Assessment of microcirculatory function with retrobulbar blood flow velocity measurement predicting cardiovascular events

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Atherosclerosis and Inflammation

Atherosclerosis first begins in the endothelium of the arterial wall, and is described as an inflammatory disease. Although atherosclerotic lesions occur in large arteries, the increased expression of adhesion molecules characteristic of endothelial cell activation, the decreased endothelium-dependent vasodilatation as well as oxidative stress are not limited to lesion-prone arteries where factors other than endothelial cell activation might progress to detect atheroma formation. Microvascular endothelial cell activation might be directly stimulated by cardiovascular risk factors with consequent release of inflammatory mediators and soluble isoforms of adhesion molecules that detect microvascular dysfunction and the atherosclerosis-associated systemic inflammatory state. The quantification of retrobulbar blood flow velocity has been used to analyze the microvascular circulation of the eye. Structural and functional changes in various microvascular beds can predict CV risk factors and diseases.

Keywords: Atherosclerosis; Microcirculation; Retrobulbar Blood Flow


Introduction

The significance of inflammatory pathway activation in the process of atherosclerosis has been demonstrated by a lot of evidence. The inflammatory stages are involved in the cascade of atheroma evolution, from the early development of endothelial dysfunction to the formation of the mature atheroma, and its vulnerability [1]. The observation that increased inflammation biomarkers predict future cardiovascular diseases in patients with various risk factors supported these findings [2, 3]. Therefore, there is an ongoing search to clarify the factors coordinating these complex stages.

Microvascular endothelial cell activation might be directly induced by cardiovascular risk factors with consequent release of inflammatory mediators and soluble isoforms of adhesion molecules that detect microvascular dysfunction and the atherosclerosis-associated systemic inflammatory state. Retinal blood flow velocity and resistivity index may be associated with microvascular function. Thus, ocular microvascular dysfunction, defined by retrobulbar blood flow velocities, may be an early manifestation of developing advanced-stage cardiovascular disease [4].

Atherosclerosis and Inflammation
Atherosclerosis firstly initiates in the endothelium of the arterial wall, and is defined as a concurrent inflammatory disease [1, 5, 6]. The first pathological change is impairment of the endothelium which is represented by increased vascular constriction and suppressed dilatation of the vessels and alterations in the mediators of thrombosis. Endothelium-derived relaxing factor [EDRF] or nitric oxide [NO] protects the endothelial vasodilatation, and inhibits the vasoconstriction triggered by angiotensin II and endothelin [5]. Inflammatory cascades are demonstrated by increased synthesis of inflammation and thrombosis mediators. Interleukin-1 [ICAM-1], monocyte chemoattractant protein-1 [MCP-1] and interleukin-6 are the major mediators [7]. Macrophages form foam cells by storing cholesterol esters [7]. The excess of cholesterol in the macrophages is managed by the transport of cholesterol regulated by ATP-binding cassette transporter A1 [ABC-A1] and transport of oxidized LDL via CD36 [7]. Fibronectin, elastin, collagens, matrix metalloproteinases [MMPs] and growth of endothelial cells and smooth muscle cells also take part in plaque formation [5, 6, 8, 9]. Recent trials demonstrated that the pathophysiology of the endothelium owing to inflammatory reactions were also regulated by cytokines and tissue factors.

C-reactive protein [CRP] that is one of the biomarkers of inflammation, produced by hepatic cells and is also regulated by interleukin 6 [IL-6], interleukin 1 [IL-1] and tumor necrosis factor alpha [TNF-α] is important [10]. Evidence suggests that elevated blood CRP level is a major predictor of cardiovascular diseases [11, 12]. It is also involved in the progression of atherosclerotic lesions by setting physiology of endothelium [5, 13, 14]. It increases the production of vascular cell adhesion molecule 1 [VCAM-1], Intercellular Adhesion Molecule 1 [ICAM-1], MCP-1 and selectins in the endothelium via initiation of strong constrictor of the vessels endothelin 1 [ET-1] and IL-6 [5, 15]. It improves the synthesis of NO via suppressing the transcription and translation of enzyme NO synthase [5, 15]. It is also associated with the activities of other cytokines and factors. CRP stimulates the synthesis and functions of plasminogen activator inhibitor-1 [PAI-1] in the endothelium [15]. It is known that PAI-1 actively plays a role in thrombosis during atherosclerosis process and hinders destruction of the fibrin clot by depressing plasminogen activation [16]. There is a direct correlation between increased blood PAI-1 concentration and mortality rate in patients with coronary artery diseases [16]. Apolipoprotein E and LDL-receptor knockout mice indicate express atherosclerotic lesions [17, 18]. The plaques in these animals also contain sizeable counts of macrophages/T cells. Cross-breeding of apolipoprotein-E knockout with T-cell knockout mice with deficient macrophages [osteoporotic op/op] displayed the impact of immune cells in the advancement of atherosclerosis [19]. Inflammatory reactions are not only associated with vascular plaques progression but also involved in the plaque rupture which converts chronic disorder into an acute thrombo-embolic disease. The cytokines, cyclooxygenase-2, matrix metalloproteinases, and tissue factors involve in the rupture of internal arterial plaques [1, 19, 20]. Experimental trials indicate that inflammatory reactions have major role in the connection between risk factors for atherosclerosis and pathophysiology of the disease [9]. Serum amyloid A [SAA] protein has also been associated with the inflammatory reactions involved in atherosclerosis and defined as a marker for cardiovascular disorders and their outcome [21]. TNF-α is an inflammatory cytokine and involves in inception as well as progression of atherosclerosis. It stimulates transcription factor nuclear factor-κB [NF-κB], a major factor in the cascades of inflammation. In the pathway of atherosclerosis, the transcription of MCP-1, VCAM-1, E-selectin and ICAM-1 is provoked by NF-κB in smooth muscle and endothelial cells of the vessels [22]. NO levels are consumed by TNF-α in the endothelium which induces reduction of endothelial dilatation and leads to endothelial dysfunction [23, 24]. TNF-α provokes apoptosis of the endothelial cells via dephosphorylation of protein kinase B [Akt] bringing about endothelial damage [25, 26]. Resistin enforces vasoactive effects and inflammatory reactions in cultured endothelial cells [5]. In atherosclerotic cascade resistin causes transcription of cellular factors like VCAM-1 and MCP-1 [27]. Endothelial cells picked out resistin consume the levels of TNF receptor-associated factor [TRAF-3]. TRAF-3 is an inhibitor of the endothelial activation [28]. Enhanced resistin concentration implies a severe endothelial dysfunction via activation of endothelial system. Moreover, resistin exposure induces transcription of ET-1 and increases ET-1 release, showing its effect on endothelial dysfunction [29]. Leptin increases both ET-1 and NO synthase synthesis in the endothelial cells and improves production of free radicals and oxidants [30, 31], causing oxidative stress [32]. The cellular growth, migration of endothelial cells [33] and smooth muscle cells are enhanced by Leptin [34]. It stimulates the synthesis of MCP-1 in the aortic endothelial cells [35]. It increases the aggregation of the platelets and vascular thrombus formation via leptin receptor pathways [30, 31]. It directly improves concentrations of monocyte colony-stimulating factor [MCSF] [36], enhances cholesterol levels in hyperglycemia [37] and induces new blood vessel formation [38]. Husain et al. in their studies showed that increased concentrations of factors associated with inflammatory cascade, namely MCP-1, TNF-α, TGF-β1, Cox-2, iNOS, and Mn-SOD in ApoE-deficient atherosclerotic mice [39, 40] proved the vascular inflammation played a significant role in the atherosclerosis.

Inflammatory state of microcirculation associated to atherogenesis
Although atherosclerotic lesions take place in large arteries, the elevated expression of adhesion molecules characteristic of EC activation, the decreased endothelium-dependent vasodilatation as well as oxidative stress are not limited to lesion-prone arteries where factors other than EC activation [e.g. elevated shear stress] might progress to detect atheroma formation. Atherosclerosis is related with a systemic inflammation featured by endothelial and blood cell activation besides elevated plasmatic concentration of inflammatory mediators and endothelial dysfunction composed of decreased endothelium-dependent vasodilation [41]. The circulating concentrations of proinflammatory factors or soluble isoforms of adhesion molecules have been suggested as biomarkers for cardiovascular [CV] risk. Increased expression of inflammatory mediators such as cytokines, chemokines and reactive oxygen species take places in the atherosclerotic lesions detection and maintaining local intramural inflammation. On the other hand, inflammatory mediators can also be released in the circulation. Alternatively, cardiovascular risk factors such as increased blood cholesterol levels, hypertension, diabetes, obesity and cigarette smoking might directly induce microvascular endothelial cell activation with consequent release of inflammatory mediators and soluble isoforms of adhesion molecules. In this way, they give rise to microvascular dysfunction and the atherosclerosis-associated systemic inflammatory state [42]. Many investigation have demonstrated that the cardiovascular risk factors including hypercholesterolemia, obesity, hypertension and diabetes stimulate microvascular responses consistent with the induction of an inflammatory phenotype [43]. In both scenarios, because of its predominant surface area, microcirculation would quantitatively characterize the main source of circulating inflammatory mediators.

Previous observations indicate that the main pathogenic mechanism underlying the advancement of atherosclerosis is oxidative stress, being defined as an imbalance between oxidants and antioxidants in favor of the former. The endothelial cells, phagocytes and smooth muscle cells produce reactive oxygen species [ROS] such as superoxide anions [O2 −], hydrogen peroxide, and hydroxyl radicals.

It is known that free radicals such as O2 − and hydroxyl radicals are very reactive and have a high oxidizing activity. ROS is generated by enzymes such as nicotinamide adenine dinucleotide phosphate [NADPH] oxidase and xanthine oxidase, as well as the mitochondrial redox cycle. Endothelial cells, vascular smooth muscle cells, and monocyte/macrophage cells possess the enzymes superoxide dismutase, catalase, and glutathione peroxidase, and non-enzymatic antioxidants, which are potent defense systems against ROS. However, excessive production of ROS can occur and cause endothelial dysfunction, inflammation of the arterial wall, and finally, atherosclerosis. Endothelial dysfunction is a very early marker of the cascade. Endothelium has a strategic position between the blood and the vessel wall. NO produced by the endothelium is not only a potent vasodilator but also highly reactive with O2. NO oxidation generates peroxynitrite, which lacks in vasodilatory properties. Impaired response to all vasodilators which act by stimulating the endothelial release of NO is seen when NO bioavailability is reduced. In subjects with cardiovascular risk factors such as diabetes, hypercholesterolemia, and hypertension and cigarette smoking, increased oxidative stress and endothelial dysfunction have been demonstrated. NO also play a role in other functions of the vascular endothelium such as the regulation of blood coagulation and the recruitment of circulating leukocytes. NO contributes to limit endothelial cell-leukocyte interaction and, subsequently, inflammation, by downregulating the surface expression of adhesion molecules. In addition, NO exerts antithrombogenic and antiproliferative effects [44]. Therefore, reduced NO levels have a significant negative impact vascular physiology. Increased ROS concentrations causes augmented oxidation of low-density lipoproteins, which are taken up by macrophages, leading to foam cell formation and vascular inflammation [45-47].


Evaluation of retrobulbar blood flow velocity has been used to examine the microvascular circulation of the eye. Changes in various microvascular circulation can give critical predictive information of future CV diseases [48]. Previous studies have shown that that microvascular beds of the eye and kidney were most often affected by systemic disease, and their structural and functional changes not only predict local complications but also CV and cerebrovascular events [49]. Especially retinopathy is a marker for microvascular dysfunction of the retinal circulation and it has been shown to be related with future vascular diseases such as coronary artery disease and stroke [4, 50, 51]. The ophthalmic artery [OA] originates from the internal carotid artery gives out central retinal artery [CRA] and posterior ciliary artery [PCA] branches [52]. The human eye is supplied by two different vascular systems, namely the retinal and uveal systems. The uveal vessels include the vascular beds of the iris, the ciliary body, and the choroid and the outer retinal layers including the photoreceptors whereas the inner layers of the retina are nourished by the retinal vessels. [53]. The major source of blood flow to the optic nerve head is in most cases derived from the PCA, whereas the CRA supplies the blood flow to the retina [54].
Doppler sonography has been used to evaluate volumetric flow in these vessels [55, 56]. As in other vascular beds, ocular blood flow is given as blood flow velocity multiplied by cross-sectional area. The resistive index (RI), a measure of distal vascular resistance, is reported to be linearly related to vascular resistance in both in vitro and in vivo studies [57, 58]. The blood flow in OA is similar to that of the internal carotid artery with a high peak systolic velocity [PSV] and a low end-diastolic velocity [EDV]. A dicrotic notch follows a steep PSV. The flow of the CRA is similar to that of the OA, with a lower systolic peak. The flow pattern of the PCA also resembles that of the CRA but the diastolic flow of the PCA is higher, reflecting low-resistance vascular channels of the choroid [59].

With improvement in Doppler technology, Doppler imaging of the OA was recently made possible. Changes in OA blood flow assessed by pulsed Doppler [OA Doppler] have provided new information about various vascular disorders including ophthalmic diseases, carotid artery stenosis, and diabetes mellitus [60].

OA Doppler is easy to obtain due to the absence of ultrasonic obstacles and the vertical angle to the transducer has advantages over the parallel-signaling of carotid artery Doppler [61, 62].

The resistive index [RI] = peak systolic velocity [PSV] - end-diastolic velocity [EDV]/PSV and pulsatility index [PI] = PSV - EDV/mean velocity can be assessed for each vessel from the Doppler signal [63].

Abnormal findings in ocular fundus are associated closely with systemic atherosclerosis, and previous studies has suggested that atherosclerotic changes in retinal arteries were potential markers of systemic cardiovascular disease [64, 65]. OA Doppler flow patterns may also reflect the severity of diabetes retinopathy.

In their study, Fukuda et al. stated that the ratio of systolic to diastolic mean velocity in OA Doppler is closely involved in age and the severity of diabetic retinopathy [66]. Assessment by Doppler sonography can be used in patients with CRA occlusion and obstruction, ocular ischemic syndrome, diabetes mellitus, Behçet disease, and glaucoma [67,68]. Sanjari et al. [69] demonstrated that non-arteritic anterior ischemic optic neuropathy multifactorial, resulting in acute ischaemia of the optic nerve head 1 2 may be associated with decreased retrobulbar flow velocities and increased carotid wall thickness. Sturge-Weber syndrome [SWS] is a disease presenting with facial cutaneous angioma, vasculararmal formations in the brain, and ocular anomalies such as glaucoma. Cerebral blood flow is diminished and ischemia have been well documented in this condition. Conway and Hosking showed that retrobulbar hemodynamics appear to be altered in participants diagnosed with SWS irrespective of their diagnosis of glaucoma [70].

Elevation of the RI and PI is seen in hypertension, indicating increased peripheral resistance or a vasospasm [63].

The treatment with Angiotensin Converting Enzyme inhibitors improves in the RI and PI of the PCA and OA suggesting the reversibility of the effects of hypertension on vessels. Thus early diagnosis is of great importance even when the eye examination is normal, the hemodynamic effects of hypertension may have started, and it may be partially reversed by antihypertensive drugs, at least in the PCA and OA [63].

Hu et al. [71] found that the flow velocities [systolic peak velocity and end-diastolic velocity] and pulsatility indices [A/B ratio and resistance index] in the ophthalmic and central retinal arteries declined as the severity of carotid stenosis increased.

Kozobolis et al. [72] found that, carotid endarterectomy [CEA] results in the improvement of retrobulbar blood flow and perimetric parameters.

Maruyoshi et al. [73] reported that the blood flow indices of OA are clinically useful for evaluating the severity of CAD and may explain the relationship between OA circulation and systemic arterial compliance.

Several reports have shown that HIV-1-infected patients have an increased risk of cardiovascular disease [74, 75]. In their study, Grima et al. [76] showed that in HIV-1 infected patients that OARI could be a clinically significant indicator of increased cardiovascular risk, reflecting vascular resistance from atherosclerotic changes.

Systemic AA amyloidosis is a long-term complication of several chronic inflammatory diseases such as rheumatoid arthritis, Crohn’s disease, malignancies, etc [77].

The extracellular deposition of proteolytic fragments of the acute-phase reactant serum amyloid A [SAA] causes organ damage. It has been noted that only a minority of patients with long-standing inflammation actually presents with this complication, indicating the presence of disease-modifying factors, the best characterized of which being SAA1 genotype. The kidneys, liver and spleen are the main target organs of AA amyloid deposits. Proteinuria, nephrotic syndrome and/or renal dysfunction constitute the major clinical problems in more than 90% of patients [77]. Keles et al. [78] demonstrated that increased OA RI that indicates retrobulbar microvascular
dysfunction is found in amyloid-valued chronic inflammatory disease patients. Amyloidosis-related retrobulbar microvascular dysfunction may occur by a variety of mechanisms: Intramural amyloid deposition forming thickening of the wall and narrowing of the lumen, external pressure on the microvasculature by amyloid deposits and autonomic and endothelial dysfunction.\(^{[51]}\)

**Conclusions**

Assessment of OARI evaluations may predict patients with significant risk for developing cardiovascular disease, and allow timely intervention.

**Conflicting interests**

The authors have declared that no conflict of interests exist.

**Contributions**

All of the authors contributed planning, conduct, and reporting of the work. All authors had full access to all data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

**Abbreviations:**

EDRF: Endothelium-derived relaxing factor; NO: nitric oxide; ICAM-1: Intercellular adhesion molecule-1; MCP-1: monocyte chemoattractant protein-1; ABCA1: ATP-binding cassette transporter A1; MMPs: matrix metalloproteinases; CRP: C-reactive protein; IL-6: interleukin 6; IL-1: interleukin 1; TNF-α: tumor necrosis factor alpha; VCAM-1, vascular cell adhesion molecule 1; ICAM-1, Intercellular Adhesion Molecule 1; ET-1: endothelin 1; PAI-1: plasminogen activator inhibitor-1; SAA: Serum amyloid A; NF-κB: factor nuclear factor-κB; TRAF-3: TNF receptor-associated factor; MCSF: monocyte colony-stimulating factor; CV: cardiovascular; ROS: reactive oxygen species; NADPH: nicotinamide adenine dinucleotide phosphate; OA: ophthalmic artery; CRA: central retinal artery; PCA: posterior ciliary artery; PSV: peak systolic velocity; EDV: end-diastolic velocity; RI: resistive index; PI: pulsatility index; SWS: Sturge-Weber syndrome; CEA: carotid endarterectomy.

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