PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Increase in the Oxidized Low-Density Lipoprotein Level by Smoking and the Possible Inhibitory Effect of Statin Therapy in Patients with Cardiovascular Disease.
AUTHORS	Ogawa, Kazuo; Tanaka, Toshikazu; Nagoshi, Tomohisa; Sekiyama, HIroshi; Arase, Satoshi; Minai, Kosuke; Ogawa, Takayuki; Yoshimrua, Michihiro

VERSION 1 - REVIEW

REVIEWER	Morihiro Matsuda Institution for Clinical Reserch, National Hospital Organization Kure Medical Center and Chugoku Cancer Center, Japan
REVIEW RETURNED	30-Aug-2014

GENERAL COMMENTS	The authors have shown that MDA-LDL level is elevated by cigarette smoking independently of LDL cholesterol level, and might be reduced by statin therapy in patients with cardiovascular disease. These data may provide interesting insight for the generation and reduction of oxidized LDL. However, there are several issues on the data presentation and analyses in this paper. 1) The authors should add other risk factors that may affect the MDA-LDL levels, including serum levels of triglycerides, HDL cholesterol and C-reactive protein and the uses of various anti-diabetic and anti-hypertensive medicines, and include them into the analyses to minimize the bias and avoid misleading the conclusion because this is an observational study but not an intervention study. 2) 2) Several confounding factors, such as age, sex, and the presence of coronary artery disease (CAD), should affect the smoking habit and MDA-LDL levels. In particular, the presence of CAD may be associated with the use of statin. When the authors compare MDA-LDL level among the subjects with varied smoking status, they should present their different background and statistically adjust for these variables to reduce the bias. 3) There should be several confounding factors associated with statin use, which may affect MDA-LDL level. The authors should present these data and statistically analyze the association between statin use and smoking-mediated elevation of MDA-LDL level independently of other factors. 4) As Brinkman index is not normally distributed, the linear regression is not suitable. Other statistical method may be

	better for assessing the relationship between Brinkman index and MDA-LDL levels. 5) The authors should describe the definition of dyslipidemia and the way to determine LDL cholesterol level in the methods.

REVIEWER	Amit Segev Chaim Sheba Medical Center Israel
REVIEW RETURNED	16-Oct-2014

GENERAL COMMENTS	The manuscript by Ogawa et al shows the correlation of smoking status with MDA-LDL levels, predictors of MDA-LDL levels and what is relatively unique is that statins positively affect MDA-LDL levels in smokers and ex-smokers. The main drawback of this paper that it lacks outcome data. What was the timing of blood sampling? If after PCI, the intervention itself might change MDA-LDL levels as compared to baseline levels. Please discuss 2 important papers on the effects of PCI on phospholipids levels and the correlation with restenosis – Tismikas S et al Circulation 2004 and Segev A et al Am Heart Journal 2005 Results page 12 line 23 – please avoid interpretation in the results section and discuss it in the Discussion Did you correlate MDA-LDL levels with the extent of CAD – 1-3 vessel, please include it in the regression analysis. Please provide details on any intervention performed – PCI, CABG I have a general suggestion on how to improve the manuscript – since the main topic is smoking I would present baseline characteristics and MDA levels according to 3 groups – current smokers, ex-smokers, and non-smokers. i.e. Table 1 and 2 Why only 55% of this patients with proven CAD received statins?

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

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

1) The authors should add other risk factors that may affect the MDA-LDL levels, including serum levels of triglycerides, HDL cholesterol and C-reactive protein and the uses of various anti-diabetic and anti-hypertensive medicines, and include them into the analyses to minimize the bias and avoid misleading the conclusion because this is an observational study but not an intervention study.

Thank you very much for your constructive comments. We understand that we need further analysis using other possible important factors affecting the MDA-LDL levels. In the former manuscript, we did statistical analysis for determination of MDA-LDL levels by using possible major contributing factors including LDL-C, Brinkman Index, BMI, age, gender, Cr, BP, HbA1c and HT; and we found that LDL-C and Brinkman index were significantly correlated with MDA-LDL levels. We suppose that these factors would be mutually independent and valid; and therefore we would like to keep it unchanged in the revised manuscript. However, your comments are appropriate and important; and we agree with that more precise analysis should be done.

Here, we have done another multivariate analysis to examine other possible contributing factors for

MDA-LDL by using triglyceride, HDL-C, the number of the vessels of coronary disease, CRP and use of statin, αGI, metformin, ACE, ARB and CCB. When selecting the factors, we respectfully referred to the former study presented by Dr, Matsuda (Reference No. 27). The additional multivariate analysis was simplified as follows. A high MDA-LDL level was defined by a median value of >109U/L; and we did multiple logistic regression analysis. As a result, while smoking, triglyceride and LDL-C affected the MDA-LDL level; however, HDL-C, anti-hypertensive drugs, oral anti-diabetic agents, number of the vessels of coronary artery disease and CRP did not. Because of your important suggestions, we here confirmed that the factors affecting MDA-LDL levels were smoking status, LDL-C (and TG).

	Odds ratio	SE	p value	Exp (B)	95% CI
Smoking	0.648	0.277	0.020	1.911	1.109-3.291
Statin	-0.209	0.277	0.451	0.812	0.472-1.396
αGI	-0.086	0.532	0.872	0.918	0.324-2.604
Metformin	0.368	0.441	0.404	1.445	0.609-3.431
TG	0.003	0.001	0.009	1.003	1.001-1.006
HDL	-0.011	0.009	0.223	0.989	0.971-1.007
LDL	0.033	0.005	0.000	1.034	1.023-1.044
Number of disease vessels	0.008	0.143	0.958	1.008	0.761-1.334
ACE	0.573	0.367	0.118	1.774	0.865-3.638
ARB	-0.016	0.291	0.955	0.984	0.556-1.741
CCB	-0.286	0.273	0.295	0.751	0.440-1.284
BB	-0.242	0.271	0.373	0.785	0.462-1.336
CRP	0.119	0.146	0.413	1.127	0.846-1.501

SE:Standard error, αGI: alpha-glucosidase inhibitor, ACE: Angiotensin-converting enzyme inhibitor, ARB: AngiotensinII Receptor Blocker, CCB: Calcium channel blocker, BB: β-blocker

These new data would be important; however, we afraid that the results would become complex. We thus hope to only include the comments in the discussion session as follows.

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 andα-glucosidase inhibitors in statin-treated diabetes patients with CAD (Reference. No 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).

2) Several confounding factors, such as age, sex, and the presence of coronary artery disease (CAD), should affect the smoking habit and MDA-LDL levels. In particular, the presence of CAD may be associated with the use of statin. When the authors compare MDA-LDL level among the subjects with varied smoking status, they should present their different background and statistically adjust for these variables to reduce the bias.

Thank you for your comment. You probably anticipated that the confounding factors would affect the present results. In particular, your concern is about that the presence of coronary artery disease (CAD) may be associated with the use of statin. Therefore, we further analyzed the coefficient of correlation regarding confounding factors related with CAD and statin. As shown in the following Table, there were several significant associations; however, there was no association between the presence of the number of the vessels of coronary disease (0, 1, 2 and 3) and use of statin.

Pearson coefficient of correlation

	CAD	smoking	statin	metformin	αGI	TG	HDL
CAD	1	0.098*	-0.021	-0.004	0.033	0.026	-0.163**
smoking	0.098*	1	-0.034	-0.067	0.060	0.122**	-0.160**
statin	-0.021	-0.034	1	0.100*	0.032	-0.017	0.002
Metformin	-0.004	-0.067	0.100*	1	0.097*	0.067	-0.022
αGI	0.033	0.060	0.032	0.097*	1	0.034	-0.042
TG	0.026	0.122**	-0.017	0.067	0.034	1	-0.203**
HDL	-0.163**	-0.160**	0.002	-0.022	-0.042	-0.203**	1

*. significant difference with <0.05 **. significant difference with <0.01
αGI: alpha-glucosidase inhibitor

The new data would be important; however, we also afraid that the results would become complex. We thus hope to only include the comments in the discussion session as follows.

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=-0.122) (Precise data not shown).

3) There should be several confounding factors associated with statin use, which may affect MDA-LDL level. The authors should present these data and statistically analyze the association between statin use and smoking-mediated elevation of MDA-LDL level independently of other factors.

As shown in our reply to your question (2), there would be no correlation between statin use and smoking-elevated of MDA-LDL level.

4) As Brinkman index is not normally distributed, the linear regression is not suitable. Other statistical method may be better for assessing the relationship between Brinkman index and MDA-LDL levels.

Thank you for an important suggestion; and we agree with your comment.
Since we could not analyze Brinkman index with logarithmic conversion due to the inclusion of “0”, we had a quaternary analysis by dividing into 4 groups shown as below. As a results, we found that there was no significant difference regarding LDL-C level regardless of the smoking status although there was significant difference regarding MDA-LDL level between non-smoking and smoking. These new figures were added to the previous ones in the revise manuscript.

5) The authors should describe the definition of dyslipidemia and the way to determine LDL cholesterol level in the methods.

Thanks for your suggestion.
We put the sentence as below which described the definition of dyslipidemia and the way to determine LDL-C level in our study.

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

Serum level of LDL cholesterol were determined enzymatically (Sekisui Medical Co., Ltd., Tokyo, Japan).

Reviewer: 2
Title: Increase in the Oxidized Low-Density Lipoprotein Level by Smoking and the Possible Inhibitory Effect of Statin Therapy in Patients with Cardiovascular Disease.

1) The main drawback of this paper that it lacks outcome data. What was the timing of blood sampling? If after PCI, the intervention itself might change MDA-LDL levels as compared to baseline levels. Please discuss 2 important papers on the effects of PCI on phospholipids levels and the correlation with restenosis – Tismikas S et al Circulation 2004 and Segev A et al Am Heart Journal 2005

Thank you for your critical and thoughtful suggestion regarding the fact that MDA-LDL level might be affected after the coronary intervention.

We would like to put the comment in regard to the timing of blood sampling in the revised manuscript. Basically we used the blood data taken on the day of the catheter exam, however, we used the data taken in outpatient clinic before coronary intervention in case patients who had already been taken in outpatient. We also would like to add the comment in the data collection section of our manuscript. Among 600 patients, we used 342 samples on the day of the catheter exam and rest of 258 samples in the clinic.

2) Results page 12 line 23 – please avoid interpretation in the results section and discuss it in the Discussion

Thanks for your suggestion.
We removed the sentence as below in our manuscript.
This suggests that MDA-LDL level will never completely recover once a subject has started smoking.

3) Did you correlate MDA-LDL levels with the extent of CAD – 1-3 vessel, please include it in the regression analysis.

Thank you for your considerate suggestion. We received the same comment from the other reviewer. Based on your suggestion, we performed logistic regression analyses. A high MDA-LDL level was defined by a median value of >109U/L. While smoking, triglyceride and LDL-C would affect to the MDA-LDL level, however, HDL-C, anti-hypertensive drugs, oral anti-diabetic agents, number of the vessels of coronary artery disease and C-reactive protein would not.

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4) Please provide details on any intervention performed – PCI, CABG

The number of the patients with PCI is 287 and that with CABG is 31.

5) I have a general suggestion on how to improve the manuscript – since the main topic is smoking I would present baseline characteristics and MDA levels according to 3 groups – current smokers, ex-smokers, and non-smokers. i.e.

We put the baseline characteristics based on the smoking status in our manuscript

6) Why only 55% of this patients with proven CAD received statins?

Thanks for your suggestion. These were the patients before the coronary angiogram who were suspected to have a coronary artery disease (CAD). Therefore, once they were proven to have significant CAD, they had a statin treatment afterwards.

VERSION 2 – REVIEW

REVIEWER	Amit Segev Chaim Sheba medical Centre Israel
REVIEW RETURNED	17-Nov-2014

GENERAL COMMENTS	The authors adequately addressed the comments raised by the 2 reviewers

REVIEWER	Morihiro Matsuda National Hospital Organization Kure Medical Center, Japan
REVIEW RETURNED	05-Dec-2014

GENERAL COMMENTS	The authors additionally performed deeper analyses as this reviewer had requested. The manuscript is well-revised.