Atherosclerosis, epigenetic modifications, and arterial stiffness

Abstract

Atherosclerosis is a chronic, inflammatory vascular disease, characterized by progressive plaque build-up in the vessel wall, leading to tissue ischemia. It is the most common cause of cardiovascular diseases and is a major epidemiologic concern in modern society. The pathophysiology of atherosclerosis includes two main events: endothelial injury and vessel wall inflammation. Recent findings indicate that the pathogenesis of atherosclerosis involves dynamic changes in epigenetic modifications and gene expression in a cell type- and stage-specific manner. Epigenetics is the study of gene expression and involves phenotypical variations without genotypic changes. Three major epigenetic mechanisms participate in atherosclerosis: DNA methylation, posttranslational modifications of histones, and non-coding RNA molecules.
DEFINITION, EPIDEMIOLOGY, AND CLINICAL SIGNIFICANCE

Atherosclerosis is a chronic, inflammatory vascular disease characterized by progressive plaque build-up in the vessel walls, leading to tissue ischemia (1). It is the most common cause of cardiovascular diseases, including coronary artery disease (CAD), cerebrovascular disease, peripheral artery disease, and thoracoabdominal aortic aneurysms (2). Cardiovascular diseases are the leading cause of death in Western countries and account for more than one-third of deaths in patients aged 35 years or more (3). Men are affected more often than women (4, 5). Several studies demonstrated the beginning of pre-atherosclerotic vessel changes in young, adolescent patients and progressive growth and plaque evolution throughout later stages of life (6-8). The traditional risk factors for atherosclerosis include genetic predisposition, arterial hypertension, dyslipidemia, diabetes mellitus, metabolic syndrome, smoking, fat-rich diets, and psychological stress (9). Proteinuria is an independent risk factor for advanced atherosclerosis and cardiovascular disease (10).

PATHOPHYSIOLOGY: THE IMPORTANCE OF EPIGENETICS

The pathophysiology of atherosclerosis is multifactorial and complex; however, it can be explained by two major events. The first is endothelial damage due to different factors, including arterial hypertension, smoking, dyslipidemia, and macrophage activation, which causes lipid aggregation in the subendothelial tissue and monocyte recruitment to the site of the injury. The recruited monocytes transform into macrophages, which then become foam cells after the phagocytosis of lipid particles in the vessel wall (11). The second event is inflammation in the vessel wall. Macrophages, smooth muscle cells, and endothelial cells oxidize intramural lipid particles, leading to the increased release of cytokines followed by chemotaxis of additional immune cells (12). Structural changes in the vessel wall are important events in atherosclerosis and include the proliferation of smooth muscle cells and the accumulation of lipids and foam cells. Plaque growth due to central hypoxia leads to the formation of a thrombogenic necrotic core, which is covered by a thin layer of smooth muscle cells, macrophages, collagen, and proteoglycans (fibroatheroma). Macrophages release metalloproteinases, which destabilize the atherosclerotic plaque, leading to plaque rupture and thrombus formation (13).
Recent findings suggest that the pathogenesis of atherosclerosis involves dynamic changes in epigenetic modifications and gene expression in a cell type- and stage-specific manner. Epigenetics is the study of gene expression and involves phenotypical variations without genotypic changes. Three epigenetic mechanisms have been proposed to participate in the pathogenesis of atherosclerosis. The first is DNA methylation, which is the addition of methyl groups to cytosine nucleotides of DNA molecules by the action of DNA methyltransferases. Valencia-Morales et al. studied the correlation between DNA hypermethylation and increased atherogenesis (14).

The second epigenetic mechanism is the posttranslational modification of histones. Cao et al. observed that increased histone acetylation was associated with advanced atherosclerosis (15).

The third epigenetic mechanism is non-coding RNA molecules, which participate in several steps of the development of atherosclerosis (16). Long non-coding RNAs (lncRNAs) are longer than 200 nucleotides and have no protein-coding function. These molecules can affect protein synthesis, maturation and transportation of RNA molecules, chromatin structure, and gene transcription (17). It has been reported that some lncRNAs, including anti-sense non-coding RNA in the cyclin-dependent kinase-4 locus (ANRIL), H19, p21, hypoxia-inducible factor 1α-antisense 1 (HIF1α-AS1), and hyaluronan synthase-2 antisense-1 (HAS2-AS1), cause increased proliferation of smooth muscle cells in the vessel wall, leading to faster plaque growth (18, 19). Increased endothelial dysfunction has been found in tissues with increased expression of the noncoding repressor of nuclear factor of activated T–cells (NRON) and metastasis-associated lung adenocarcinoma transcript−1 (MALAT−1) lncRNAs. The lncRNAs ALIEN, TERMINATOR, and PUNISHER have a role in the development of cardiac cell lineages and endothelial dysfunction (19). The increased expression of two lncRNAs—hepatocellular carcinoma up-regulated (HULC−1) lncRNA and apolipoprotein A1 anti-sense (APOA1−AS)—have been correlated with increased lipid accumulation in the liver and other tissues (20). The lncRNA dynein light chain roadblock 2 type 2 (DYNLRB2−2) facilitates cholesterol efflux from cells in cases of hyperlipidemia and is also associated with lower levels of proinflammatory cytokines, including interleukin−1β (IL−1β), tumor necrotizing factor−α (TNF−α), and interleukin−6 (IL−6) (19).

Micro RNAs (miRNAs) are 18−24 nucleotides long RNA molecules that affect different cellular processes. Several miRNAs are involved in the pathophysiology of atherosclerosis (21). MiRNA−223 is associated with lower cholesterol synthesis and decreased liver uptake of high-density lipoproteins (HDL) (22). Several other miRNAs affect lipid metabolism, including miRNA−122, −27b, −30c, −33, −148a, −128−1, −130b, and −301b (21). MiRNA−181b and −146a inhibit the synthesis of adhesion molecules and cytokines, reducing diapedesis and inflammatory changes in the vessel wall (23). Increased endothelial dysfunction has been found in tissues with increased expression of miRNA−92 (24). MiRNA−712 and −205 inhibit the action of metalloproteinases and lead to increased plaque stability (25). Several miRNAs, including miRNA−33, −21, −147, −146a, −143, and −145, affect the function of intramural macrophages and smooth muscle cells (26).

The expression of non-coding RNA molecules can be measured in peripheral blood, urine, and saliva using microarrays, indicating the potential of these markers as diagnostic tools and therapeutic targets (21).

**ARTERIAL STIFFNESS**

Arterial stiffness is increasingly recognized as a surrogate endpoint for cardiovascular disease. This complication is the result of several structural alterations in the arterial walls, pathophysiologically similar to those in atherosclerosis, leading to reduced distensibility and decreased buffering capacity of arteries to pulsatile cardiac ejection (27).

The histological features of arterial stiffness include degradation of elastic fibers, deposition of collagen, loss of endothelial integrity, increased number of smooth muscle cells and macrophages in the vessel walls, and a higher degree of vessel wall inflammation. In addition to higher collagen levels, the increased deposition of chondroitin sulfate, fibronectin, and heparin sulfate increases vessel wall rigidity. Fragmented
elastic fibers are prone to calcium and phosphate deposition and non-enzymatic glycation, which leads to the increased production of free radicals and proinflammatory cytokines and endothelial dysfunction (28).

**THE ROLE OF APPLANATION TONOMETRY AND ARTERIAL STIFFNESS PARAMETERS**

Applanation tonometry is a non-invasive, easily reproducible technique used for measuring arterial stiffness. It enables us to perform pulse wave analysis (PWA), most commonly on the radial artery. Several central hemodynamic parameters can be derived from PWA, including pulse pressure (PP), augmentation pressure (AP), augmentation index (Alx), adjusted Alx for heart rate 75 beats per minute (Alx @ 75), ejection duration (ED), and ejection duration index (EDI). The heart rate affects Alx; for this reason, Alx @ 75 is used to compare individuals with different heart rates. Slower heart rate is associated with an early systolic return of the pressure wave, and faster heart rate is associated with later systolic or diastolic return of the pressure wave to the heart. An additional parameter derived from PWA is the Buckberg index or subendocardial viability ratio (SEVR). Buckberg et al. demonstrated that SEVR was closely correlated with the blood supply to the subendocardium, cardiac function, and oxygen consumption and energy supply to the heart (29).

Pulse wave velocity (PWV) is considered the most precise approach to noninvasively estimate arterial stiffness in humans. Aortic PWV is related to the intrinsic stiffness of the aorta and is a major determinant of the pressure load on the heart via aortic compliance and transmission of the forward and reflected pressure wave. Aortic PWV cannot be measured directly by non-invasive means, except by expensive non-portable techniques such as phase contrast magnetic resonance imaging (MRI). Therefore, aortic PWV is usually evaluated between the carotid and femoral artery and in this case is termed carotid-femoral PWV (cfPWV) (30).

The definitions of the most commonly used arterial stiffness parameters are shown in Table 1.

**Table 1. Arterial stiffness parameters and their definitions (29, 30).**

<table>
<thead>
<tr>
<th>Arterial stiffness parameter</th>
<th>Definition</th>
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<tbody>
<tr>
<td><strong>PWA-derived parameters</strong></td>
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<tr>
<td>Pulse pressure (mmHg)</td>
<td>Difference between systolic and diastolic pressure.</td>
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<tr>
<td>Augmentation pressure (mmHg)</td>
<td>Difference between systolic and inflection pressure.</td>
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<tr>
<td>Augmentation index</td>
<td>Augmentation pressure divided by pulse pressure.</td>
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<tr>
<td>Alx @ 75/minute</td>
<td>Augmentation index adjusted for heart rate at 75 beats per minute.</td>
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<tr>
<td>Ejection duration (ms)</td>
<td>Duration of left ventricular systolic ejection.</td>
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<tr>
<td>Ejection duration index (%)</td>
<td>Ratio of the duration of systolic ejection to the total duration of the heart cycle.</td>
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<tr>
<td>Subendocardial viability ratio (%)</td>
<td>Diastolic area under the curve divided by the systolic area under the curve, derived from pulse wave curve.</td>
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<tr>
<td>cfPWV (m/s)</td>
<td>Pulse wave distance between two measuring sites (carotid and femoral artery) divided by pulse transit time (measured by electrocardiographic monitoring).</td>
</tr>
</tbody>
</table>

Legend: PWA – pulse wave analysis; Alx @ 75 – augmentation index, adjusted for heart rate; cfPWV – carotid-femoral pulse wave velocity.

**CLINICAL SIGNIFICANCE OF INCREASED ARTERIAL STIFFNESS**

Increased arterial stiffness is a hallmark of several different pathological states. Weber et al. prospectively enrolled 465 male patients undergoing coronary angiography for the assessment of suspected CAD. Their results indicated that Alx, AP, and Alx @ 75 were strong, independent risk markers for premature CAD (31). Prskalo et al. evaluated 160 patients undergoing coronary angiography and found a significant correlation between PWV, Alx, and CAD. Patients with in-stent restenosis and left main CAD had higher PWV compared to other patient groups (32). Gaszner et al. found that patients with CAD had higher cfPWV and Alx (33).
Laurent et al. observed that higher cfPWV was independently associated with a higher risk of fatal stroke (34). Gasecki et al. demonstrated that lower cfPWV and AIx improved the functional outcome after stroke (35). A prospective Rotterdam study involving 2,835 patients reported that patients with increased arterial stiffness determined by cfPWV presented a higher risk of coronary and cerebrovascular events (36). A population Hoorn study included 261 healthy patients and 358 patients with prediabetes or diabetes and found that patients with glucose metabolism impairment had significantly higher values of cfPWV and AIx (37).

Prenner et al. performed a meta-analysis in 2015 and found that increased arterial stiffness (determined by cfPWV and AIx in most of the included studies) was correlated with higher mortality and more extensive target organ damage (nephropathy, retinopathy, and neuropathy) in patients with diabetes (38). Dyslipidemia is associated with increased arterial stiffness (39). The meta-analysis by Upala et al. indicated that a short–term statin therapy reduced central aortic PWV (40).

The study by Tsiachris et al. included 36 patients with arterial hypertension and without known macrovascular CAD and found that patients with decreased coronary flow reserve (determined by the flow change after intracoronary application of adenosine and nitroglycerine) presented lower SEVR, indicating the importance of SEVR in assessing microvascular bed impairment (41). Laugesen et al. performed a study on 86 patients and found that female patients with diabetes had the lowest SEVR values (42).

Chronic kidney disease (CKD) is an independent risk factor for advanced atherosclerosis and cardiovascular disease. High PWV is an independent risk factor for coronary events and higher mortality in patients with CKD not subjected to renal replacement therapy and those on renal replacement therapy with peritoneal dialysis, hemodialysis, and kidney transplantation (43).

Briet et al. included 95 patients with CKD (glomerular filtration measured by 51Cr–EDTA clearance) and 121 hypertensive patients with normal kidney function (glomerular filtration rate estimated by the Modification Diet in Renal Disease – MDRD study equation) and found that patients with CKD presented a higher cfPWV and that a lower glomerular filtration rate (GFR) was associated with higher cfPWV and vice versa (44).

Sedaghat et al. found that the correlation between CKD and arterial stiffness is reciprocal because increased blood pulsatility could damage the kidney microcirculation and further decrease renal function. These authors analyzed data from 3,666 patients and found that higher cfPWV was correlated with a faster decrease in the estimated GFR using the Chronic Kidney Disease and Epidemiology Collaboration – CKD–EPI equation (45).

Anemia is a common complication of CKD and involves a decrease in the myocardial oxygen supply and an increase in the incidence of cardiovascular diseases. A study reported that SEVR was independently associated with hemoglobin levels in nondialysis CKD patients. CKD patients with lower hemoglobin had lower SEVR (46).

Proteinuria is a major risk factor for early mortality, acute myocardial infarction, and faster progression of kidney disease, independently of GFR (47). The results of a post hoc analysis of the Chronic Renal Insufficiency Cohort (CRIC) study by Weir et al. indicated that higher proteinuria was correlated with higher cfPWV and increased arterial stiffness (48). Furthermore, albuminuria is associated with lower SEVR and higher ED in nondialysis CKD patients, highlighting the impaired cardiac perfusion in these patients (49). Moreover, SEVR is significantly impaired in highly proteinuric CKD patients with a low estimated GFR (below 30 ml/min/1.73m²), calculated with the CKD–EPI equation (50).

Di Micco et al. performed a prospective 36–month study involving patients with CKD stages 3 and 4. During the study period, 34 patients died, 29 because of cardiovascular events. These authors found that patients with lower SEVR had higher mortality. Post-mortem evaluation showed a higher degree of coronary vessel wall calcification and larger myocardial mass in patients with previously lower levels of SEVR (47).
CONCLUSION

Atherosclerosis is one of the most significant causes of morbidity and mortality in developed countries. New evidence suggests an important role of epigenetic processes in the pathophysiology of this disease, opening up additional diagnostic and therapeutic options. Arterial stiffness is closely associated with atherosclerosis and can be used as a non-invasive tool for assessing cardiovascular status and the risk of mortality and morbidity in patients with coronary disease, cerebrovascular disease, diabetes, arterial hypertension, and kidney disease.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this study.

REFERENCES

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