The Association of Lipoprotein(a) and Intraplaque Neovascularization in Patients With Carotid Stenosis: A Retrospective Study

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Research Article

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The association of lipoprotein(a) and intraplaque neovascularization in patients with carotid stenosis: a retrospective study

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Abstract

Background: Lipoprotein(a) is increasingly recognised as a major risk factor for atherothrombotic cardiovascular disease. We examined whether plasma lipoprotein(a) concentrations were associated with intraplaque neovascularization (IPN) levels in patients with carotid stenosis and in terms of increasing plaque susceptibility to haemorrhage and rupture.

Methods: We included 85 patients diagnosed with carotid stenosis as confirmed using carotid ultrasound who were treated at Guangdong General Hospital. The IPN level was determined using contrast-enhanced ultrasound through the movement of the microbubbles. Univariate and multivariate binary logistic regression analyses were used to determine whether lipoprotein(a) affected IPN levels, and whether lipoprotein(a), cholesterol, triglyceride, and low-density lipoprotein cholesterol affected IPN levels, respectively.

Results: Lipoprotein(a) was a significant predictor of higher IPN levels in binary logistic regression before adjusting for other risk factors (P = 0.031, odds ratio [OR]: 1.238, 95% confidence interval [CI]: 1.020,1.503) whereas cholesterol, triglyceride, and low-density lipoprotein cholesterol were not predicators of IPN in univariate analysis. After adjusting for other risk factors, including age, diabetes mellitus, and smoking status, lipoprotein(a) remained statistically significant in predicting IPN (P = 0.012, OR: 1.335, 95% CI: 1.065,1.674).

Conclusions: Plasma lipoprotein(a) concentrations were found to be independently associated with higher IPN levels in patients with carotid stenosis, but not cholesterol,

triglyceride, or low-density lipoprotein cholesterol. Lowering plasma lipoprotein(a) levels may decrease the risk of cardiovascular and cerebrovascular events.

Keywords: contrast-enhanced ultrasound, lipoprotein(a), intraplaque neovascularization, carotid stenosis, cardiovascular events

Background

Due to recent medical developments, many carotid artery wall imaging methods are available to diagnose and assess carotid stenosis, such as ultrasound, computed tomography, magnetic resonance imaging, and even catheter-based angiography [1]. However, few methods can detect intraplaque neovascularization (IPN), which increases the susceptibility to haemorrhage and rupture of the plaque [2] that are also expensive and inconvenient to deal with in terms of repeated examinations. Contrast-enhanced ultrasound (CEUS) can reliably detect IPN [3] through identifying the movement of the contrast microbubbles within the plaque [4].

Lipoprotein(a) (Lp(a)), which is formed from an apolipoprotein (apo) B-100 covalently linked to apo(a), is a low-density lipoprotein (LDL)-like protein and increasingly recognised as a major risk factor for atherothrombotic cardiovascular disease (CVD) [5]. Klein JH and his colleagues found that Lp(a) was able to independently predict carotid stenosis and occlusion, but not in terms of carotid plaque area, suggesting that atherogenesis and cardiovascular risk caused by Lp(a) is

based on thrombosis and impaired fibrinolysis [6]. It appears there may be an interesting link between Lp(a) and IPN in that Lp(a) may accelerate IPN formation and affect plaque stability.

This research aimed to study the association between IPN and plasma Lp(a) concentrations and analyse the role of Lp(a) in relation to the carotid artery in patients with carotid stenosis using CEUS.

Methods

Study design and population

This retrospective study enrolled 85 consecutive patients who were diagnosed with carotid stenosis using carotid artery ultrasonography in Guangdong General Hospital, China, from January 2017 to January 2020. The inclusion criteria were as follows: (1) carotid stenosis confirmed using carotid artery ultrasonography, (2) absence of clinical contraindications for CEUS, and (3) ≥18 years of age. The exclusion criteria were: (1) having undergone previous carotid endarterectomy, (2) no outcome data concerning Lp(a), and (3) declining to be involved initially or in follow-up. All patients underwent CEUS after being diagnosed with carotid stenosis using carotid artery ultrasonography. Clinical histories, along with demographic and clinical data, were recorded for all patients at admission. All patients provided written informed consent. This retrospective investigation was approved by the local institutional review board as well as performed in accordance with the Declaration of Helsinki.

Laboratory Measurements

All fasting venous blood samples were collected during hospital admission before undergoing CEUS. Serum Lp(a) levels were measured through a murine monoclonal antibody (E022-1-1, Bioroyee, Beijing, China) involving latex turbidimetric method. Cholesterol (CHOL), triglyceride (TRIG), and low-density lipoprotein cholesterol (LDLC) levels were determined by chemiluminescence method using an auto-analyser.

CEUS examinations of the carotid artery

Carotid CEUS examinations were performed by a researcher who is blinded to the patients' histories and characteristics, using a GE Vivid E95 or Philips IU elite diasonograph contrast model and a high-frequency superficial probe. CEUS was performed with an ultrasound contrast agent, SonoVue. An initial bolus injection of 1.6 ml of contrast agent was quickly administered into the median cubital vein in 2 to 3 seconds, immediately followed by 3 mL of 0.9% normal saline solution at the same speed. Ultrasound cine-loops were then recorded over 15 to 30 seconds. The images at 3 s before and 5 min after contrast agent was introduced into the carotid artery lumen were stored for real-time dynamic analysis. IPN levels were determined using CEUS scores as follows: 0, no visible microbubbles in the plaque; 1, minimal microbubbles restricted to adventitial side or shoulder of the plaque; or 2, microbubbles spread all over the plaque [7].

Statistical analysis

All descriptive data consistent with normal distribution are expressed as mean value ± standard deviation, with the rest expressed as median (interquartile spacing).

Discrete data are presented as frequencies and percentages. A Student's t-test was used to evaluate continuous variables showing a normal distribution, and a Mann–Whitney U-test was used to evaluate variables that did not show a normal distribution. We first stratified participants into one of two groups based on their CEUS score, that is, a CEUS score on both sides that added up to greater than or equal to 2 points was used to define an IPN group whereas a CEUS score on both sides that added up to fewer than 2 points was used to define a no IPN group. Univariate and multivariate binary logistic regression analyses were then used to determine the association between Lp(a) and IPN levels, as well as between CHOL, TRIG, and LDLC, and IPN levels, respectively. Other variables assessed included age, smoking history, and diabetes mellitus, where P-values were less than 0.2. All analyses were performed with SPSS version 22.0 for Windows, and a two-sided P-value of less than 0.05 was considered to indicate statistical significance.

Results

Participant Characteristics

The characteristics of the participants are shown in Table 1. All 85 consecutive patients, that we had complete data for the binary logistic regression analysis, were seen in Guangdong General Hospital from January 2017 to January 2020. More than 70% of the participants were male, with 92.9% of the participants receiving statin therapy. Approximately 20% were smokers, with a similar proportion suffering from diabetes mellitus and cerebral infarction. Slightly fewer than half of the participants had coronary heart disease, and 70% had hypertension. (Table 1)

Table 2 shows that Lp(a) was a significant predictor of IPN in binary logistics regression before adjusting for the other risk factors (P = 0.031, odds ratio [OR]: 1.238, 95% confidence interval [CI]: 1.020, 1.503) whereas CHOL, TRIG, and LDLC were not predictors of IPN. After adjusting for other risk factors, including age, diabetes mellitus, and smoking status, Lp(a) remained statistically significant in predicting IPN (P = 0.012, OR: 1.335, 95% CI: 1.065,1.674) (see Fig. 1).

Discussion

Our study showed an association between plasma Lp(a) concentrations and IPN of the carotid artery in patients with carotid stenosis, independent of conventional risk factors, such as age, diabetes mellitus, and smoking status. A higher plasma Lp(a) concentration was found to be significantly related to a higher risk of IPN, while other lipid parameters, such as CHOL, TRIG, and LDLC, were not found to be significant in this regard.

Plaque instability and progression are largely related to extensive IPN, which adds plaque susceptibility to rupture or haemorrhage [8]. Lp(a) has been associated with carotid stenosis and plaque stability [5]. Our study found that plasma Lp(a) was linked to IPN levels, suggesting that a higher plasma Lp(a) concentration may accelerate IPN formation and affect plaque stability, leading to cardiovascular and cerebrovascular events [4,9,10].

Furthermore, Johri Amer et al. reported that in those patients with severe coronary lesions (whose coronary artery stenosis ≥70%), IPN level of the carotid

artery was associated with coronary lesion degree and complexity [11]. A recent study has demonstrated that carotid plaque neovascularization could predict significant and complex coronary artery disease (CAD) and future cardiovascular events after investigating carotid IPN in 459 stable angina patients referred for coronary angiography [4]. When those results are considered alongside the findings of this study, it seems that Lp(a) accelerates not only carotid IPN formation, but also coronary artery plaque, which corresponds with previous studies showing that plaque instability frequently co-exists at multiple vascular bed [8,12,13]. In our study, when we classified patients into two groups in terms of whether the CEUS scores on both sides of the carotid IPN added up to at least 2 points or not, we found that 83.8% of participants in the ≥2 points group were affected on both sides, indicating that most of the patients had plaque instability at multiple sites.

Our results showed that IPN was not associated with other lipid parameters, including CHOL, TRIG, and LDLC, which seemed inconsistent with conventional understanding. On the one hand, almost all these patients were being treated with statins that would reduce plasma lipids while not influencing the Lp(a) level [14]. On the other hand, Lp(a) has a stronger likelihood of causing atherogenesis than LDLC, because Lp(a) is not only structured similarly to LDLC but also consists of apo(a), which can also facilitate the development of atherogenesis [14]. Hence, Lp(a) may be more sensitive to predict IPN than LDLC. For these reasons, our study could not assess the association between CHOL, TRIG, and LDLC, and IPN. Further large cohort studies are needed to determine whether Lp(a) is more sensitive than other

lipid parameters, especially LDLC (an independent risk factor for CVD) in predicting IPN as well as cardiovascular and cerebrovascular diseases before statin treatment.

In addition, our results support that lower Lp(a) would be worthy of attention to prevent cardiovascular and cerebrovascular events, especially given that statins, the most used lipid-lowering drugs, cannot reduce Lp(a) levels. Proprotein convertase subtilisin/Kexin type 9 (PCSK9) inhibitors have been confirmed to lower Lp(a) and should be considered as an independent treatment after acute coronary syndrome [15,16]. Clinical trials, however, have shown that Lp(a) levels have only been reduced by 20% to 30% [17–19]. Other traditional Lp(a)-lowering approaches, such as the use of niacin, mipomerson, lomitapide, and so on, have been showed that the limited and non-specific effect to lower Lp(a) with intolerable side effects, invasive procedures, and high expense [20]. However, the apo(a) ASO IONIS-Apo(a)-LRX has recently been shown to significantly reduce Lp(a) levels in phase 2 clinical trials with good tolerance, and may become a promising drug for the management of elevated Lp(a) in the future [21]. To date, large scale randomized controlled trials have yet to be conducted to determine the precise cardiovascular benefits of lowering Lp(a) and further research is needed.

Limitations

This study had several limitations. First, this was an observational study, with limited possibilities to draw causal inferences. Second, it was a single-centre study, consisting of only 85 patients with carotid stenosis, and all the patients were diagnosed and treated at Guangdong General Hospital. Therefore, our study findings

cannot be readily generalized, and future studies with populations of different ethnicities and comprising multiple centres are recommended. Third, our study did not take genetic variants into consideration, although plasma Lp(a) levels are mainly determined by genetic factors while are not significantly reduced through lifestyle interventions. Therefore, further studies using genetic approaches are warranted. Finally, follow-up data were not collected for cardiovascular and cerebrovascular disease events. However, the relationship between Lp(a) levels and prognosis with carotid stenosis deserves further study.

Conclusions

Plasma Lp(a) concentrations were found to be independently associated with IPN in patients with carotid stenosis. As the concentration of Lp(a) increases, the risk of IPN increases. Lowering plasma Lp(a) levels may decrease the risks of cardiovascular and cerebrovascular events through slowing down IPN formation, as assessed using CEUS. Large prospective studies assessing the utility of Lp(a) to predict IPN in the clinical setting are required. Randomised clinical trials are needed to test whether substantial reductions in Lp(a) concentrations using the various treatments identified, most notably, the apo(a) ASO IONIS-Apo(a)-LRX, may facilitate improved management of individuals with high Lp(a) levels. Furthermore, IPN is also associated with plaque stability, so plasma Lp(a) concentrations may be a predictor of plaque stability. In other words, controlling Lp(a) may help protect plaque from rupturing.

Availability of data and materials

The datasets generated and analysed during the current study are not publicly available due to the privacy protection and a number of researches on Lp(a) may be continued, but are available from the corresponding author (e-mail:gdghllw@163.com) on reasonable request.

List of abbreviations

Lp(a): lipoprotein(a); CVD: cardiovascular disease; IPN: intraplaque neovascularization; CEUS: contrast-enhanced ultrasound; CHOL: cholesterol; TRIG: triglyceride; LDLC: low-density lipoprotein cholesterol; LDL: low-density lipoprotein; apo: apolipoprotein; OR: odds ratios; CAD: coronary artery disease

Declarations

Ethics approval and consent to participate

This retrospective investigation was approved by the Research Ethics Committee of Guangdong General Hospital, Guangdong Academy of Medical Sciences. [No.GDREC2017172h].Each participant provides written informed consent to collect all data before the study and was anonymized before analyses.

Consent for publication

Not applicable.

Availability of data and materials

The processed data required to reproduce these findings cannot be shared at this time as the data also form part of an ongoing study.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Dr. Shuang Xia contributed to the conception of the study. Dr. Weida Qiu performed the data analyses and wrote the manuscript. Professor Liwen Li provided valuable guidance at every stage in the writing of this thesis. Dr. Bo Kong performed the measurements on contrast-enhanced ultrasound. Dr. Lan Xu and Dr. Zejia Wu entered and edited much of the data used in these analyses.

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Not applicable.

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Figures

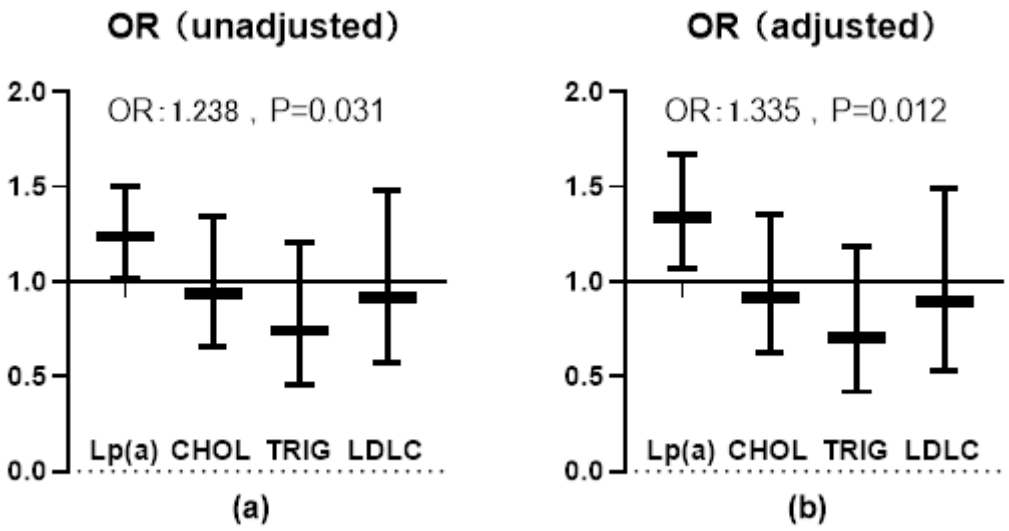

Figure 1

Lipoprotein(a) unadjusted and adjusted odds ratios (ORs) in relation to various risk factors Lipoprotein(a), (Lp(a)); cholesterol (CHOL); triglyceride, (TRIG), and low-density lipoprotein cholesterol (LDLC) unadjusted (1a) and adjusted (1b) for age, sex, hypertension, diabetes, smoking status, coronary heart disease, and cerebral infarction.