RESEARCH ARTICLE

The Correlation between Lipoprotein-Associated Phospholipase A2 and Atherosclerosis (ox-LDL) in Centrally Obese Men

Priscilla Dian Ari[1,2]*, Ellis Susanti[2,4], Ilham Jaya Patellongi[3],

[1]Prodia Clinical Laboratory, Jl. A. Yani, Kompleks Cenderawasih Trade Center, Blok A-6, Samarinda, Indonesia
[2]Post Graduate Program in Clinical Biochemistry, Hasanuddin University, Jl. Perintis Kemerdekaan Km.10. Makassar, Indonesia
[3]Faculty of Medicine, Hasanuddin University, Jl. Perintis Kemerdekaan Km.10. Makassar, Indonesia
[4]Prodia Clinical Laboratory, Jl. Kramat Raya 150, Jakarta, Indonesia
*Correspondence: priscilla.dian@prodia.co.id

Abstract

BACKGROUND: Obesity is closely associated with atherosclerosis. Obesity and atherosclerosis are closely associated with inflammatory disease. Atherosclerosis constitutes a multifactorial disorder affecting the arterial wall, which is initiated by dyslipidemia and exacerbated by inflammation. Plasma levels of lipoprotein-associated phospholipase A_2 (Lp-PLA_2) and oxidized low density lipoprotein (ox-LDL) have been identified as risk factors for cardiovascular disease. Lp-PLA_2 is the sole enzyme responsible for the hydrolysis of oxidized phospholipids (oxPL) on LDL particles in atherosclerosis plaque. Plasma level of oxLDL is associated with inflammation and plays an important role in the development of atherosclerosis. The aim of this study was to assess the correlation between Lp-PLA_2 and atherosclerosis (oxLDL) in centrally obese men.

METHODS: This was a cross-sectional study involving 71 men with central obesity with waist circumference > 90 cm, aged 30-60 years old. Lp-PLA_2 measurement was done by sandwich enzyme immunoassay. oxLDL measurement was done by ELISA method.

Abstrak

LATAR BELAKANG: Obesitas terkait erat dengan aterosklerosis. Obesitas dan aterosklerosis terkait erat dengan inflamasi. Aterosklerosis merupakan gangguan multifaktorial yang mempengaruhi dinding arterial, yang diinisiasi oleh dislipidemia dan diperkuat oleh adanya inflamasi. *Lipoprotein-associated phospholipase* A_2 (Lp-PLA_2) dan *oxidized low density lipoprotein* (ox-LDL) telah diidentifikasi merupakan faktor risiko kardiovaskular. Lp-PLA_2 merupakan enzim yang terlibat dalam hidrolisis oxidized phospholipids (oxPL) pada partikel LDL dalam plak aterosklerosis. Konsentrasi oxLDL terkait dengan inflamasi dan berperan penting dalam perkembangan aterosklerosis. Studi ini bertujuan untuk menilai korelasi antara Lp-PLA_2 dan aterosklerosis (oxLDL) pada pria obesitas sentral.

METODA: Studi ini menggunakan metoda *cross-sectional* yang melibatkan 71 pria dengan obesitas sentral yang ditandai dengan lingkar perut/*waist circumference* (WC) > 90 cm, berusia 30-60 tahun. Konsentrasi Lp-PLA2 ditentukan menggunakan metoda *sandwich enzyme immunoassay*. Penentuan konsentrasi oxLDL dilakukan menggunakan metoda ELISA.

RESULTS: Results of this study showed that central obesity correlated positively with oxLDL (r = 0.258*; p = 0.040) and Lp-PLA_2 > 422 ng/mL correlated positively with oxLDL (r = 0.331*; p = 0.042).

CONCLUSIONS: We conclude that there is a correlation of Lp-PLA_2 with atherosclerosis (oxLDL) in men with central obesity.

KEYWORDS: obesity, Lp-PLA_2, oxLDL, atherosclerosis

Indones Biomed J 2012; 4 (2): 101–106

RESULTS: Hasil studi menunjukkan bahwa obesitas sentral berhubungan positif dengan oxLDL (r = 0,258*; p = 0,040) dan Lp-PLA_2 > 422 ng/mL berkorelasi positif dengan oxLDL (r = 0,331*; p = 0,042).

KESIMPULAN: Hasil studi ini menyimpulkan bahwa terdapat korelasi antara Lp-PLA_2 dengan aterosklerosis (oxLDL) pada pria dengan obesitas sentral.

KATA KUNCI: obesitas, Lp-PLA_2, oxLDL, aterosklerosis

Indones Biomed J 2012; 4 (2): 101–106

Introduction

One of the most important recent developments in the study on obesity is the emergence of the concept that obesity is characterized by chronic mild inflammation – paralleling the situation with other diseases. The basis for this view is that the circulating level of several cytokines and acute phase proteins associated with inflammation is increased in obese individuals (1). Previous studies have demonstrated that enlargement of adipocytes is associated with substantial changes in metabolic functions, *e.g.* in lipid metabolism (2). It has been hypothesized that such alteration may contribute to the health-related risks of obesity. Obesity is linked to a variety of metabolic disorders such as insulin resistance and atherosclerosis. Abnormal regulation of fat production– derived secretory factors, adipocytokines, is partly responsible for obesity – linked metabolic disorders (3).

Lipoprotein-associated phospholipase A_2 (Lp-PLA^2), also known as platelet-activating factor acetylhydrolase (PAF-AH), is a phospholipase A_2 enzyme. In the blood it travels mainly with small-dense low density lipoprotein (sdLDL). It is an enzyme produced by inflammatory cells and hydrolyzes oxidized phospholipids in LDL. Lp-PLA^2 is involved in the development of atherosclerosis. Hydrolysis of phospholipids that is associated with lipoprotein particles by the phospholipase A_2 family of enzymes leads to modulation of lipoprotein particle phospholipid content and size as well as production of both pro- and antiinflammatory intermediates, all of which may serve as a basis for involvement of these enzymes in the development of atherosclerosis. In human atherosclerotic lesions, two main sources of Lp-PLA_2 can be identified, including that which is brought into the intima bound to LDL (from the circulation), and that which is synthesized *de novo* by plaque inflammatory cells (macrophages, T cells, mast cells). It is used as a marker for cardiac disease. A meta-analysis involving a total of 79.036 participants in 32 prospective studies showed that Lp-PLA_2 levels are positively correlated with increased risk of developing coronary heart disease and stroke (4). Lipid-lowering agents, particularly statins, lower Lp-PLA_2 mass and activity; therefore, Lp-PLA_2 may represent an important target of lipid-lowering therapy for reducing the inflammatory nature of atherosclerosis and plaque vulnerability (16). Lp-PLA_2 results are categorized into three progressive levels of cardiovascular risk: low < 310 ng/mL; moderate 310-422 ng/mL; high > 422 ng/mL (17).

The mechanisms underlying the pathogenesis of atherosclerosis are complex and affected by interaction among several biological pathways, including those of inflammation, metabolic disorder, and oxidative stress (5). The process of inflammation has been shown pathohistologically in advanced atherosclerotic plaques. Inflammation is mediated by the increasing quantities of proinflammatory cytokines. An early event in the progression of the disease is accumulation of LDL that may become oxidized. LDL can be oxidized enzymatically. With further oxidization, recognition by scavenger receptors occurs, leading to foam cell formation (6).

Oxidative modification of LDL is regarded as a key step in the formation of atherosclerosis. OxLDL involved in atherosclerogenic steps such as endothelial dysfunction, migration of macrophage and smooth muscle

cells, and release of inflammatory cytokines, induces oxidative stress and incorporated with macrophage transformation into foam cells and atherosclerotic plaque formation (7). Growing evidence indicates a relationship between circulating oxLDL and pathogenic processes of cardiovascular disease. Thus, circulating oxLDL has been established as a biomarker of atherosclerosis (8). The presence of circulating antibodies against oxLDL suggests its availability as an antigen outside the vascular system. Cholesterol crystals, detected not only in necrotic cores but also in the subendothelial areas in early atherosclerotic settings, trigger inflammasome activation, leading to interleukin-1β secretion. Their possible source may be the circulation (6).

Methods

SUBJECTS

This study was observational with a cross-sectional design done on a group of centrally obese men (n=71). The subjects' ages were between 30-60 years; they had central obesity with waist circumference ≥ 90 cm. Before commencement of the study, all subjects signed an informed consent. None of the subjects had acute inflammation (hsCRP ≥ 10 mg/L), kidney disorder, and nor consuming anti-inflammatory drug. All subjects underwent standardized interviews and physical and laboratory examinations.

ASSAY OF BIOCHEMICAL MARKERS

The plasma levels of Lp-PLA_2 were measured using two highly specific monoclonal antibodies for direct measurement of concentration. The PLAC™ Test Regent Kit (diaDexus Inc. manufacturer, South San Fransisco), a turbidimetric immunoassay, was used. oxLDL in EDTA plasma/serum/plasma heparin (oxLDL) was measured by sandwich /capture ELISA based on the mouse monoclonal antibody 4E6 (Mercodia, Uppsala, Sweden).

STATISTICAL ANALYSIS

Statistical analysis was done by SPSS for Windows v 17 with a level at $p < 0.05$. The correlations between biomarkers were assessed by Pearson or Spearman's rho correlation test.

Results

Table 1 provides information on the general and biochemical characteristics of the study subjects. In general, the population characteristics of the subjects were in compliance with the inclusion and exclusion criteria. On each subject's characteristics we did normal distribution test (Kolmogorov Smirnov Test).

Table 1. General Characteristics of Subjects

Variable	Min	Max	Mean ± SD	p
Age (year)	30.00	60.00	43.90 ± 8.69	0.165
WC (cm)	90.00	125.00	97.45 ± 7.26	0.016
hsCRP (mg/L)	0.29	8.53	1.84 ± 1.59	0.935
Creatinine (mg/dL)	0.60	1.20	0.90 ± 0.12	0.855
eGFR (ng/mL)	64.00	132.00	91.00 ± 5.15	0.029
Lp-PLA2 (ng/mL)	18.00	682.00	423.60 ± 4.35	0.082
oxLDL (U/L)	53.00	245.00	98.70 ± 2.70	0.271

WC = Waist Circumference, hsCRP = high sensitivity C – Reactive Protein; eGFR = estimate glomerolus filtration rate.

Pearson or Spearman's correlations test was performed to assess correlation between parameters. The result showed positive correlation of oxLDL with central obesity based on waist circumference 90-110 cm (p=0.040), hsCRP with oxLDL also showed linear correlation (p=0.047). This suggests that an increased waist circumference in inflammatory conditions contribute to the pathogenesis of atherosclerosis (Table 2).

The statistical analyses in this study showed a potential correlation of increased Lp-PLA2 > 422 ng/mL with atherosclerosis (oxLDL) in the group of centrally obese men. This finding indicated that high concentration of Lp-PLA2 contributes to the pathogenesis of atherosclerosis. This result is presented in a scatter graph, as shown in Figure 1.

Table 2. Correlation between WC, hs-CRP with oxLDL in Centrally Obese Men

Variable	oxLDL	
	r	p
WC	0.258*	0.040
hsCRP	0.248*	0.047

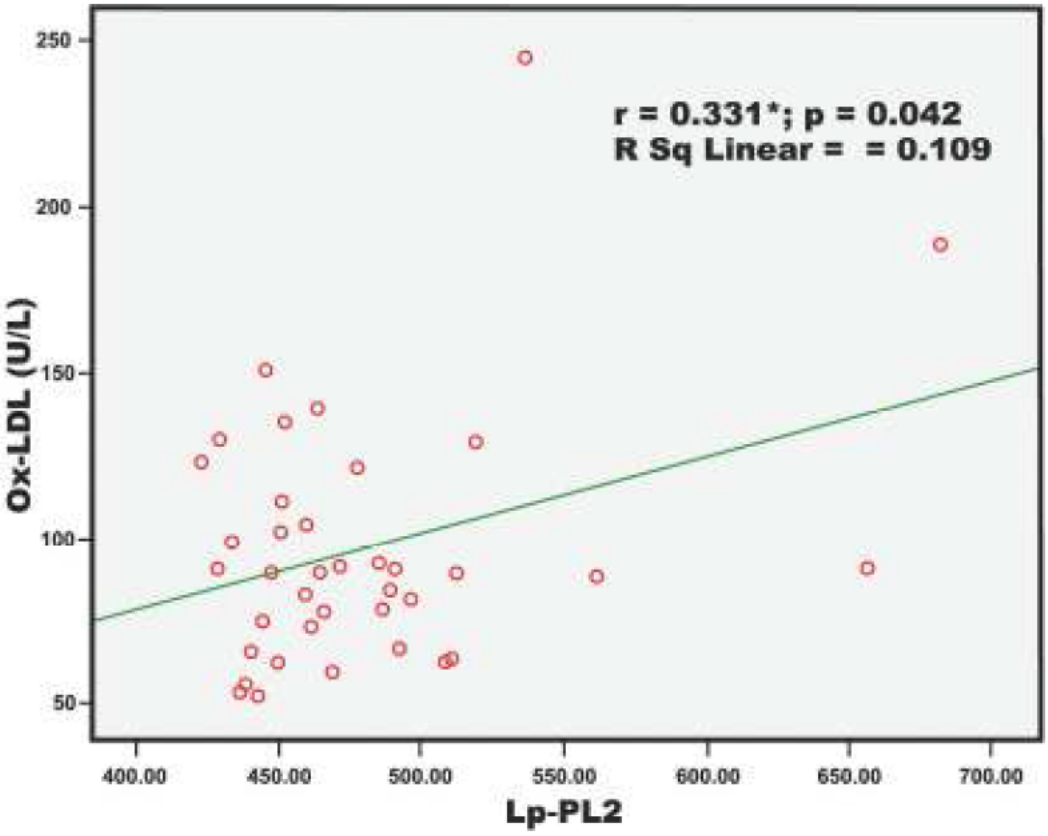

Figure 1. Correlation of Lp-PLA2 with oxLDL.

Discussions

Results of this study showed relationship between central obesity and inflammatory condition with the pathogenesis of atherosclerosis as shown by the linear analysis results ($p<0.05$). In central obesity, adipocytes are stimulated to synthesize proinflammatory cytokines such as TNF-α and Interleukin-6. These cytokines constitute the main regulators of CRP synthesis. The level of hsCRP can be regarded as an atherosclerosis predictor. Obesity is associated with chronic low-grade inflammatory condition resulting from the adipose tissues. Inflammation triggered by obesity may accelerate the process of atherosclerosis (9).

In this study we found no correlation of hsCRP with Lp-PLA_2. It is conceivable that these two biomarkers relate to different atherogenesis mechanisms. Jenny *et al.* examined the influence of CRP on Lp-PLA_2 associations with cardiac events. In all of the models tested, there were no statistically significant multiplicative interactions of CRP with Lp-PLA_2 mass or activity. Addition of CRP (by cutpoints, < 1 mg/L, 1–3 mg/L, ≥ 3 mg/L) to the multivariable models for Lp-Lp-PLA_2 mass or activity did not appreciably alter associations of these factors with CVD events. CRP and Lp-PLA_2 were independent predictors of events and there was a significant additive effect when the biomarkers were combined (10).

In this study we found that Lp-PLA_2 > 422 ng/mL had a linear correlation with oxLDL ($p = 0.042$). This finding supports the previous studies that showed Lp-PLA_2 was related to stroke occurrence (11). Other study found Lp-PLA_2 to be correlated with the increased incidence of heart attack and other death-causing vascular diseases (12). Lp-PLA_2 hydrolyzes oxidized phospholipid in oxLDL that results in lysophosphatylcholine and oxidixed fatty acid, which are proinflamatory substance and act to accelerate atherosclerosis process (13).

The most abundant oxPL in oxLDL is oxidized phosphatidylcolin (oxPC), which typically carries a truncated sn-2 acyl chain of nine carbons. oxPC is a specific substrate for the enzyme Lp-PLA_2, which release an oxidized short-chain fatty acid and lysophosphatidylcholine (LysoPC). LysoPC is a highly atherogenic lipid which induces multiple deleterious processes in the atherosclerotic plague (13).

Perssons *et al.* determined the relationship between Lp-PLA_2, the metabolic syndrome and incident CVD in 4480 individuals. Lp-PLA_2 mass was significantly higher in the individuals with Mets. The combination of both MetS and increased Lp-PLA_2 could, therefore be a prognostic marker for high-risk individuals (14).

Lp-PLA_2 is secreted predominantly by the macrophage. Its expression and secretion significantly increase during differentiation of human monocytes into macrophages as well as dramatically increase during activation of macrophages in the atherosclerotic lesion. It is thought that in plasma, Lp-PLA_2 is circulating bound to LDL (80%) and HDL (20%). Inhibiton of Lp-PLA_2 activity will abolish the inflammatory response of the cells (15).

Vickers *et al.* observed that Lp-PLA_2 was significantly more abundant in atherosclerotic lesions than in normal tissue ($p < 0.05$). This might explain why no correlation is found between low concentration of Lp-PLA_2 (> 422 ng/mL) and oxLDL in centrally obese men. Plasma Lp-PLA_2 levels represent risk related to the total burden of vascular inflammation and not just the burden arising from particular atheroma. Likewise, the plasma Lp-PLA_2 level reflects the contributing factors deriving from a single lesion, but mainly from multiple atherosclerotic sites (13).

Conclusion

This study showed a strong correlation between Lp-PLA_2 > 422 ng/mL and oxLDL in centrally obese men ($r = 0.331^*$; $p = 0.042$).

Acknowledgement:

We would like to thank the Prodia Education and Research Institute, Research Support Department of Prodia Clinical Laboratory, Prodia Clinical Laboratory – Makassar, and Prodia Clinical Laboratory – Bandung, for their invaluable supports to this research.

References:

1. Trayhurn P. Adipocyte Biology. Obes Rev 2007; 8 (suppl. 1): 41-4.
2. Engfeldt P, Arner P. Lipolysis in human adipocytes, effects of cell size, age and of regional differences. Horm Metab Res Suppl. 1988; 19: 26-9.
3. Hosogai N, Fukuhara A, Oshima K, Miyata Y, Tanaka S, Segawa K, *et al.* Adipose tissue hypoxia in obesity and its impact on adipocytokine dysregulation. Diabetes 2007; 56: 901-11
4. Thompson A, Gao P, Orfei L, Watson S, Di Angelantonio E, Kaptoge S *et al.* Lipoprotein-associated phospholipase A2 and risk of coronary disease, stroke, and mortality: collaborative analysis of 32 prospective studies. Lancet 2010; 375: 1536-44.

5. Ishigaki Y, Oka Y, Katagiri H. Circulating oxidized LDL: a biomarker and a pathogenic factor. Curr Opin Lipidol 2009; 20: 363-9
6. Grundtman C, Wick G. The autoimmune concept of atherosclerosis. Curr Opin Lipidol 2011; 22: 327-34
7. Galle J, Hansen-Hagge T, Wanner C, Seibold S. Impact of oxidized low density lipoprotein on vascular cells. Atherosclerosis 2006; 185: 219-26
8. Tsimikas S. Oxidized low-density lipoprotein biomarkers in atherosclerosis. Curr Atheroscler Rep 2006; 8: 55-61
9. Lyon CJ, Law RE, Hsueh WA. Minireview ; Adiposity, Inflammation, and Atherogenesis. Endocrinology. 2003;144: 2195-200
10. Jenny NS, Solomon C, Cushman M, Tracy RP, Nelson JJ, Psaty BM, *et al.* Lipoprotein-associated phospholipase A(2) (Lp-PLA(2)) and risk of cardiovascular disease in older adults: results from the Cardiovascular Health Study. Atherosclerosis 2010; 209: 528-32
11. Saenger AK, Christenson RH. Stroke biomarkers: progress and challenges for diagnosis, prognosis, differentiation, and treatment. Clin Chem 2010; 56: 21-33
12. Corsetti JP, Rainwater DL, Moss AJ, Zareba W, Sparks CE. High Lipoprotein-Associated Phospholipase A2 Is a Risk Factor for Recurrent Coronary Events in Postinfarction Patients. Clin Chem 2006; 52: 1331-8.
13 Vickers KC, Maguire CT, Wolfert R, Burns AR, Reardon M, Geis R *et al.* Relationship of lipoprotein-associated phospholipase A2 and oxidized low density lipoproteinvin carotid atherosclerosis. J Lipid Res 2009; 50: 1735–42
14. Wrahle KWJ, Heys SD. Atherosclerosis : cell biology and lipoprotein. Curr Opin Lipidol 2008; 19: 435-7
15. Mohler ER, Ballantyne CM, Davidson MH, Hanefeld M, Ruilope LM, Johnson JL, *et al.* The effect of darapladib on plasma lipoprotein-associated phospholipase A2 activity and cardiovascular biomarkers in patients with stable coronary heart disease or coronary heart disease risk equivalent: the results of a multicenter, randomized, double-blind, placebo-controlled study". J. Am. Coll. Cardiol. 2008; 51: 1632–41
16. O'Donoghue M, Morrow DA, Sabatine MS, Murphy SA, McCabe CH, Cannon CP, *et al.* Lipoprotein-associated phospholipase A2 and its association with cardiovascular outcomes in patients with acute coronary syndromes in the PROVE IT-TIMI 22 (PRavastatin Or atorVastatin Evaluation and Infection Therapy-Thrombolysis In Myocardial Infarction) Trial. Circulation 2006; 113: 1745-52.
17. Ballantyne CM, Hoogeveen RC, Bang H, *et al.* Lipoprotein-associated Phospholipase A2, High-Sensitivity C-Reactive Protein, and Risk for Incident Ischemic Stroke in Middle-aged Men and Women in the Atherosclerosis Risk in Communities (ARIC) Study. Arch Intern Med 2005; 165: 2479-84.