (pg/mL)	0.741	0.658-0.825	<.001	129.38 pg/mL	69.3	78.4

Abbreviations: AUC, area under curve; GSH-Px, glutathione peroxidase; LPO, lipid hydroperoxides; MDA, malondialdehyde.

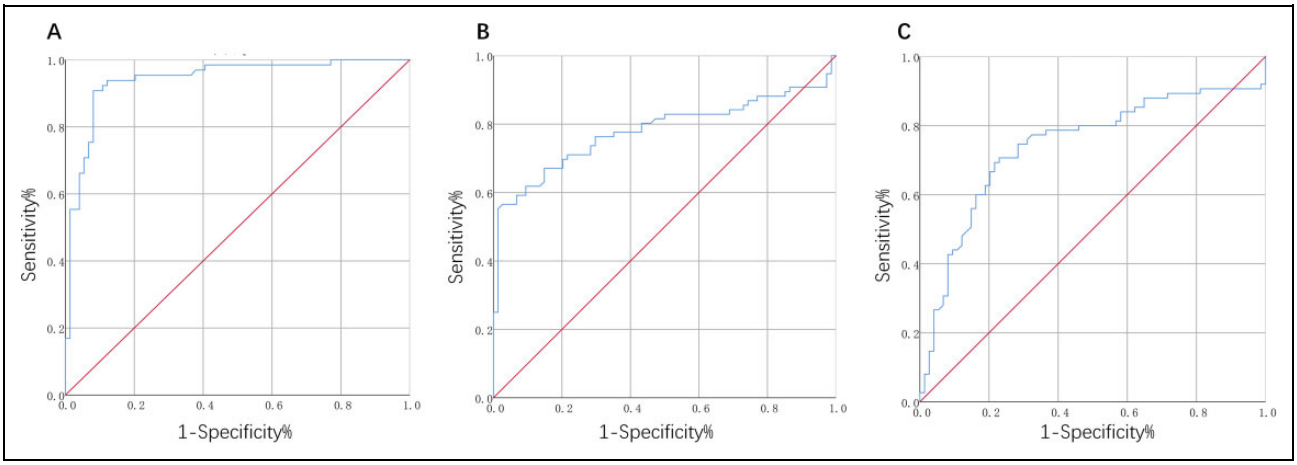

Figure 3. Receiver operating characteristic curve for serum malondialdehyde (A), lipid hydroperoxides (B), and glutathione peroxidase (C) levels to predict AAA.

Table 6. Multiple Logistic Regression Analysis of MDA, LOP, and GSH-Px Levels for AAA Risk.[a]

	MDA (nmol/mL)		LPO (ummol/L)		GSH-Px (pg/mL)	
Adjusted variable	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
Model 1	13.308 (6.056-29.241)	<.001	2.022 (1.551-2.636)	<.001	0.998 (0.995-1.002)	.388
Model 2	12.870 (5.734-28.885)	<.001	1.895 (1.456-2.467)	<.001	0.998 (0.994-1.002)	.246
Model 3	13.706 (5.888-31.909)	<.001	1.891 (1.451-2.464)	<.001	0.998 (0.994-1.002)	.236

Abbreviations: GSH-Px, glutathione peroxidase; LPO, lipid hydroperoxides; MDA, malondialdehyde; OR, odds ratio.
[a]Model 1: age and gender were adjusted; Model 2: Model 1 plus heart rate, BMI and smoking status; Model 3: Model 2 plus hypertension, type 2 diabetes, prior coronary heart disease and prior stroke status.

abdomen, were incorporated into the multivariate logistic regression analyses. The results suggested that, only serum MDA level (OR = 2.536; 95% CI: 1.037-6.203; $P = .041$) was significantly correlated with AAA rupture (Table 7) after adjusting for confounding factors. Nevertheless, rAAA showed no correlation with any variable included in the current study.

Table 7. Univariate and Multivariate Logistic Regression Analyses for AAA Rupture.[a]

	Model 1		Model 2	
	OR (95% CI)	P value	OR (95% CI)	P value
MDA, nmol/mL	2.010 (0.951-4.251)	.068	2.536 (1.037-6.203)	**.041**[b]
Maximum diameter, mm	1.010 (0.960-1.062)	.707	1.030 (0.962-1.103)	.392
Hypertension, %	3.571 (0.406-31.409)	.251	2.949 (0.253-34.407)	.388
Current smoker, %	0.490 (0.055-4.368)	.523	1.069 (0.091-12.605)	.958
Pulsating sensations in the abdomen, %	1.400 (0.177-11.083)	.750	1.724 (0.109-27.366)	.700
AST, U/L	0.703 (0.481-1.029)	.070	0.120 (0.009-1.635)	.112
TBIL, μmol/L	1.184 (0.983-1.427)	.075	1.153 (0.882-1.507)	.299
LDH, U/L	1.015 (0.998-1.033)	.093	1.331 (0.001-47.916)	.996
MO, $\times 10^9$/L	1.533 (0.910-2.584)	.109	1.452 (0.739-2.856)	.279
RDW, %	1.039 (0.459-2.351)	.927	2.582 (0.449-14.839)	.288
MPV, fL	3.139 (0.876-11.24)	.079	1.651 (0.405-6.727)	.484

Abbreviations: AAA, abdominal aortic aneurysm; AST, aspartate aminotransferase; BMI, body mass index; CI, confidence interval; LDH, lactate dehydrogenase; MDA, malondialdehyde; MO, monocyte; MPV, mean platelet volume; OR, odds ratio; RDW, red blood cell volume distribution width; TBIL, total bilirubin.
[a]Model 1 no adjustments, Model 2 adjusted for age, gender, BMI
[b]$P < .05$.

Discussion

Oxidative stress is widely acknowledged to play a major role in the development of aneurysmal diseases.[29,30] Oxidative burden has been reported to potentially worsen the cellular degeneration. Certain molecules, such as MDA, are well investigated to be able to enhance oxidative stress through lipid peroxidation.[31] The indirect detection of oxygen free radicals is possible by measuring their oxidative attack on lipids and proteins, which lead to products such as aldehydes, hydroperoxides, and conjugated dienes. Our present findings suggested higher MDA and LPO levels in either AAA or rAAA group than those in the control group. Inversely, GSH-Px level was significantly decreased in patients with AAA than that in the control group. Intriguingly, patients with rAAA were also more likely to have higher MDA levels than patients with AAA overall, possibly depending on their different wall mechanics and arterial hemodynamics. This outcome supports the contributive role of enhanced lipid oxidative stress to the pathogenesis of AAA.[19]

Subgroup comparisons further revealed that patients with rAAA had higher serum MDA level compared with controls in univariate analysis. In addition, serum MDA level was found to be significantly related to AAA rupture after adjusting for confounding factors. Malondialdehyde, one of the most common and harmful products of lipid peroxidation, would cause cell damaging, react with the free amino groups of proteins and nucleic acids, with the target mutagenic activity at guanine site in DNA sequence.[32,33] Thus, the intensity of oxidative stress or lipid peroxidation-triggered damage could be assessed by determination of MDA. This study is similar with a previous study concentrating on determining oxidative stress based on aortic media tissue. Billaud et al determined the levels of superoxide anion and lipid peroxidation marker MDA, and activity of peroxidase and SOD in aortic media tissue, revealing that elevated hemodynamic stress caused by bicuspid aortic valve could result in increased oxidative stress parameters.[34] Their findings indicate that the characteristic absence of SMCs in bicuspid aortic valve aortopathy might be a result of superoxide-mediated cell damage. However, there is barely any information about the biochemical alterations in blood or their correlation with clinical stage of the AAA disease.

In the present study, we also found that serum LPO level was significantly elevated among AAA cases than those in control patients. The levels of F2-isoprostane, specific markers of LPO, revealed that rAAAs populations were burdened with 2 phases of oxidative injury, including before arrival at hospital and postoperation.[35] Moreover, the significant correlation of postoperatively increased level of F2-isoprostane with neutrophil oxidant production indicates the occurrence of neutrophils in the oxidative injury after rAAA. Therefore, novel therapeutic interventions by decreasing neutrophil-mediated oxidant injury during reperfusion might relieve organ failure, thereby ultimately decreasing mortality in rAAAs patients.[35] However, there are still certain limitations in the predictive models in consideration of the incomplete understanding of precise molecular mechanisms underlying AAA rupture. Novel understanding of the pathomechanism of aneurysm rupture would definitely assist decision-making in clinical management and further enhance the development of new noninvasive therapeutic strategies.

Intracellular decreased GSH is converted into oxidized glutathione by GSH-Px, which catalyzes the reduction of peroxides.[36] GSH-Px eliminates H_2O_2 by using glutathione as a hydrogen donor and converts organic hydroperoxides to the corresponding alcohol.[37,38] Nevertheless, there are only limited studies on glutathione system and GSH-Px levels of aneurysm, and no investigations concerning the specific region of the above systems for rAAA group and small AAA.

Nevertheless, certain limitations should be noted in the current research. To begin with, in this single-centered study, the sample size was relatively small. Therefore, multicenter cohort

study with a large sample size should be conducted by enrolling a large number of patients with AAA, to investigate the relationships of serum lipid peroxidation content with AAA. Secondly, the lipid peroxidation contents were only measured in some transverse time periods. However, lipid peroxidation at different stage and in-hospital or postoperative period during the identical AAA pathological process remains unclear. Therefore, future study should be performed to dynamically detect MDA, LPO, and GSH-Px levels at various AAA stages, which would contribute to more accurate judgment of the lipid peroxidation contents/changes among patients with AAA. Additionally, few women were included in our study, typical of studies in patients with AAA. This is particularly important because being female is an important predictor of AAA rupture,[39] and a third of all deaths caused by AAA rupture are in women.[40] Additionally, histological specimens and lipid peroxidation biomarker tissue expression were not examined in this study.

Conclusion

Collectively, in the present study, we demonstrated a positive correlation between serum MDA, LPO, and AAA. Serum MDA and LPO concentrations could be used to predict disease severity in patients with AAA. Moreover, serum MDA may serve as the candidate biomarker for diagnosis of AAA and accurate identification of increased risks of AAA rupture. These findings indicated that lipid peroxidation might be critically involved in the initiation and progression of AAA, while the prognostic significance of lipid peroxidation requires further investigation. In addition, all these speculations can be important issues topics in lipid peroxidation biology and AAA pathobiology, which deserve further investigation.

Authors' Note

The data used in this study are accessible from the corresponding author upon reasonable request. Feng Shi and Yanshuo Han conducted the experiment. Feng Shi and Chao Ji performed the analysis and drafted the manuscript. Changcheng Ma collected the laboratory data. Feng Shi collected the clinical data. Feng Shi and Yanshuo Han designed the study and revised the manuscript. All authors read and approved the final manuscript. The study protocol was approved by the Ethics Committee of Shengjing Hospital of China Medical University (CMU) according to the Declaration of Helsinki.

Declaration of Conflicting Interests

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ORCID iD

Yanshuo Han https://orcid.org/0000-0002-4897-2998

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