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Short communication

Perilipin 2 levels are increased in patients with in-stent neoatherosclerosis: A clue to mechanisms of accelerated plaque formation after drug-eluting stent implantation

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ABSTRACT

Background: Perilipin 2 (PLIN2) is a protein that potentially facilitates atherogenesis in native coronary arteries or arteries with an implanted drug-eluting stent (DES). The aim of the study was to determine PLIN2 protein levels in peripheral monocytes of enrolled subjects and compare them between patients with native coronary artery disease (CAD) and those with an in-stent restenosis (ISR) due to neoatherosclerosis occurring >1 year after DES implantation.
Methods: Forty-two patients were prospectively enrolled in the study in 3:1 fashion and underwent coronary catheterization. Both groups were angiographically matched for CAD burden with respect to the number of diseased vessels. Neoatherosclerosis was determined by intracoronary optical coherence tomography (OCT) among patients with ISR.
Results: Patients with ISR due to neoatherosclerosis had significantly higher PLIN2 protein levels in peripheral blood monocytes compared to patients with native CAD (342.47 ± 75.63[SE] versus 119.51 ± 20.95, $p < 0.001$). PLIN2 protein levels did not significantly differ between unstable and stable disease phenotype (125.59 ± 131.02 vs. 146.14 ± 111.87, $p = 0.109$).
Conclusions: In this explorative study, PLIN2 protein levels are significantly increased in patients with neoatherosclerosis, irrespective of clinical presentation, implicating that it might play a pathogenetic role in accelerated atherosclerosis after DES implantation. Further larger clinical studies are warranted to confirm these initial findings.

1. Introduction

Neoatherosclerosis is a special type of coronary atherosclerosis that occurs late after drug-eluting stent (DES) implantation [1]. This pathomorphological substrate was found to occur more frequently and earlier in DES compared to bare metal stents (BMS), after implantation [2]. A persistent endothelial dysfunction, lack of re-endothelialization and chronic inflammation within stent struts, appear to play a role in the pathogenesis of neoatherosclerosis [3,4]. This problem is of clinical importance since thrombosis of neoatherosclerotic etiology, as confirmed by intracoronary optical coherence tomography (OCT), carries a high risk for severe acute myocardial infarction (AMI) and portends poor prognosis [5].

However, the knowledge on exact mechanisms of neoatherosclerosis after DES deployment is insufficient at this point. Among mechanisms of plaque growth, the role of perilipin 2 (PLIN2, also known as *adipophilin*) has been recently studied in the context of lipid droplet (LD) regulation and gatekeeping of intracellular lipolysis [6,7]. PLIN2 favors increased cholesterol retention and reduced cholesterol efflux from lipid-laden macrophages [8]. Moreover, the modified low-density lipoprotein (LDL) induces its expression [9,10]. Since the abundance of lipid-laden macrophages within neointima is the hallmark of stent-associated neoatherosclerosis, we hypothesized that PLIN2 might have a distinguishable role in accelerated atherogenesis among patients with an implanted DES compared to patients with native coronary artery disease (CAD) that were stent-naïve. Therefore, the main goal of this explorative and hypothesis-generating study was to determine and compare PLIN2

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protein levels in peripheral monocytes of patients with native CAD and patients with late neoatherosclerosis occurring >1-year after DES implantation.

2. Methods

The information on methods used in the making of this study and relevant ethical disclosures are provided in the Appendix A submitted as supplemental file along with this manuscript.

3. Results

3.1. Baseline clinical and laboratory characteristics of studied population

A total of 42 patients were included in the final analysis (mean age 71 ± 11, 89% males, 11% females). In 31 patients (74%) symptoms were associated with obstructive stenoses in native CAD, while in 11 patients (26%) symptoms were associated with late ISR caused by neoatherosclerosis as confirmed by OCT. Regarding the clinical presentation, 14 patients (33%) had stable disease phenotype in the form of stable angina (SA), 18 (43%) patients had a non-ST elevation acute coronary syndrome (NSTE-ACS) and 10 (24%) patients had ST-elevation myocardial infarction (STEMI). No significant differences between the two matched groups were observed in terms of age, sex, cardiovascular risk factors and comorbidities. The number of diseased vessels at coronary angiography was similar between the two groups (1.27 ± 0.467 vs. 1.29 ± 0.693, p = 0.938). Moreover, no significant differences were observed in baseline plasma levels of total cholesterol, cholesterol fractions – LDL-c, HDL-c, triglycerides, and creatinine. The use of acetylsalicylic acid, beta-blockers, angiotensin-converting enzyme inhibitors/angiotensin receptor blockers and statins was comparable between the groups (Table 1).

Table 1
Baseline characteristics of patients with in-stent neoatherosclerosis that developed >1 year after DES implantation versus patients with a native coronary artery disease (CAD).

	Neoatherosclerosis group (2nd generation DES ISR due to neoatherosclerosis)	Native CAD group (stent-naïve)	p
Clinical characteristics			
Number [n, (%)]	11	31	–
Male [n, (%)]	8 (76.92%)	27 (77.41%)	0.973
Female [n, (%)]	3 (27.27%)	4 (12.90%)	0.277
Age (aa ± SD)	78 ± 11.41	72.94 ± 10.67	0.181
Smoking	5 (9.09%)	5 (16.11%)	0.572
Arterial Hypertension	11 (100%)	30 (96.77%)	0.551
Diabetes mellitus	4 (36.36%)	7 (22.58%)	0.377
Hypercholesterolemia	8 (72.72%)	17 (54.83%)	0.304
CRF	0 (0%)	2 (18.18%)	0.133
Positive family history of ACS events	4 (36.36%)	6 (19.35%)	0.260
Mean Number of the diseased vessels as assessed by coronary angiography	1.27 ± 0.467	1.29 ± 0.693	0.938
Disease presentation			
Stable angina	5 (45.45%)	9 (29.03%)	0.326
NSTE-ACS	4 (36.36%)	14 (45.16%)	0.616
STEMI	2 (18.18%)	8 (25.80%)	0.614
Laboratory data			
Triglycerides (mg/dL)	110.67 ± 59.33	123.5 ± 64.80	0.603
Total Cholesterol (mg/dL)	173.2 ± 72.73	165.4 ± 39.69	0.678
LDL-c (mg/dL)	102.38 ± 56.58	102.38 ± 40.27	0.991
HDL-c (mg/dL)	41.75 ± 9.63	41.46 ± 10.63	0.955
Creatinine (mg/dL)	1.06 ± 0.20	1.01 ± 0.3	0.603
Pharmacotherapy			
ASA	11 (100%)	28 (90.32%)	0.290
Beta-blockers	7 (63.64%)	17 (54.84)	0.616
ACE-I/ARBs	10 (90.90%)	23 (74.19%)	0.251
Statins	9 (81.81%)	21 (67.74%)	0.380

Abbreviations: ACS-acute coronary syndrome; ACE-I-angiotensin-converting enzyme inhibitor; ARBs-angiotensin II receptor blockers; ASA-acetylsalicylic acid; CRF-chronic renal failure; DES-drug-eluting stent; HDL-c-high density lipoprotein cholesterol; ISR-in-stent restenosis, LDL-c-low density lipoprotein cholesterol; NSTE-ACS-non-ST-elevation acute coronary syndrome; STEMI-ST-elevation myocardial infarction.

3.2. PLIN2 in neoatherosclerosis and native coronary atherosclerosis

Patients with neoatherosclerosis had a significantly higher PLIN2 protein levels in their peripheral blood mononuclear cells (PBMCs) when compared to patients with native CAD (342.47 ± 75.63[SE] versus 119.51 ± 20.95, p < 0.001) (Fig. 1). No significant differences in PLIN2 protein levels were observed between patients with acute and stable disease phenotype (28/42 patients presenting with acute coronary syndromes vs. 14/42 patients presenting with stable angina) with respective values being 125.59 ± 131.02 vs. 146.14 ± 111.87, p = 0.109.

4. Discussion

In this hypothesis-generating study, we report for the first time in humans that perilipin 2 (PLIN2), a protein involved in plaque growth and lipid retention, is significantly increased in circulating monocytes of patients with in-stent restenosis caused by neoatherosclerosis compared to circulating monocytes of patients with significant obstructions due to a native CAD.

PLIN2 is a protein implicated in lipid accumulation in adipocytes, monocytes and found to be upregulated in macrophage foam cells [8,11]. Intracellularly, PLIN2 hindered lipase access to LDs thus promoting lipid retention and decreasing lipid transport from the cell [12]. In a preclinical study by Paul et al., when PLIN2 deficiency was induced in the apolipoprotein E-deficient mice, significant reduction in the number of LDs in foam cells and decrease in lesion atherosclerotic burden were achieved [13].

Importantly, in our study, we measured PLIN2 protein levels in circulating peripheral monocytes, not resident cardiac macrophages. However, peripheral monocytes significantly contribute and correlate with resident myocardial macrophage content in the situations of acute infarction, inflammation or after induction of macrophage death in preclinical models [14,15]. Mechanistically, it is, therefore, plausible that PLIN2 content in peripheral monocytes might correlate with that in resident macrophages within the coronary vessel lesions since these monocytes differentiate into macrophages after being recruited to lesion site [16].

Second important point is that atherosclerosis of native blood vessels and in-stent neoatherosclerosis share some similar characteristics, but they have some relevant differences [4]. Of note, neoatherosclerosis occurs within months to years' post-stent implantation and is driven by non-traditional and stent-specific risk factors as opposed to atherosclerosis of native arteries that develops gradually over the decades enhanced by the traditional risk factors thus being a considerably slower pathophysiological process [1]. This is important difference because it implicates different proatherogenic mechanisms between these two entities. Both drug and polymer of DES may be involved in the process of early accelerated neoatherosclerosis with persistent inflammation favoring lack of endothelial coverage of stent struts, endothelial dysfunction, and neorevascularization with deposition of circulating cholesterol [17].

Third important point is that plasma levels of lipids and statin use were similar between two studied groups, suggesting that despite similar levels of circulating cholesterol, a significant 3-fold increase in PLIN2 protein levels was observed in patients with neoatherosclerosis when compared to those with native CAD. The effects of statin use on PLIN2 levels have not been previously described in the literature. However, our results suggest that intracellular LD retention within resident foamy macrophages at the lesion and within monocytes