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Increase in the Oxidized Low-Density Lipoprotein Level by Smoking and the Possible Inhibitory Effect of Statin Therapy in Patients with Cardiovascular Disease.

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Keywords: MDA-LDL; Smoking; Oxidative stress; Coronary artery disease; Statin therapy

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Abstract

Objectives:

MDA-LDL level is a marker of oxidative stress and is linked to progression of arteriosclerosis; however, the clinical factors affecting to the oxidized LDL level have not been elucidated. We herein investigated various factors to identify correlation with MDA-LDL level in high risk patients requiring catheter intervention.

Setting:

Secondary care (cardiology), single center study

Participants:

600 patients who were admitted to our hospital and underwent cardiac catheterization

Primary and secondary outcome measures:

Blood samples were obtained to measure lipid profiles and MDA-LDL level.

Results:

With regard to smoking status, MDA-LDL level was significantly higher in ex-/current smokers compared with non-smokers. Of note, there was no improvement of MDA-LDL level even in patients who quitted smoking. Multiple regression analysis showed that MDA-LDL level was positively correlated with LDL-cholesterol level, Brinkman index and male gender. The correlation between smoking status and either MDA-LDL or LDL-C level was investigated in

two groups; namely, patients, with or without statin treatment. In non-statin group, MDA-LDL level and MDA-LDL/LDL-C ratio were significantly higher in ex-/current smokers compared with non-smoker, while no significant correlation was observed between smoking status and LDL-C level. In contrast, in statin group, there were no significant correlations between smoking status and all any of these cholesterol parameters.

Conclusions:

We found that MDA-LDL level was affected by multiple factors, such as smoking status, LDL-C level and male gender. The present findings give additional evidence that smoking should be prohibited from MDA-LDL standpoint. Furthermore, statin therapy might have a beneficial effect on the reduction of MDA-LDL level.

Trial registration:

N/A

Main strengths

Although oxidative LDL is associated with the marker of oxidative stress and the progression of atherosclerosis, clinical factor which affects the oxidative LDL remains uncertain. Our study revealed that MDA-LDL was associated with smoking and the MDA-LDL level would never decrease with smoking cessation. However, MDA-LDL level was decreasing even in smokers with statin therapy.

Study limitations

Smoking cessation was not found to be effective for reducing MDA-LDL level in this study; however, favorable effects of smoking cessation would likely occur with regard to other parameters than MDA-LDL level. Thus smoking cessation is recommended at any time, even after long-term smoking, and is considered to provide cardiovascular health benefits.

This was a retrospective study, and the true effects of a statin on MDA-LDL level remain unclear. Finally, we did not examine the prognosis of the study population and therefore, the effects of smoking cessation and/or statin therapy remains unclear especially in terms of their impact on MDA-LDL level. Prospective studies are required to obtain answers regarding there topics.

Introduction

The malondialdehyde modified low-density lipoprotein (MDA-LDL; oxidized LDL) is LDL that has been modified by MDA, leading to the production of a large amount of aldehyde when LDL becomes degenerated and oxidized [1].

It is known that MDA-LDL level is elevated in patients with dyslipidemia and diabetes mellitus (DM), both risks factors for atherosclerotic disease [2 3]. Since MDA-LDL level has a positive correlation with the serum LDL level, the ratio of MDA-LDL/LDL-C (M/L) is used to evaluate the severity of oxidization of LDL; in some reports, not only MDA-LDL level, but also M/L ratio has been shown to increase in patients with DM compared with controls [4 5].

In patients with coronary artery disease (CAD), MDA-LDL level and M/L ratio have been shown to increase even when there are no other differences in the other lipid profiles [6]. In addition, it has been shown that the measurement of MDA-LDL level might be useful as a predictor of restenosis after percutaneous coronary intervention in patients with DM [7]. Based on these findings, it has been speculated that MDA-LDL level might be important marker of the progression of arteriosclerosis; however, the clinical factors possibly affecting MDA-LDL level have not been elucidated. Therefore, in the present study, we investigated “the clinical factors” affecting MDA-LDL level in high risk patients requiring catheter intervention.

Methods

Study patients

The study protocol (24-150[6916]) was approved by the ethics committee of the Jikei University School of Medicine. Six hundred consecutive patients who underwent cardiac catheterization from March 2010 to September 2011 were examined in this study. The baseline patient characteristics, including the clinical parameters and the biochemical data, were collected retrospectively from the hospital medical records. In addition, the results of the catheterization (i.e. the number of occluded or narrowed vessels), body weight, body mass index (BMI), coronary risk factors and medication profiles were also investigated. The patients taking eicosapentaenoic acid (EPA) were excluded since it has been demonstrated that EPA is major antihyperlipidemic agents with potent antioxidant effects.

Data collection

Blood sampling was performed to examine the serum MDA-LDL, serum creatinine, hemoglobin A1c (HbA1c), B-type (brain) natriuretic peptide (BNP) and low-density lipoprotein cholesterol (LDL-C) levels. MDA-LDL level was measured by an ELISA using an anti-MDA-LDL monoclonal antibody (ML25) and β-galactosidase anti-apoB monoclonal antibody (AB16) [1]. It is well known that the combination of ML25 and AB16 can accurately detect MDA-LDL [1].

The concentration of MDA-LDL is defined at 1 mg/L of MDA-LDL produced artificially, which shows the same signal as 1U/L of MDA-LDL in the serum. Diabetes mellitus (DM), hypertension, dyslipidemia and smoking were defined as described previously [8 9]. The definition of smoking status was as follows: current smokers were those who were smoking at the time of the study or who had smoked in the past year; the subjects who had quit smoking more than one year before the study were defined as ex-smokers and those who had never smoked were defined as non-smokers. Brinkman Index was used to evaluate the smoking status of current/ex-smokers [10].

Statistical analysis

Comparisons between MDA-LDL level and LDL-C level, age, BMI, HbA1c, Cr, BNP and Brinkman index were performed with a linear regression analysis. Comparison between Brinkman index and LDL-C was also performed with a linear regression analysis. Comparisons of MDA-LDL levels between males and females, subjects with or without hypertension, non-smokers versus ex-smokers versus current smokers, and among the various patient groups after smoking cessation were performed with Mann-Whitney U test. Multiple factors, which were considered to possibly modify MDA-LDL level were evaluated with a stepwise multiple regression analysis. Comparisons of LDL-C and MDA-LDL level and M/L ratio between non-

smokers and smokers were performed with Mann-Whitney U test.

Statistical significance was defined as a value of $p<0.05$.

Results

Baseline characteristics

The baseline characteristics of the patients in this study are shown in Table 1. The average age was 64.8 ±11.4 years old and 80.3% of them were male. The percentages of non-smokers, ex-smokers and current smokers were 32%, 42% and 26%, respectively. The average LDL-C level and MDA-LDL level was 106.1 ± 30.8 mg/dl and 119.2 ± 48.7 U/L, respectively. The percentage of patients taking statin therapy was 55.3%.

Table 1-1 Patient's Characteristics

n=600	mean ± SD
Age, years	64.8±11.4
Male, gender (%)	80.3
Height, cm	165.4±25.1
Weight, kg	66.8±13.3
BMI, kg/m^2	24.4±3.74
Non-smoker,n(%)	192(32.4)
Ex-smoker,n(%)	247(41.7)
Current smoker,n(%)	153(25.8)
Cr, mg/dL	1.38±1.94
eGFR, mL/min/1.73m^2	62.6±21.6
HbA1c, %	6.4±1.1
BNP, pg/mL	140±263
LDL-C,mg/dℓ	106.1±30.8
MDA-LDL,U/L	119.2±48.7
M/L	1.16±0.47

BMI: body mass index Cr: creatinine, eGFR: estimated glomerular filtration rate, BNP: B-type natriuretic peptide, LDL-C:low-density lipoprotein cholesterol ,MDA-LDL: Malondialdehyde modified low density lipoprotein. M/L: Malondialdehyde modified low density lipoprotein/ low density lipoprotein cholesterol,

Table 1-2 Patient's Characteristics

Disease	n(%)
Diabetes Mellitus	252(42.0)
Hypertension	455(75.5)

Dyslipidemia	441(73.5)
Medicine	n(%)
statin	332(55.3)
Ezetimibe	31(5.2)
fibrate	13(2.1)
Coronary Artery Disease	n(%)
0VD	215(35.8)
1VD	249(41.5)
2VD	84(14.0)
3VD	52(8.7)

0VD: 0-vessel disease, 1VD: single-vessel disease, 2VD: double-vessel disease, 3VD: triple-vessel disease

Clinical factors affecting MDA-LDL level

To elucidate the determinants of MDA-LDL level, we first performed a simple regression analysis (*Fig. 1*). MDA-LDL level showed a significantly positive correlation with LDL-C level (*Fig. 1A*) and a negative correlation with age (*Fig. 1B*). In addition, MDA-LDL levels were significantly higher in males as well as the patients without hypertension (*Figs. 1C, D*). BMI, HbA1c, Cr and BNP level had no impact on MDA-LDL level (*Figs. 1E-H*).

Correlation of smoking status with MDA-LDL level

We next examined the impact of smoking status on MDA-LDL level. MDA-LDL, but not LDL-C, showed a significantly positive correlation with the smoking profiles indicated by Brinkman

index *(Fig. 2-A, B*). The patients were subsequently divided into three groups according to their smoking status: Non-, ex- and current smokers. MDA-LDL level was significantly higher in both ex- and current smokers compared to that in non-smokers *(Fig. 2-C)*. Moreover, when patients were compared based on the number of years after smoking cessation, not only the current smoking group, but also the group that had quit smoking within 10 years and that where the patients had quit smoking more than 21 years earlier showed higher MDA-LDL levels than did the non-smoking group (*Fig. 2-D*). This suggests that MDA-LDL level will never completely recover once a subject has started smoking.

Clinical factors affecting MDA-LDL level identified in multiple regression analysis

To assess the independent determinants of MDA-LDL level, a multiple regression analysis was performed. After removing the confounding factors, MDA-LDL level was shown to be positively correlated with LDL-C level ($p<0.001$), Brinkman index ($p=0.009$) and a male gender ($p=0.019$) (Table 2).

Table 2 Multiple Regression Analysis

Significant variable	Regression coefficients	Standard error	Standard regression coefficients	F	p
LDL-C	0.675	0.06	0.429	128.089	<0.001

Brinkman index	0.008	0.003	0.105	71.502	0.009
Gender	11.511	4.908	0.94	49.901	0.019

Objective variable: MDA-LDL

Explanatory variable: BMI, Age, Gender, brinkman index, Cr, BNP, LDL-C, HbA1c, HT

No significant variables: BMI, Age, Cr, BNP, HbA1c, HT

Effects of statin therapy on the correlations of smoking status with MDA-LDL or LDL-cholesterol level

The correlations of the smoking status with MDA-LDL/LDL-C level were investigated after patients were divided into two groups; those with or without statin treatment (*Fig. 3*). In non-statin-treated group, M-LDL level as well as MDA-LDL/LDL-C ratio, was significantly increased in ex-/current smokers compared to those in non-smokers, although there was no significant differences in LDL-C levels between the subjects with the different smoking status. LDL-C level was not significantly different in non-statin-treated and statin-treated groups.

Discussion

In this study, we investigated the factors associated with MDA-LDL level in high risk patients requiring cardiac catheterization. According to a multivariate analysis, Brinkman index, as well as the LDL-C level and gender were found to be significantly associated with MDA-LDL level. Furthermore, we found that smoking cessation was not effective for reducing MDA-LDL level,

even after the patients had quit smoking for many years. However, we found evidence that statin treatment may reduce MDA-LDL level, which could possibly help in the treatment of smokers.

It has been reported that smoking may affect susceptibility of plasma LDL to peroxidative modification. Modified LDL has been shown to be the preferred substrate for macrophages and induces their subsequent transformation into foam cells [11 12]. Thus, oxidative stress is very important for synthesis of modified-LDL. However, it is noteworthy that MDA-LDL level was emphatically influenced by smoking but not by obesity (estimated by BMI), hypertension, diabetes mellitus (estimated by HbA1c level), renal failure (estimated by Cr level) or heart failure (estimated by BNP level) by the multivariate analysis in this study, though all of these clinical characteristics have been shown to increase oxidative stress. The precise mechanisms by which smoking increases MDA-LDL level remain unclear at present.

The current study clearly showed that smoking was substantially harmful with regard to increasing MDA-LDL level. When we examined the effect of smoking status on MDA-LDL level among the non-smokers, ex-smokers and current smokers, we found that MDA-LDL level was still higher in the ex-smokers than in the non-smokers and was unexpectedly similar to the level in the current smokers. Furthermore, we examined the effects of the period of smoking cessation and the analysis indicated that even many years after smoking cessation,there was no

significant reduction of MDA-LDL level. This result suggests that smoking should never be started, and that if started, it is important to quit smoking as soon as possible before becoming a heavy smokerss.

The present results suggests that smoking keeps MDA-LDL level elevated for a long time. Therefore, a method for lowering MDA-LDL level is especially needed for smokers. One possible candidate would be statin therapy. Statins facilitate the LDL uptake in hepatocytes, decrease old LDL (which is easily oxidized),and thereby reduce the risk of LDL oxidization [13]. The decreases in fatty acids and cholesterol in the lipoprotein are also likely to lead to a decrease in oxidization [14]. In the current study, we examined the effects of statin treatment on MDA-LDL levels between non-smokers and smokers. MDA-LDL level was found to be significantly higher in smokers than in non-smokers in the stain (-) group. On the other hand, the effect of smoking on LDL-C level was not seen in the statin (+) group, the levels were similar between smokers and non-smokers. The ratio of MDA-LDL/LDL-C showed a similar result. These results may suggest that statin therapy can reduces MDA-LDL level in smokers to a level similar to that in non-smokers.

It has been reported that cigarette smoking is one of the risk factors for organic stenosis but that it does not act alone in contributing to the progression of atherosclerosis [14 15]. In addition atherosclerosis was not produced by smoking alone in animal models [16]. However, cigarette

smoking acts in concert with other risk factors such as hypercholesterolemia to accelerate atherosclerosis[14 15 17 18 19]. The current study is in agreement with the previous reports. Importantly, it has been shown that among other risk factors cigarette smoking alone is a highly significant risk factor for coronary spasm [20 21 22 23 24] and treatment with fluvastatin reduced the coronary spasm [25]. Thus, smoking cessation combined with statin therapy would be beneficial for prevention of ischemic heart disease by reducing progression of atherosclerosis and suppressing coronary spasm.

Conclusion

We found that MDA-LDL level was affected by multiple factors such as smoking status (as indicated by Brinkman index), LDL-C level and gender. In addition to its other health effects, smoking should be strongly prohibited due to its harmful effects from MDA-LDL standpoint. We recommend that patients should never smoke, but that once smoking has started, it is essential to quit smoking as early as possible and to cut back on the number of cigarettes consumed. Furthermore, statin therapy might have a beneficial effect on the reduction of MDA-LDL level.

Contributorship Statement:

KO collected the data, performed the statistical analyses, and wrote the manuscript.

TT and TN conceived of the research hypothesis and analyses, wrote and edited the manuscript. HS and SA performed the statistical analyses and edited the manuscript. KM and TO participated in the design and coordination of the study and collected the data. MY conceived of the study, and participated in its coordination and edited the manuscript. All authors read and approved the final manuscript.

Competing Interests: None

Data Sharing Statement: No additional data are available

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Figure legends

Fig. 1 Correlations of MDA-LDL level with various clinical factors

The clinical factors affecting MDA-LDL level are shown in (A)-(H).

Fig. 2 Correlation of smoking status with MDA-LDL and LDL-cholesterol levels

Correlation between MDA-LDL level and Brinkman index (A) and, between LDL-C level and Brinkman index (B) was determined with a linear regression analysis. Comparison of MDA-LDL levels among non-smokers, ex-smokers and current smokers (C). Comparison of MDA-LDL levels in each patient group among non-smokers and ex-smokers who had quit more than 21 years earlier, ex-smokers who had quit 11 to 20 years ago, ex-smokers who had quit less than 10 years ago and current smokers (D).

Fig. 3 Effects of the statin therapy on the correlations of smoking status with MDA-LDL or LDL-cholesterol level

The correlations of the smoking status with MDA-LDL and LDL-C levels were investigated after dividing the patients into two groups; with (A) or without (B) statin treatment.

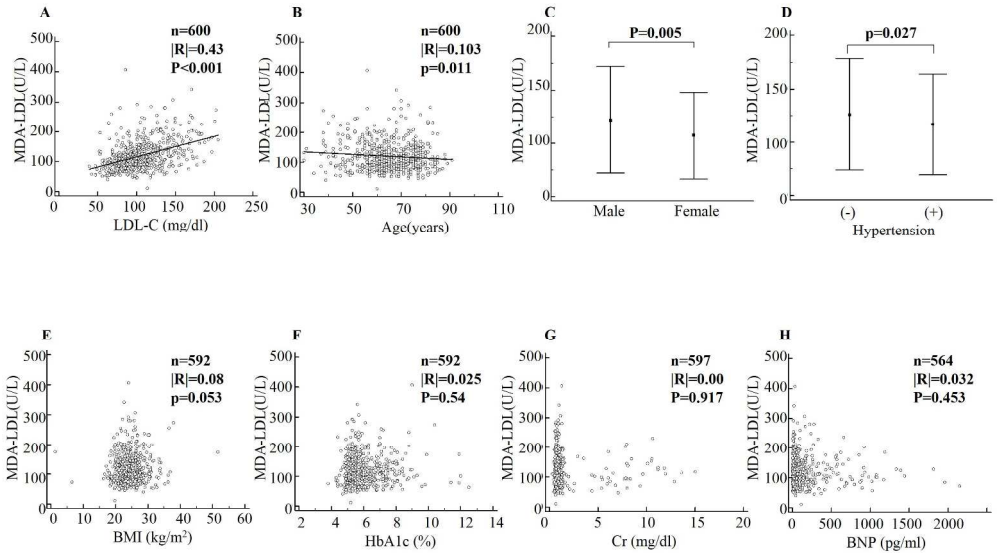

Figure 1

Fig. 1 Correlations of MDA-LDL level with various clinical factors
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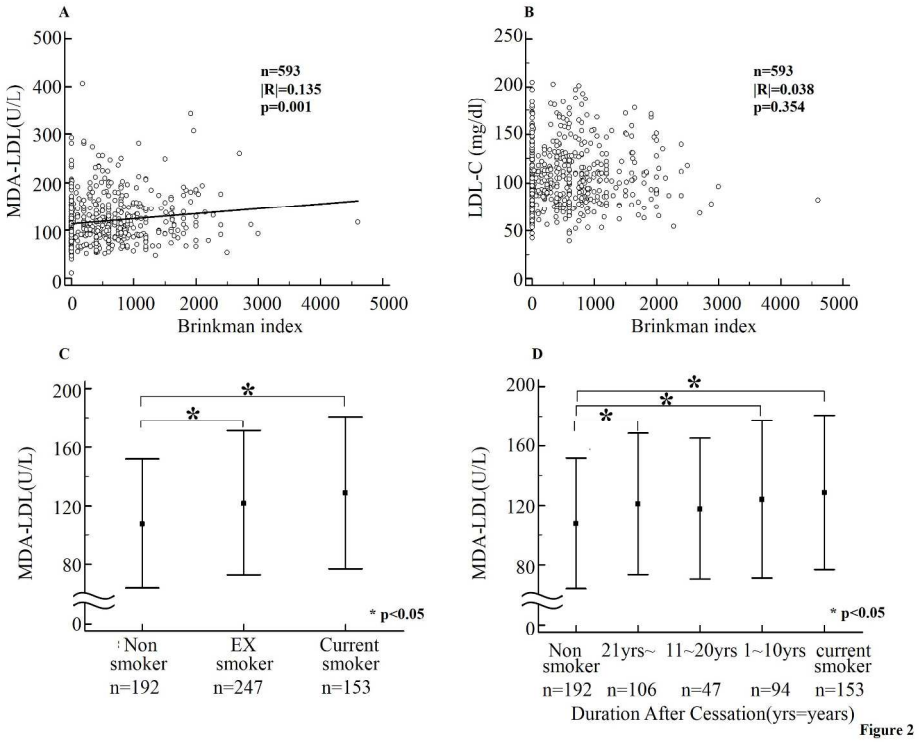

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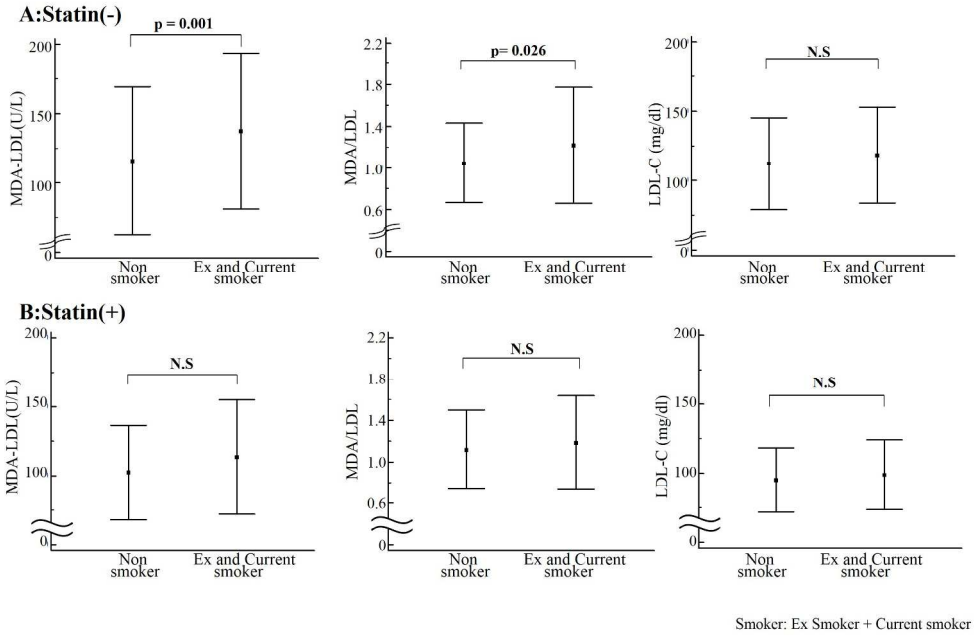

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Increase in the Oxidized Low-Density Lipoprotein Level by Smoking and the Possible Inhibitory Effect of Statin Therapy in Patients with Cardiovascular Disease: a Retrospective Study

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This author takes responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation

Keywords: MDA-LDL; Smoking; Oxidative stress; Coronary artery disease; Statin therapy

Word count: 2650

Abstract

Objectives:

MDA-LDL level is a marker of oxidative stress and is linked to progression of arteriosclerosis; however, the clinical factors affecting to the oxidized LDL level have not been elucidated. We herein investigated various factors to identify correlation with MDA-LDL level in high risk patients requiring catheter intervention.

Setting:

Secondary care (cardiology), single center study

Participants:

600 patients who were admitted to our hospital and underwent cardiac catheterization

Primary and secondary outcome measures:

Blood samples were obtained to measure lipid profiles and MDA-LDL level.

Results:

With regard to smoking status, MDA-LDL level was significantly higher in ex-/current smokers compared with non-smokers. Of note, there was no improvement of MDA-LDL level even in patients who quitted smoking. Multiple regression analysis showed that MDA-LDL level was positively correlated with LDL-cholesterol level, Brinkman index and male gender. The correlation between smoking status and either MDA-LDL or LDL-C level was investigated in

two groups; namely, patients, with or without statin treatment. In non-statin group, MDA-LDL level and MDA-LDL/LDL-C ratio were significantly higher in ex-/current smokers compared with non-smoker, while no significant correlation was observed between smoking status and LDL-C level. In contrast, in statin group, there were no significant correlations between smoking status and all any of these cholesterol parameters.

Conclusions:

We found that MDA-LDL level was affected by multiple factors, such as smoking status, LDL-C level and male gender. The present findings give additional evidence that smoking should be prohibited from MDA-LDL standpoint. Furthermore, statin therapy might have a beneficial effect on the reduction of MDA-LDL level.

Trial registration: N/A

Main strengths

Although oxidative LDL is associated with the marker of oxidative stress and the progression of atherosclerosis, clinical factor which affects the oxidative LDL remains uncertain. Our study revealed that MDA-LDL was associated with smoking and MDA-LDL level would never decrease with smoking cessation. However, MDA-LDL level was decreasing even in smokers with statin therapy.

Study limitations

Smoking cessation was not found to be effective for reducing MDA-LDL level in this study; however, favorable effects of smoking cessation would likely occur with regard to other parameters than MDA-LDL level. Thus smoking cessation is recommended at any time, even after long-term smoking, and is considered to provide cardiovascular health benefits.

This was a retrospective study, and the true effect of statin on MDA-LDL level remains uncertain. Finally, we did not examine the prognosis of the study population and therefore, the effect of smoking cessation and/or statin therapy remains uncertain, especially in terms of their impact on MDA-LDL level. Prospective studies should be required to obtain answers regarding their topics.

Introduction

The malondialdehyde modified low-density lipoprotein (MDA-LDL; oxidized LDL) is LDL that has been modified by MDA, leading to the production of a large amount of aldehyde when LDL becomes degenerated and oxidized [1].

It is known that MDA-LDL level is elevated in patients with dyslipidemia and diabetes mellitus (DM), both risks factors for atherosclerotic disease [2 3]. Since MDA-LDL level has a positive correlation with the serum LDL level, the ratio of MDA-LDL/LDL-C (M/L) is used to evaluate the severity of oxidization of LDL; in some reports, not only MDA-LDL level, but also M/L ratio has been shown to increase in patients with DM compared with controls [4 5].

In patients with coronary artery disease (CAD), MDA-LDL level and M/L ratio have been shown to increase even when there are no other differences in the other lipid profiles [6]. In addition, it has been shown that the measurement of MDA-LDL level might be useful as a predictor of restenosis after percutaneous coronary intervention in patients with DM [7]. Based on these findings, it has been speculated that MDA-LDL level might be important marker of the progression of arteriosclerosis; however, the clinical factors possibly affecting MDA-LDL level have not been elucidated. Therefore, in the present study, we investigated “the clinical factors” affecting MDA-LDL level in high risk patients requiring catheter intervention.

Methods

Study patients

Six hundred consecutive patients who underwent cardiac catheterization from March 2010 to September 2011 were examined in this study. The baseline patient characteristics, including the clinical parameters and the biochemical data, were collected retrospectively from the hospital medical records. In addition, the results of the catheterization (i.e. the number of occluded or narrowed vessels), body weight, body mass index (BMI), coronary risk factors and medication profiles were also investigated. The patients taking eicosapentaenoic acid (EPA) were excluded since it has been demonstrated that EPA is major lipid-lowering agents with potent antioxidant effects. This study was approved by the ethics committee of the Jikei University School of Medicine (Study protocol: 24-150[6916]); and we complied with the routine ethical regulation of our institution as follows. This is a retrospective study and the informed consent could not be obtained from each patient. Instead of informed consent from each patient, we publicly posted a notice about the study design and contact information at a publicly-known space in our institution.

Data collection

Blood sampling was performed to examine the serum MDA-LDL, serum creatinine,

hemoglobin A1c (HbA1c), B-type (brain) natriuretic peptide (BNP) and low-density lipoprotein cholesterol (LDL-C) levels. The previous study reported by Tismikas et al.[8] encompassed that PCI would affect the oxidative LDL level. To avoid the modification of LDL level by PCI, we tried to take a blood draw immediately before the cardiac catheterization or at the outpatient clinic before admission. MDA-LDL level was measured by an ELISA using an anti-MDA-LDL monoclonal antibody (ML25) and β-galactosidase anti-apoB monoclonal antibody (AB16) [1]. It is well known that the combination of ML25 and AB16 can accurately detect MDA-LDL [1]. The concentration of MDA-LDL is defined at 1 mg/L of MDA-LDL produced artificially, which shows the same signal as 1U/L of MDA-LDL in the serum. Serum levels of LDL cholesterol was determined enzymatically. (Sekisui Medical Co., Ltd., Tokyo, Japan)

Diabetes mellitus (DM), hypertension and smoking were defined as described previously [9 10]. Dyslipidemia was diagnosed with the use of lipid-lowering agents, the presence of 1 or more of the following 3 lipid disorders at first fasting blood sampling or both: a low-density lipoprotein (LDL) cholesterol level ≥140 mg/dL, a triglyceride level ≥150 mg/dL, and a high-density lipoprotein (HDL) cholesterol level <40 mg/dL[9]. Blood sampling was performed on the day of the catheter examination, except for the case that has already been performed at outpatient clinic. Among 600 patients, 342 were performed on the day of the catheter examination, and remaining 258 were at outpatient.

The definition of smoking status is as follows: current smokers were those who were smoking at the time of the study or who had smoked in the past year; the subjects who had quit smoking more than one year before the study were defined as ex-smokers and those who had never smoked were defined as non-smokers. Brinkman Index was used to evaluate the smoking status of current/ex-smokers [11].

Statistical analysis

Comparisons between MDA-LDL level and LDL-C level, age, BMI, HbA1c, Cr, BNP and Brinkman index were performed with a linear regression analysis. Comparison between Brinkman index and LDL-C was also performed with a linear regression analysis. Comparisons of MDA-LDL level between males and females, subjects with or without hypertension, non-smokers versus ex-smokers versus current smokers, and among the various patient groups after smoking cessation were performed with Mann-Whitney U test. Kruskal Wallis test was performed to evaluate the difference of MDA-LDL (C) and LDL-C (D) among 4 groups divided by Brinkman index. Multiple factors, which were considered to possibly modify MDA-LDL levels were evaluated with a stepwise multiple regression analysis. Comparison of LDL-C and MDA-LDL level and M/L ratio between non-smokers and smokers were performed with Mann-Whitney U test.

A value of $p<0.05$ was considered to be statistically significant for all data that were statistically analyzed using the SPSS software package, version 21.0 (SPSS Inc., Chicago, IL).

Results

Baseline characteristics

The baseline characteristics of the patients in this study were shown in Table 1. The average age was 64.8 ±11.4 years old and 80.3% of them were male. The percentages of non-smokers, ex-smokers and current smokers were 32%, 42% and 26%, respectively. The average LDL-C level and MDA-LDL level were 106.1 ± 30.8 mg/dl and 119.2 ± 48.7 U/L, respectively. The percentage of patients taking statin therapy was 55.3%. In addition, patient characteristics divided by smoking status into 3 groups (non-smokers, ex-smokers, current smokers) were shown in Table 1-3.

Table 1-1 Patient's Characteristics

N=600	mean ± SD
Age, years	64.8±11.4
Male, gender (%)	80.3
Height, cm	165.4±25.1
Weight, kg	66.8±13.3
BMI, kg/m^2	24.4±3.74
Non-smoker, N (%)	192(32.4)
Ex-smoker, N (%)	247(41.7)
Current smoker, N (%)	153(25.8)
Cr, mg/dL	1.38±1.94
eGFR, mL/min/1.73m^2	62.6±21.6
HbA1c, %	6.4±1.1
BNP, pg/mL	140±263
LDL-C, mg/dℓ	106.1±30.8
MDA-LDL,U/L	119.2±48.7
M/L	1.16±0.47

BMI: body mass index, Cr: creatinine, eGFR: estimated glomerular filtration rate, BNP: B-type natriuretic peptide, LDL-C: low-density lipoprotein cholesterol, MDA-LDL: malondialdehyde modified low density lipoprotein, M/L: malondialdehyde modified low density lipoprotein/ low density lipoprotein cholesterol

Table 1-2 Patient's characteristics

Disease	N (%)
Diabetes Mellitus	252(42.0)
Hypertension	455(75.5)
Dyslipidemia	441(73.5)
Medicine	N (%)
Statin	332(55.3)
Ezetimibe	31(5.2)
Fibrate	13(2.1)
Coronary Artery Disease	N (%)
0VD	215(35.8)
1VD	249(41.5)
2VD	84(14.0)
3VD	52(8.7)

0VD: 0-vessel disease, 1VD: single-vessel disease, 2VD: double-vessel disease, 3VD: triple-vessel disease

Table 1-3 Patient's characteristics divided by smoking status

	Non-smoker	Ex-smoker	Current smoker
Number of patients (%)	192(32.4)	250(42.2)	151(25.4)
Age	66.9±12.4	65.5±9.5	60.9±12.2
Male, gender (%)	110(57.3)	228(91.2) *	138(91.4)
Height, cm	160.3±11.0	166.0±11.8*	167.4±7.3
Weight, kg	62.7±14.0	67.7±10.9*	70.6±14.8
BMI, kg/m^2	24.2±3.6	24.3±3.0	25.0±4.9
Cr, mg/dL	1.2±1.5	1.6±2.1	1.3±2.1

HbA1c, %	6.3±0.9	6.4±1.1	6.4±1.1
BNP, pg/mL	167.1±302.0	133.2±264.8	120.7±201.1
LDL-C, mg/dℓ	102.3±29.1	104.2±28.4	114.8±35.2**
MDA-LDL, U/L	108.0±43.6	122.1±49.2*	129.3±52.1
M/L	2.06±0.85	2.26±0.82	2.6±0.99
LVEF, %	60.2±10.0	56.7±9.6*	54.4±12.1
Diabetes Mellitus	72(37.5)	112(44.8)	65(43.0) *
Hypertension	155(80.7)	191(76.4)	104(68.9) *
Dyslipidemia	134(69.8)	185(74.0)	116(76.8)
Statin	111(57.8)	146(58.4)	73(48.3)
Ezetimibe	10(3.6)	14(5.6)	7(4.6)
Fibrate	2(1.0)	6(2.4)	5(3.3)
Coronary Artery Disease			
0VD	82(42.7)	83(33.2)	49(32.5)
1VD	69(35.9)	105(42.0)	71(47.0) *
2VD	26(13.5)	34(42.0)	22(14.6)
3VD	15(7.8)	28(7.2)	9(6.0)

*: P<0.05 vs. Non-smoker, **: P<0.05 vs. Ex-smoker

Clinical factors affecting MDA-LDL level

To elucidate the determinants of MDA-LDL level, a simple regression analysis was performed. (*Fig. 1*) MDA-LDL level showed a significantly positive correlation with LDL-C level (*Fig. 1A*) and a negative correlation with age. (*Fig. 1B*) In addition, MDA-LDL level was

significantly higher in male as well as patient without hypertension. (*Figs. 1C, D*) BMI, HbA1c, Cr and BNP level had no impact on MDA-LDL level. (*Figs. 1E-H*)

Correlation of smoking status with MDA-LDL level

Next, the impact of smoking status on MDA-LDL level was performed. MDA-LDL, but not LDL-C, showed a significantly positive correlation with the smoking profiles indicated by Brinkman index. *(Fig. 2-1-A, B*) However, considering the bias of the Brinkman distribution, in addition to Fig. 2-1-A, B, MDA-LDL level and LDL-C level were analyzed with dividing by Brinkman index into 4 groups. There was a significant difference of MDA-LDL level between non-smoker and current-smoker, whereas there was no significant difference of LDL-C regardless of the smoking status. (Fig. 2-1C, D)

The patients were subsequently divided into three groups according to their smoking status: Non-, ex- and current smokers. MDA-LDL level was significantly higher in both ex- and current smokers compared to that in non-smokers. *(Fig. 2-2-E)* Moreover, when patients were compared based on the number of years after smoking cessation, not only current smoking group, but also group that had quit smoking within 10 years and that where the patients had quit smoking more than 21 years earlier showed higher MDA-LDL levels than did non-smoking group (*Fig. 2-2-F*).

Clinical factors affecting MDA-LDL level identified in multiple regression analysis

To assess the independent determinants of MDA-LDL level, a multiple regression analysis was performed. After removing the confounding factors, MDA-LDL level was shown to be positively correlated with LDL-C level ($p<0.001$), Brinkman index ($p=0.009$) and a male gender ($p=0.019$) (Table 2).

Table 2 Multiple Regression Analysis

Significant variable	Regression coefficients	Standard error	Standard regression coefficients	F	p
LDL-C	0.675	0.06	0.429	128.089	<0.001
Brinkman index	0.008	0.003	0.105	71.502	0.009
Gender	11.511	4.908	0.94	49.901	0.019

Objective variable: MDA-LDL

Explanatory variable: BMI, Age, Gender, brinkman index, Cr, BNP, LDL-C, HbA1c, HT

No significant variables: BMI, Age, Cr, BNP, HbA1c, HT

Effects of statin therapy on the correlation of smoking status with MDA-LDL or LDL-cholesterol level

The correlations of smoking status with MDA-LDL/LDL-C level was investigated after patients were divided into two groups; those with or without statin treatment (*Fig.3*). In

non-statin-treated group, MDA-LDL level as well as MDA-LDL/LDL-C ratio, was significantly increased in ex-/current smokers compared to those in non-smokers, although there was no significant difference in LDL-C level between the subjects with the different smoking status. LDL-C level was not significantly different in non-statin-treated and statin-treated groups.

Discussion

In this study, we investigated the factors associated with MDA-LDL level in high risk patients requiring cardiac catheterization. According to a multivariate analysis, Brinkman index, as well as the LDL-C level and gender were found to be significantly associated with MDA-LDL level. Furthermore, we found that smoking cessation was not effective for reducing MDA-LDL level, even after the patients had quit smoking for many years. However, we found evidence that statin treatment may reduce MDA-LDL level, which could possibly help in the treatment of smokers.

It has been reported that smoking may affect susceptibility of plasma LDL to peroxidative modification. Modified LDL has been shown to be the preferred substrate for macrophages and induces their subsequent transformation into foam cells [12 13]. Thus, oxidative stress is very important for synthesis of modified-LDL. However, it is noteworthy that MDA-LDL level was emphatically influenced by smoking but not by obesity (estimated by BMI), hypertension, diabetes mellitus (estimated by HbA1c level), renal failure (estimated by Cr level) or heart

failure (estimated by BNP level) by the multivariate analysis in this study, though all of these clinical characteristics have been shown to increase oxidative stress. The precise mechanism by which smoking increases MDA-LDL level remains unclear at present.

The current study clearly showed that smoking was substantially harmful with regard to increasing MDA-LDL level. When we examined the effect of smoking status on MDA-LDL level among non-smokers, ex-smokers and current smokers, we found that MDA-LDL level was still higher in ex-smokers than in non-smokers and was unexpectedly similar to the level in current smokers. Furthermore, we examined the effects of the period of smoking cessation and the analysis indicated that even many years after smoking cessation, there was no significant reduction of MDA-LDL level. This result suggests that smoking should never be started, and that if started, it is important to quit smoking as soon as possible before becoming a heavy smoker.

The present result suggests that smoking keeps MDA-LDL level elevated for a long time. Therefore, a method for lowering MDA-LDL level is especially needed for smokers. One possible candidate might be statin therapy. Statins facilitate the LDL uptake in hepatocytes, decrease old LDL (which is easily oxidized), and thereby reduce the risk of LDL oxidization [14]. The decreases in fatty acids and cholesterol in the lipoprotein are also likely to lead to a decrease in oxidization [15]. In the current study, we examined the effect of statin treatment on

MDA-LDL level between non-smokers and smokers. MDA-LDL level was found to be significantly higher in smokers than in non-smokers in stain (-) group. On the other hand, the effect of smoking on LDL-C level was not seen in statin (+) group, the level was similar between smokers and non-smokers. The ratio of MDA-LDL/LDL-C showed a similar result. These results may suggest that statin therapy would reduce MDA-LDL level in smokers to a level similar to that in non-smokers.

It has been reported that cigarette smoking is one of the risk factors for organic stenosis but that it does not act alone in contributing to the progression of atherosclerosis [15 16]. In addition atherosclerosis was not produced by smoking alone in animal models [17]. However, cigarette smoking acts in concert with other risk factors such as hypercholesterolemia to accelerate atherosclerosis[15 16 18 19 20]. The current study is in agreement with the previous reports. Importantly, it has been shown that among other risk factors cigarette smoking alone is a highly significant risk factor for coronary spasm [21 22 23 24 25] and treatment with fluvastatin reduced the coronary spasm [26]. Thus, smoking cessation combined with statin therapy would be beneficial for prevention of ischemic heart disease by reducing progression of atherosclerosis and suppressing coronary spasm.

In the present study, we performed multivariate analysis for determination of MDA-LDL by using the factors of LDL-C level, age, BMI, HbA1c, Cr, BNP and Brinkman index; however,

there may be other factors associated with MDA-LDL in a direct or indirect manner. As a matter of fact, the previous study by Matsuda et al. showed that MDA-LDL level was correlated with triglyceride, HDL-C, metformin and α-glucosidase inhibitors in statin-treated diabetes patients with CAD [27]. Then, we performed another multivariate analysis. As a result, it revealed that MDA-LDL level was correlated with LDL-C, triglyceride and smoking, however, not with HDL-C, anti-hypertensive drugs, anti-diabetes agents such as metformin and α-glucosidase inhibitors, number of the vessels with CAD and CRP. (Precise data not shown) Furthermore, there may be confounding factors among them; and the correlation among each factors were also investigated. As a result, there were only slight correlation between CAD and HDL-C (R=-0.163), between CAD and smoking (R=0.098), between triglyceride and HDL-C (R=0.203) and between triglyceride and smoking (R=-0.163) (Precise data not shown). The reason of the difference between the previous study and ours may be due to the different study population. In any case, it would be safe to say that smoking affected MDA-LDL level in a fairly direct manner.

Conclusion

We found that MDA-LDL level was affected by multiple factors such as smoking status (as indicated by Brinkman index), LDL-C level and gender. In addition to its other health effects, smoking should be strongly prohibited due to its harmful effect from a MDA-LDL standpoint.

We recommend that patients should never smoke, but that once smoking has started, it is essential to quit smoking as early as possible and to cut back on the number of cigarettes consumed. Furthermore, statin therapy might have a beneficial effect on the reduction of MDA-LDL level.

Footnotes

Contributors: Conceived and designed the experiments: KO TT KM MY. Performed the experiments: KO HS SA TN TO. Performed the statistical analysis: KO HS KM MY. Contributed reagents/materials/analysis tools: KO TT TN KM. Wrote the paper: KO TT KM MY.

Competing interests: None

Funding: None

Ethics approval: The study protocol (24-150[6916]) was approved by the ethics committee of the Jikei University School of Medicine.

Provenance and peer review: Not commissioned; externally peer reviewed

Data sharing: No additional data are available.

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Figure legends

Fig. 1 Correlations of MDA-LDL level with various clinical factors

The clinical factors affecting MDA-LDL level are shown in (A)-(H).

Fig. 2 Correlation of smoking status with MDA-LDL and LDL-cholesterol levels

Correlation between MDA-LDL level and Brinkman index (A) and, between LDL-C level and Brinkman index (B) was determined with a linear regression analysis. Kruskal Wallis test was performed to evaluate the difference of MDA-LDL (C) and LDL-C (D) among 4 groups divided by Brinkman index. Comparison of MDA-LDL levels among non-smokers, ex-smokers and current smokers (E). Comparison of MDA-LDL levels in each patient group among non-smokers and ex-smokers who had quit more than 21 years earlier, ex-smokers who had quit 11 to 20 years ago, ex-smokers who had quit less than 10 years ago and current smokers (F).

Fig. 3 Effects of the statin therapy on the correlations of smoking status with MDA-LDL or LDL-cholesterol level

The correlations of the smoking status with MDA-LDL and LDL-C levels were investigated after dividing the patients into two groups; with (A) or without (B) statin treatment.

Increase in the Oxidized Low-Density Lipoprotein Level by Smoking and the Possible Inhibitory Effect of Statin Therapy in Patients with Cardiovascular Disease.

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Keywords: MDA-LDL; sSmoking; oOxidative stress; cCoronary artery disease; sStatin therapy

Word count: 27692650

Abstract

Objectives:

MDA-LDL level is a marker of oxidative stress and is linked to progression of arteriosclerosis; however, the clinical factors affecting to the oxidized LDL level have not been elucidated. We herein investigated various factors to identify correlation with MDA-LDL level in high risk patients requiring catheter intervention.

Setting:

Secondary care (cardiology), single center study

Participants:

600 patients who were admitted to our hospital and underwent cardiac catheterization

Primary and secondary outcome measures:

Blood samples were obtained to measure lipid profiles and MDA-LDL level.

Results:

With regard to smoking status, MDA-LDL level was significantly higher in ex-/current smokers compared with non-smokers. Of note, there was no improvement of MDA-LDL level even in patients who quitted smoking. Multiple regression analysis showed that MDA-LDL level was positively correlated with LDL-cholesterol level, Brinkman index and male gender. The correlation between smoking status and either MDA-LDL or LDL-C level was investigated in

two groups; namely, patients, with or without statin treatment. In non-statin group, MDA-LDL level and MDA-LDL/LDL-C ratio were significantly higher in ex-/current smokers compared with non-smoker, while no significant correlation was observed between smoking status and LDL-C level. In contrast, in statin group, there were no significant correlations between smoking status and all any of these cholesterol parameters.

Conclusions:

We found that MDA-LDL level was affected by multiple factors, such as smoking status, LDL-C level and male gender. The present findings give additional evidence that smoking should be prohibited from MDA-LDL standpoint. Furthermore, statin therapy might have a beneficial effect on the reduction of MDA-LDL level.

Trial registration:

N/A

Main strengths

- Although oxidative LDL is associated with the marker of oxidative stress and the progression of atherosclerosis, clinical factor which affects the oxidative LDL remains uncertain. Our study revealed that MDA-LDL was associated with smoking and ~~the~~ MDA-LDL level would never decrease with smoking cessation. However, MDA-LDL level was decreasing even in smokers with statin therapy.

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Study limitations

Smoking cessation was not found to be effective for reducing MDA-LDL level in this study; however, favorable effects of smoking cessation would likely occur with regard to other parameters than MDA-LDL level. Thus smoking cessation is recommended at any time, even after long-term smoking, and is considered to provide cardiovascular health benefits.

This was a retrospective study, and the true effects of ~~a~~ statin on MDA-LDL level remains uncertain~~lear~~. Finally, we did not examine the prognosis of the study population and therefore, the effects of smoking cessation and/or statin therapy remainss uncertain~~lear~~, ~~-~~especially in terms of their impact on MDA-LDL level. Prospective studies should be~~are~~ required to obtain answers regarding their~~there~~ topics.

Introduction

The malondialdehyde modified low-density lipoprotein (MDA-LDL; oxidized LDL) is LDL that has been modified by MDA, leading to the production of a large amount of aldehyde when LDL becomes degenerated and oxidized [1].

It is known that MDA-LDL level is elevated in patients with dyslipidemia and diabetes mellitus (DM), both risks factors for atherosclerotic disease [2,3]. Since MDA-LDL level has a positive correlation with the serum LDL level, the ratio of MDA-LDL/LDL-C (M/L) is used to evaluate the severity of oxidization of LDL; in some reports, not only MDA-LDL level, but also M/L ratio has been shown to increase in patients with DM compared with controls [4,5].

In patients with coronary artery disease (CAD), MDA-LDL level and M/L ratio have been shown to increase even when there are no other differences in the other lipid profiles [6]. In addition, it has been shown that the measurement of MDA-LDL level might be useful as a predictor of restenosis after percutaneous coronary intervention in patients with DM [7]. Based on these findings, it has been speculated that MDA-LDL level might be important marker of the progression of arteriosclerosis; however, the clinical factors possibly affecting MDA-LDL level have not been elucidated. Therefore, in the present study, we investigated "the clinical factors" affecting MDA-LDL level in high risk patients requiring catheter intervention.

Methods

Study patients

The study protocol (24-150[6916]) was approved by the ethics committee of the Jikei University School of Medicine. Six hundred consecutive patients who underwent cardiac catheterization from March 2010 to September 2011 were examined in this study. The baseline patient characteristics, including the clinical parameters and the biochemical data, were collected retrospectively from the hospital medical records. In addition, the results of the catheterization (i.e. the number of occluded or narrowed vessels), body weight, body mass index (BMI), coronary risk factors and medication profiles were also investigated. The patients taking eicosapentaenoic acid (EPA) were excluded since it has been demonstrated that EPA is major lipid-lowering~~antihyperlipidemic~~ agents with potent antioxidant effects.

Data collection

Blood sampling was performed to examine the serum MDA-LDL, serum creatinine, hemoglobin A1c (HbA1c), B-type (brain) natriuretic peptide (BNP) and low-density lipoprotein cholesterol (LDL-C) levels. The previous study reported by Tismikas et al.[8] encompassed that PCI would affect the oxidative LDL level. To avoid the modification of LDL level by PCI, we tried to take a blood draw immediately before the cardiac catheterization or at the outpatient

clinic before admission. MDA-LDL level was measured by an ELISA using an anti-MDA-LDL monoclonal antibody (ML25) and β-galactosidase anti-apoB monoclonal antibody (AB16) [1]. It is well known that the combination of ML25 and AB16 can accurately detect MDA-LDL [1]. The concentration of MDA-LDL is defined at 1 mg/L of MDA-LDL produced artificially, which shows the same signal as 1U/L of MDA-LDL in the serum. Serum levels of LDL cholesterol were determined enzymatically. (Sekisui Medical Co., Ltd., Tokyo, Japan)

Diabetes mellitus (DM), hypertension, dyslipidemia and smoking were defined as described previously [9 10]. Dyslipidemia was diagnosed with the use of lipid-lowering agents, the presence of 1 or more of the following 3 lipid disorders at first fasting blood sampling or both: a low-density lipoprotein (LDL) cholesterol level ≥140 mg/dL, a triglyceride level ≥150 mg/dL, and a high-density lipoprotein (HDL) cholesterol level <40 mg/dL[9]. Blood sampling was performed on the day of the catheter examination, except for the case that has already been performed at outpatient clinic. Among 600 patients, 342 were performed on the day of the catheter examination, and remaining 258 were at outpatient.

The definition of smoking status iwas as follows: current smokers were those who were smoking at the time of the study or who had smoked in the past year; the subjects who had quit smoking more than one year before the study were defined as ex-smokers and those who had never smoked were defined as non-smokers. Brinkman Index was used to evaluate the smoking

status of current/ex-smokers [11].

Statistical analysis

Comparisons between MDA-LDL level and LDL-C level, age, BMI, HbA1c, Cr, BNP and Brinkman index were performed with a linear regression analysis. Comparison between Brinkman index and LDL-C was also performed with a linear regression analysis. Comparisons of MDA-LDL levels between males and females, subjects with or without hypertension, non-smokers versus ex-smokers versus current smokers, and among the various patient groups after smoking cessation were performed with Mann-Whitney U test. Kruskal Wallis test was performed to evaluate the difference of MDA-LDL (C) and LDL-C (D) among 4 groups divided by Brinkman index. Multiple factors, which were considered to possibly modify MDA-LDL levels were evaluated with a stepwise multiple regression analysis. Comparisons of LDL-C and MDA-LDL level and M/L ratio between non-smokers and smokers were performed with Mann-Whitney U test.

A value of $p < 0.05$ was considered to be statistically significant for all data that were statistically analyzed using the SPSS software package, version 21.0 (SPSS Inc., Chicago, IL). ~~Statistical significance was defined as a value of $p<0.05$.~~

Results

Baseline characteristics

The baseline characteristics of the patients in this study were~~are~~ shown in Table 1. The average age was 64.8 ± 11.4 years old and 80.3% of them were male. The percentages of non-smokers, ex-smokers and current smokers were 32%, 42% and 26%, respectively. The average LDL-C level and MDA-LDL level were~~as~~ 106.1 ± 30.8 mg/dl and 119.2 ± 48.7 U/L, respectively. The percentage of patients taking statin therapy was 55.3%. In addition, patient characteristics divided by smoking status into 3 groups (non-smokers, ex-smokers, current smokers) were shown in Table 1-3.

Table 1-1 Patient's Characteristics

N ~~n~~= 600	mean ± SD
Age, years	64.8 ± 11.4
Male, gender (%)	80.3
Height, cm	165.4 ± 25.1
Weight, kg	66.8 ± 13.3
BMI, kg/m^2	24.4 ± 3.74
Non-smoker, N ~~n~~(%)	192 (32.4)
Ex-smoker, N ~~n~~(%)	247 (41.7)
Current smoker, N ~~n~~(%)	153 (25.8)
Cr, mg/dL	1.38 ± 1.94
eGFR, mL/min/1.73m^2	62.6 ± 21.6
HbA1c, %	6.4 ± 1.1
BNP, pg/mL	140 ± 263
LDL-C, mg/dℓ	106.1 ± 30.8
MDA-LDL, U/L	119.2 ± 48.7
M/L	1.16 ± 0.47

BMI: body mass index Cr: creatinine, eGFR: estimated glomerular filtration rate, BNP: B-type natriuretic peptide, LDL-C: low-density lipoprotein cholesterol, MDA-LDL: m~~M~~alondialdehyde modified low density lipoprotein, M/L: m~~M~~alondialdehyde modified low density lipoprotein/ low density lipoprotein cholesterol.

Table 1-2 Patient's Characteristics

Disease	N ~~n~~(%)
Diabetes Mellitus	252 (42.0)
Hypertension	455 (75.5)
Dyslipidemia	441 (73.5)
Medicine	N ~~n~~(%)
S~~s~~tatin	332 (55.3)
Ezetimibe	31 (5.2)
F~~f~~ibrate	13 (2.1)
Coronary Artery Disease	N ~~n~~(%)
0VD	215 (35.8)
1VD	249 (41.5)
2VD	84 (14.0)
3VD	52 (8.7)

0VD: 0-vessel disease, 1VD: single-vessel disease, 2VD: double-vessel disease, 3VD: triple-vessel disease

Table 1-3 Patient's characteristics divided by smoking status

	Non-smoker	Ex-smoker	Current smoker
Number of patients (%)	192 (32.4)	250 (42.2)	151 (25.4)
Age	66.9 ± 12.4	65.5 ± 9.5	60.9 ± 12.2
Male, gender (%)	110 (57.3)	228 (91.2) *	138 (91.4)

Height, cm	160.3 ± 11.0	166.0 ± 11.8*	167.4 ± 7.3
Weight, kg	62.7 ± 14.0	67.7 ± 10.9*	70.6 ± 14.8
BMI, kg/m^2	24.2 ± 3.6	24.3 ± 3.0	25.0 ± 4.9
Cr, mg/dL	1.2 ± 1.5	1.6 ± 2.1	1.3 ± 2.1
HbA1c, %	6.3 ± 0.9	6.4 ± 1.1	6.4 ± 1.1
BNP, pg/mL	167.1 ± 302.0	133.2 ± 264.8	120.7 ± 201.1
LDL-C, mg/dℓ	102.3 ± 29.1	104.2 ± 28.4	114.8 ± 35.2**
MDA-LDL, U/L	108.0 ± 43.6	122.1 ± 49.2*	129.3 ± 52.1
M/L	2.06 ± 0.85	2.26 ± 0.82	2.6 ± 0.99
LVEF, %	60.2 ± 10.0	56.7 ± 9.6*	54.4 ± 12.1
Diabetes Mellitus	72 (37.5)	112 (44.8)	65 (43.0) *
Hypertension	155 (80.7)	191 (76.4)	104 (68.9) *
Dyslipidemia	134 (69.8)	185 (74.0)	116 (76.8)
Statin	111 (57.8)	146 (58.4)	73 (48.3)
Ezetimibe	10 (3.6)	14 (5.6)	7 (4.6)
Fibrate	2 (1.0)	6 (2.4)	5 (3.3)
Coronary Artery Disease			
0VD	82 (42.7)	83 (33.2)	49 (32.5)
1VD	69(35.9)	105(42.0)	71(47.0) *
2VD	26 (13.5)	34 (13.6)	22 (14.6)
3VD	15 (7.8)	28 (7.2)	9 (6.0)
~~—~~	~~Non-smoker~~	~~Ex-smoker~~	~~Current smoker~~
~~Number of patients (%)~~	~~192 (32.4)~~	~~250 (42.2)~~	~~151 (25.4)~~

Age	66.9 ± 12.4	65.5 ± 9.5	60.9 ± 12.2
Male, gender (%)	110 (57.3)	228 (91.2) *	138 (91.4)
Height, cm	160.3 ± 11.0	166.0 ± 11.8*	167.4 ± 7.3
Weight, kg	62.7 ± 14.0	67.7 ± 10.9*	70.6 ± 14.8
BMI, kg/m^2	24.2 ± 3.6	24.3 ± 3.0	25.0 ± 4.9
Cr, mg/dL	1.2 ± 1.5	1.6 ± 2.1	1.3 ± 2.1
HbA1c, %	6.3 ± 0.9	6.4 ± 1.1	6.4 ± 1.1
BNP, pg/mL	167.1 ± 302.0	133.2 ± 264.8	120.7 ± 201.1
LDL-C, mg/dℓ	102.3 ± 29.1	104.2 ± 28.4	114.8 ± 35.2**
MDA-LDL, U/L	108.0 ± 43.6	122.1 ± 49.2*	129.3 ± 52.1
M/L	2.06 ± 0.85	2.26 ± 0.82	2.6 ± 0.99
LVEF, %	60.2 ± 10.0	56.7 ± 9.6*	54.4 ± 12.1
Diabetes Mellitus	72 (37.5)	112 (44.8)	65 (43.0) *
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Dyslipidemia	134 (69.8)	185 (74.0)	116 (76.8)
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~~Coronary Artery Disease~~			
~~0VD~~	~~82 (42.7)~~	~~83 (33.2)~~	~~49 (32.5)~~
~~1VD~~	~~69(35.9)~~	~~105(42.0)~~	~~71(47.0) *~~
~~2VD~~	~~26 (13.5)~~	~~34 (42.0)~~	~~22 (14.6)~~
~~3VD~~	~~15 (7.8)~~	~~28 (7.2)~~	~~9 (6.0)~~

*: P <0.05 vs. Non-smoker, **: P <0.05 vs. Ex-smoker

Clinical factors affecting MDA-LDL level

To elucidate the determinants of MDA-LDL level, ~~we first performed~~ a simple regression analysis was performed~~.~~ (*Fig. 1*)~~.~~ MDA-LDL level showed a significantly positive correlation with LDL-C level (*Fig. 1A*) and a negative correlation with age (*Fig. 1B*). In addition, MDA-LDL level~~s~~ was~~ere~~ significantly higher in male~~s~~ as well as ~~the~~ patient~~s~~ without hypertension (*Figs. 1C, D*). BMI, HbA1c, Cr and BNP level had no impact on MDA-LDL level (*Figs. 1E-H*).

Correlation of smoking status with MDA-LDL level

NWe next, examined the impact of smoking status on MDA-LDL level was performed. MDA-LDL, but not LDL-C, showed a significantly positive correlation with the smoking profiles indicated by Brinkman index *(Fig. 2-1-A, B)*. However, considering the bias of the Brinkman distribution, in addition to Fig. 2-1-A, B, MDA-LDL level and LDL-C level were analyzed with dividing by Brinkman index into 4 groups. There was a significant difference of MDA-LDL level between non-smoker and current-smoker, whereas there was no significant difference of LDL-C regardless of the smoking status *(Fig. 2-1C, D)*.

The patients were subsequently divided into three groups according to their smoking status: Non-, ex- and current smokers. MDA-LDL level was significantly higher in both ex- and current smokers compared to that in non-smokers *(Fig. 2-C2-E)*. Moreover, when patients were compared based on the number of years after smoking cessation, not only the current smoking group, but also the group that had quit smoking within 10 years and that where the patients had quit smoking more than 21 years earlier showed higher MDA-LDL levels than did the non-smoking group (*Fig. 2-2-FD*).

~~This suggests that MDA-LDL level will never completely recover once a subject has started smoking.~~

Clinical factors affecting MDA-LDL level identified in multiple regression analysis

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To assess the independent determinants of MDA-LDL level, a multiple regression analysis was performed. After removing the confounding factors, MDA-LDL level was shown to be positively correlated with LDL-C level ($p<0.001$), Brinkman index ($p=0.009$) and a male gender ($p=0.019$) (Table 2).

Table 2 Multiple Regression Analysis

Significant variable	Regression coefficients	Standard error	Standard regression coefficients	F	p
LDL-C	0.675	0.06	0.429	128.089	<0.001
Brinkman index	0.008	0.003	0.105	71.502	0.009
Gender	11.511	4.908	0.94	49.901	0.019

Objective variable: MDA-LDL
Explanatory variable: BMI, Age, Gender, brinkman index, Cr, BNP, LDL-C, HbA1c, HT
No significant variables: BMI, Age, Cr, BNP, HbA1c, HT

Effects of statin therapy on the correlations of smoking status with MDA-LDL or LDL-cholesterol level

The correlations of ~~the~~ smoking status with MDA-LDL/LDL-C level wasere investigated after patients were divided into two groups; those with or without statin treatment (*Fig.–3*). In non-statin-treated group, MDA-LDL level as well as MDA-LDL/LDL-C ratio, was significantly increased in ex-/current smokers compared to those in non-smokers, although there was no

significant differences in LDL-C levels between the subjects with the different smoking status. LDL-C level was not significantly different in non-statin-treated and statin-treated groups.

Discussion

In this study, we investigated the factors associated with MDA-LDL level in high risk patients requiring cardiac catheterization. According to a multivariate analysis, Brinkman index, as well as the LDL-C level and gender were found to be significantly associated with MDA-LDL level. Furthermore, we found that smoking cessation was not effective for reducing MDA-LDL level, even after the patients had quit smoking for many years. However, we found evidence that statin treatment may reduce MDA-LDL level, which could possibly help in the treatment of smokers.

It has been reported that smoking may affect susceptibility of plasma LDL to peroxidative modification. Modified LDL has been shown to be the preferred substrate for macrophages and induces their subsequent transformation into foam cells [12 13]. Thus, oxidative stress is very important for synthesis of modified-LDL. However, it is noteworthy that MDA-LDL level was emphatically influenced by smoking but not by obesity (estimated by BMI), hypertension, diabetes mellitus (estimated by HbA1c level), renal failure (estimated by Cr level) or heart failure (estimated by BNP level) by the multivariate analysis in this study, though all of these

clinical characteristics have been shown to increase oxidative stress. The precise mechanisms by which smoking increases MDA-LDL level remains unclear at present.

The current study clearly showed that smoking was substantially harmful with regard to increasing MDA-LDL level. When we examined the effect of smoking status on MDA-LDL level among ~~the~~ non-smokers, ex-smokers and current smokers, we found that MDA-LDL level was still higher in ~~the~~ ex-smokers than in ~~the~~ non-smokers and was unexpectedly similar to the level in ~~the~~ current smokers. Furthermore, we examined the effects of the period of smoking cessation and the analysis indicated that even many years after smoking cessation, there was no significant reduction of MDA-LDL level. This result suggests that smoking should never be started, and that if started, it is important to quit smoking as soon as possible before becoming a heavy smoker~~ss~~.

The present result~~s~~ suggests that smoking keeps MDA-LDL level elevated for a long time. Therefore, a method for lowering MDA-LDL level is especially needed for smokers. One possible candidate might~~would~~ be statin therapy. Statins facilitate the LDL uptake in hepatocytes, decrease old LDL (which is easily oxidized), and thereby reduce the risk of LDL oxidization [14]. The decreases in fatty acids and cholesterol in the lipoprotein are also likely to lead to a decrease in oxidization [15]. In the current study, we examined the effect~~s~~ of statin treatment on MDA-LDL level~~s~~ between non-smokers and smokers. MDA-LDL level was found

to be significantly higher in smokers than in non-smokers in the stain (-) group. On the other hand, the effect of smoking on LDL-C level was not seen in the statin (+) group, the levels was~~ere~~ similar between smokers and non-smokers. The ratio of MDA-LDL/LDL-C showed a similar result. These results may suggest that statin therapy would~~ean~~ reduce~~s~~ MDA-LDL level in smokers to a level similar to that in non-smokers.

It has been reported that cigarette smoking is one of the risk factors for organic stenosis but that it does not act alone in contributing to the progression of atherosclerosis [15 16]. In addition, atherosclerosis was not produced by smoking alone in animal models [17]. However, cigarette smoking acts in concert with other risk factors such as hypercholesterolemia to accelerate atherosclerosis[15 16 18 19 20]. The current study is in agreement with the previous reports. Importantly, it has been shown that among other risk factors cigarette smoking alone is a highly significant risk factor for coronary spasm [21 22 23 24 25] and treatment with fluvastatin reduced the coronary spasm [26]. Thus, smoking cessation combined with statin therapy would be beneficial for prevention of ischemic heart disease by reducing progression of atherosclerosis and suppressing coronary spasm.

In the present study, we performed multivariate analysis for determination of MDA-LDL by using the factors of LDL-C level, age, BMI, HbA1c, Cr, BNP and Brinkman index; however, there may be other factors associated with MDA-LDL in a direct or indirect manner. As a matter

of fact, the previous study by Matsuda et al. showed that MDA-LDL level was correlated with triglyceride, HDL-C, metformin and α-glucosidase inhibitors in statin-treated diabetes patients with CAD [27].

Then, we performed another multivariate analysis. As a result, it revealed that MDA-LDL level was correlated with LDL-C, triglyceride and smoking, however, not with HDL-C, anti-hypertensive drugs, anti-diabetes agents such as metformin and α-glucosidase inhibitors, number of the vessels with CAD and CRP. (Precise data not shown) Furthermore, there may be confounding factors among them; and the correlation among each factors were also investigated. As a result, there were only slight correlation between CAD and HDL-C (R=-0.163), between CAD and smoking (R=0.098), between triglyceride and HDL-C (R=0.203) and between triglyceride and smoking (R=-0.163) (Precise data not shown). The reason of the difference between the previous study and ours may be due to the different study population. In any case, it would be safe to say that smoking affected MDA-LDL level in a fairly direct manner.

Conclusion

We found that MDA-LDL level was affected by multiple factors such as smoking status (as indicated by Brinkman index), LDL-C level and gender. In addition to its other health effects, smoking should be strongly prohibited due to its harmful effects from a MDA-LDL standpoint.

We recommend that patients should never smoke, but that once smoking has started, it is essential to quit smoking as early as possible and to cut back on the number of cigarettes consumed. Furthermore, statin therapy might have a beneficial effect on the reduction of MDA-LDL level.

Footnotes

Contributors: Conceived and designed the experiments: KO TT KM MY. Performed the experiments: KO HS SA TN TO. Performed the statistical analysis: KO HS KM MY. Contributed reagents/materials/analysis tools: KO TT TN KM. Wrote the paper: KO TT KM MY.

Competing interests: None

Funding: None

Ethics approval: The study protocol (24-150[6916]) was approved by the ethics committee of the Jikei University School of Medicine.

Provenance and peer review: Not commissioned; externally peer reviewed

Data sharing: No additional data are available.

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Figure legends

Fig. 1 Correlations of MDA-LDL level with various clinical factors

The clinical factors affecting MDA-LDL level are shown in (A)-(H).

Fig. 2 Correlation of smoking status with MDA-LDL and LDL-cholesterol levels

Correlation between MDA-LDL level and Brinkman index (A) and, between LDL-C level and Brinkman index (B) was determined with a linear regression analysis.

Kruskal Wallis test was performed to evaluate the difference of MDA-LDL (C) and LDL-C (D) among 4 groups divided by Brinkman index. Comparison of MDA-LDL levels among non-smokers, ex-smokers and current smokers (E). Comparison of MDA-LDL levels in each patient group among non-smokers and ex-smokers who had quit more than 21 years earlier, ex-smokers who had quit 11 to 20 years ago, ex-smokers who had quit less than 10 years ago and current smokers (F).

~~Comparison of MDA-LDL levels among non-smokers, ex-smokers and current smokers (C).~~

~~Comparison of MDA-LDL levels in each patient group among non-smokers and ex-smokers who had quit more than 21 years earlier, ex-smokers who had quit 11 to 20 years ago, ex-smokers who had quit less than 10 years ago and current smokers (D).~~

Fig. 3 Effects of the statin therapy on the correlations of smoking status with MDA-LDL or

LDL-cholesterol level

The correlations of the smoking status with MDA-LDL and LDL-C levels were investigated after dividing the patients into two groups; with (A) or without (B) statin treatment.

Table 2 Multiple Regression Analysis

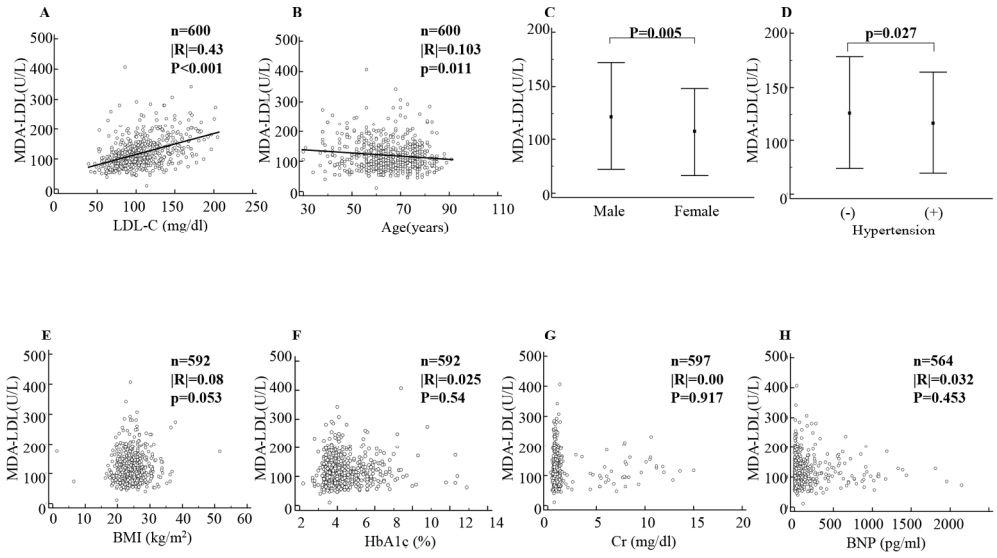

Figure 1

173x130mm (300 x 300 DPI)

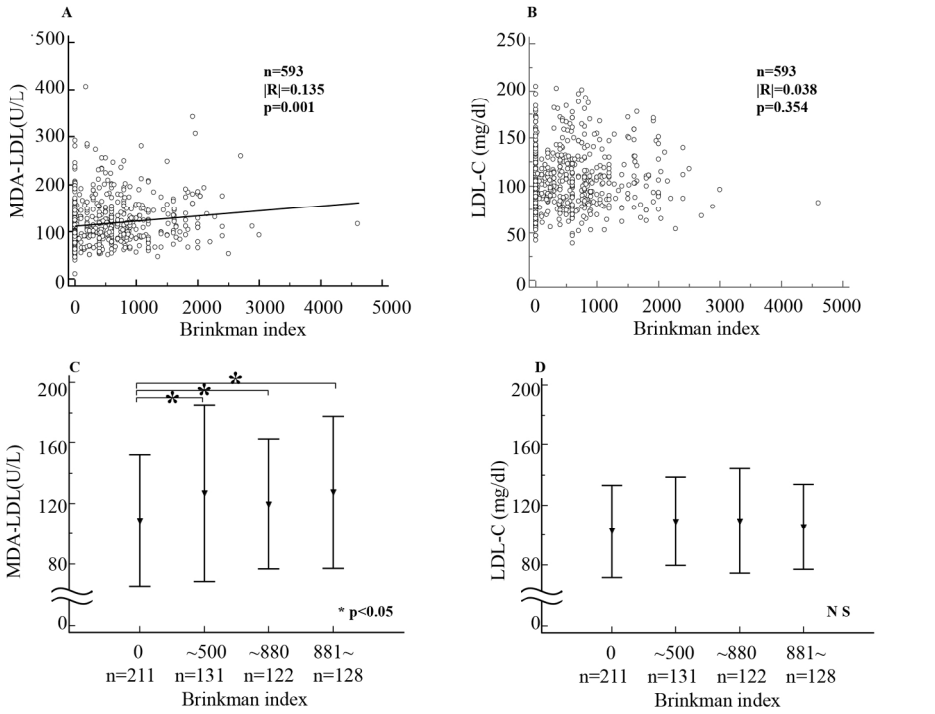

Figure 2-1

173x130mm (300 x 300 DPI)

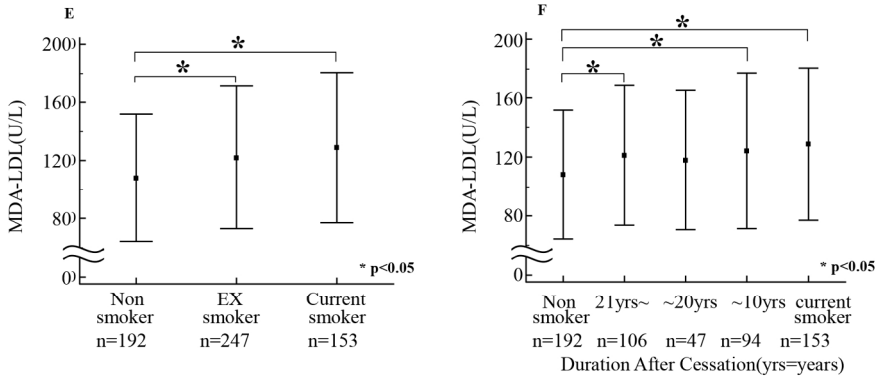

Figure 2-2

173x130mm (300 x 300 DPI)

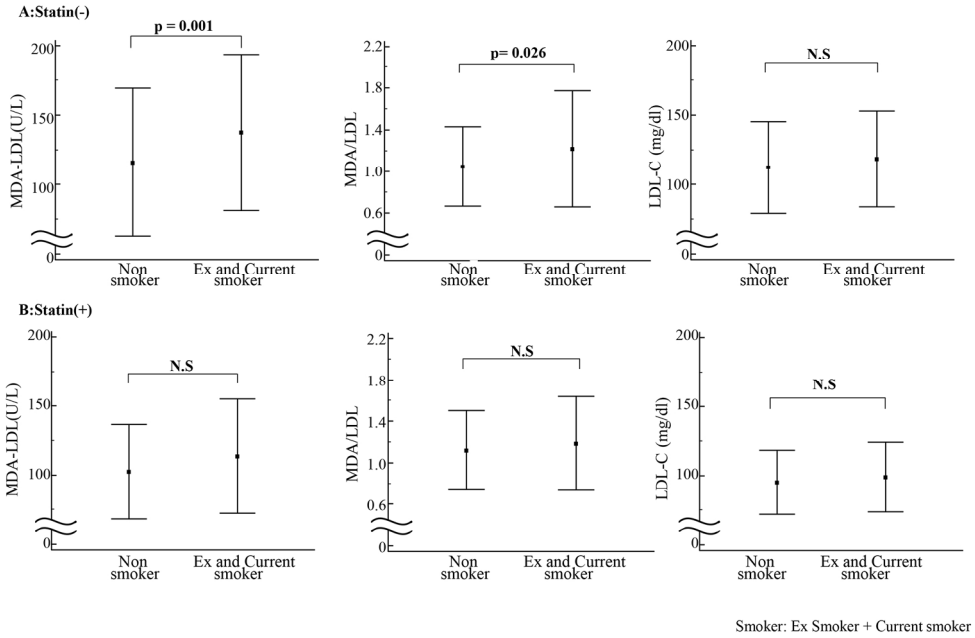

Figure 3

173x130mm (300 x 300 DPI)