Oxidation of LDL and its clinical implication

Eiji Matsuura a,⁎, Graham R.V. Hughes b, Munther A Khamashta b

a Department of Cell Chemistry, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama, Japan, 2-5-1 Shikata-cho, Okayama 700-8558, Japan

b Lupus Research Unit, The Rayne Institute, St Thomas' Hospital, King's College University, London SE1 7EH, UK

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Abstract

Oxidative modification of low-density lipoprotein (LDL) is one of the earliest events in atherosclerosis. Oxidized LDL (oxLDL) represents a variety of modification of both lipid and apolipoprotein B (apoB) components by lipid peroxidation. This promotes atherosclerosis through inflammatory and immunologic mechanisms that lead to the formation of macrophage foam cells. Recent findings also suggest that oxLDL forms complexes with β2-glycoprotein I (β2GPI) and/or C-reactive protein (CRP) within atherosclerotic lesions and that these complexes appear in the circulation. Autoantibodies (auto-Abs) against oxLDL/β2GPI complexes occur in patients with systemic lupus erythematosus (SLE) and/or antiphospholipid syndrome (APS). These autoantibodies significantly correlate with arterial thrombosis. IgG auto-Abs having similar specificity emerge spontaneously in NZW×BXSB F1 mice, which generally are considered to be an animal model of APS, and these mice produce a monoclonal IgG auto-Ab (WB-CAL-1) against oxLDL/β2GPI complexes. WB-CAL-1 significantly increased the in vitro uptake of oxLDL/β2GPI complexes by macrophages, which suggests that such IgG auto-Abs are pro-atherogenic. In contrast, IgM anti-oxLDL natural Abs found in the atherosclerosis-prone mice have been proposed to be protective. The presence of such Abs in humans has been documented in many publications but their exact pathophysiological significance remains unclear. In this article, we review recent progress in our understanding of the clinical significance of oxidation of LDL, formation of oxLDL complexes, and Abs in atherosclerotic and/or autoimmune disease.

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⁎ Corresponding author. Tel.: +81 86 235 7402; fax: +81 86 235 7404.
E-mail address: eijimatu@med.okayama-u.ac.jp (E. Matsuura).

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1. Introduction

Atherosclerosis is a pathophysiological condition in which arteries undergo gradual intima thickness, causing decreasing elasticity, narrowing, and reduced blood supply. This affects the arterial wall and leads to angina pectoris, myocardial infarction and cerebral infarction. A typical feature of atherosclerotic lesions is the appearance of lipid-laden foam cells. Oxidation of low-density lipoprotein (LDL) triggers the generation of a series of oxidation byproducts. They play important roles in the early development of atherosclerosis through the recruitment of monocyte-derived macrophages into the arterial wall, and by promoting the intracellular accumulation of cholesteryl esters in these cells, resulting in the formation of foam cells [1].

Antiphospholipid syndrome (APS) is an autoimmune disease characterized by the presence of a heterogeneous group of antiphospholipid antibodies (aPL), with arterial and/or venous thromboembolic complications, and/or pregnancy morbidity [2]. Arterial involvement in APS includes coronary heart disease, stroke and peripheral vascular disease, all with common underlying features of atherosclerosis and thrombus formation. In many cases, the term, “antiphospholipid antibodies (aPL)”, is a misnomer since some, probably most, of the Abs in APS do not recognize phospholipids directly but they recognize phospholipid-binding plasma proteins, such as β2-glycoprotein I (β2GPI) and prothrombin complexed with negatively charged phospholipids, or with oxidized LDL [oxLDL] in the case of β2GPI [3–8].

Circulating oxLDL/β2GPI complexes as one of the major autoantigens were found in sera of patients with systemic autoimmune and/or atherosclerotic disease. Examples of such diseases are APS, systemic lupus erythematosus (SLE), systemic sclerosis (SSc) and type 2 diabetes mellitus (DM). Anti-β2GPI Abs against oxLDL/β2GPI complexes are frequently present in patients with SLE and/or APS [9]. Our previous reports indicated that the in vitro macrophage uptake of oxLDL/β2GPI complexes increased significantly by IgG anti-β2GPI Abs [5,6,8], suggesting that at least some APS-derived anti-β2GPI Abs are pro-atherogenic. In contrast, IgM anti-oxLDL natural Abs are found in the atherosclerosis-prone ApoE−/− and LDL-R−/− mice, and are thought to provide protection against proinflammatory oxidized moieties. IgG anti-oxLDL Abs are widely detected in patients with cardiovascular diseases (CVD), but their exact atherogenic role and clinical significance remains under investigation [10].

C-reactive protein (CRP) is an acute-phase protein that belongs to the highly conserved “pentraxin” family of plasma proteins. It binds to phosphorylcholine on oxLDL in a calcium-dependent manner to form another type of atherogenic oxLDL complexes that are called CRP/oxLDL complexes [11]. Most recently, we demonstrated the presence of CRP/oxLDL/β2-GPI complexes in DM patients [12]. These contain at least one of the major isoforms of high sensitivity CRP (hsCRP) detectable by conventional nephelometry used in a trial for diagnosing CVD in clinical laboratories.

This article will review recent progress concerning the pathophysiological significance of the oxidation of LDL, the formation of oxLDL complexes, and the appearance of atherosclerotic lesions in autoimmune diseases.

2. LDL and its oxidative modification

LDL is the major lipid carrier in plasma, and consists of cholesteryl ester, phospholipids, free cholesterol and triglyceride, and apolipoprotein B100 (apoB100). ApoB100 is a 500 kDa single peptide chain synthesized in liver, which is one of the largest monomeric proteins and is highly insoluble in aqueous solutions and cannot exchange with other lipoprotein particles.

LDL circulates in plasma, a portion traverses the subendothelial space and can be removed from the general circulation. It is also believed that LDL oxidation does not take place in the circulation because of its antioxidant properties. In the subendothelial space, the presumed site of LDL oxidation in vivo, LDL may be exposed more frequently to cell-derived oxidants. The bidirectional transit of LDL across this space may also result in a small amount of circulating oxLDL [13].

During LDL oxidation, both the lipids and apoB100 present in LDL are modified. Reactive oxygen species induce fragmentation of apoB, producing peptides ranging from 14 kDa–500 kDa. The polyunsaturated fatty acids in cholesteryl esters, phospholipids and triglycerides are also subjected to free radical-initiated oxidation to yield a broad array of smaller fragments, including aldehydes and ketones that can become conjugated to amino lipids or to apoB. The fragments further participate in chain reactions that propagate and amplify the damage.

OxLDL has been shown to be present in atherosclerotic lesions of laboratory animals and humans [14]. Incubating LDL with a monolayer of arterial endothelial cells in the presence of transition metals converted the lipoprotein to a form that was taken up much more rapidly by macrophages than native LDL. The avid uptake of oxLDL by macrophage scavenger receptors leads to lipid-laden foam cells and fatty streak development in the arterial wall, which is one of the earliest steps in the progression of the atherosclerotic...
plaque. Macrophages express structurally different scavenger receptors, such as scavenger receptors A (SR-A) and B (SR-BI), CD36, CD68, lectin like oxLDL receptor (LOX-1), and a scavenger receptor that binds to phosphatidylserine (SR-PSOX) [15]. SR-A and CD36 have been reported to be the principal receptors responsible for the uptake of modified LDL, leading to lipid loading in macrophages. CD36 was initially identified as the platelet integral membrane glycoprotein receptor for thrombospindin-1 and has also been shown to bind to oxLDL. SR-BI is another member of the class B scavenger receptor family, and similar to CD36, it is a heavily N-glycosylated protein and can bind to typical scavenger receptor ligands, including acetyl LDL and oxLDL, AGE, apoptotic cells and anionic phospholipids. Furthermore, SR-BI can also bind to native HDL and plays an important role in reverse cholesterol transport.

3. Oxidized components in LDL

Fatty acids and fatty acid-derived compounds are natural ligands for peroxisome proliferator-activated receptors (PPARs). PPARs are nuclear receptors that regulate gene expression in response to the binding of fatty acids and their metabolites. PPARs regulate the expression of genes that control lipid metabolism by binding as heterodimers with retinoid X receptors (RXR) to PPAR response elements in the promoter and/or enhancer regions of target genes. Natural eicosanoids derived from arachidonic acid via the lipoxygenase pathway, such as 8-S-hydroxytetraenoic acid and leukotriene B4, and oxidized phospholipids from oxidized lipoproteins activate PPARα. Conversely, PPARγ is a receptor for eicosanoid metabolites formed via the cyclooxygenase pathway, e.g., prostaglandins and some polyunsaturated fatty acids such as linoleic acid and its derivatives [16]. Among lipid oxidation products of oxLDL, oxidized forms of linoleic and arachidonic acids, 9-hydroxyoctadecenoic acid (9-HODE), 13-HODE, and 15-hydroxyeicosatetraenoic acid (15-HETE), were efficient stimulants of PPARγ-mediated reporter gene transcription. Thus, oxLDL regulates macrophage gene expression through ligand activation of PPARγ.

Another class of oxidized lipids is short-chain phosphatidylcholine (PC) formed by cleavage of a double bond in 18–20 carbon polyunsaturated acyl chains of the lipid. The cleavage of PC shortens the fatty acid chain and introduces an oxidized carbon containing a peroxide or carboxylate group at the former site of the double bond. When PC molecules containing linoleic acid at the sn-2 position are oxidized, the short chain PC products containing an aldehyde group (9-CHO PC) or a carboxylic acid group (9-COOH PC) at the position 9 carbon are generated. 9-CHO PC is recognized by DLH3 monoclonal Ab, which was established against human atheroma [17]. Similarly, peroxidation of arachidonoyl-containing PC produces oxidized PC with a position 5-carbon aldehyde or carboxylic acid. These short chain molecules are sometimes referred to as platelet activating factor-like oxidized PC because of their structural similarity to the platelet activating factor lipid. These oxidized PC molecules also have the ability to form Schiff bases with proteins and react with each other and form aldol condensates [18].

When LDL undergoes oxidation in vitro, a number of changes in lipid composition occur, including a substantial loss of free and esterified cholesterol and generation of oxidation products of cholesterol, i.e., oxysterols. Oxysterols possess many potent and diverse biological activities in vitro, several of which may be important in the initiation and/or development of atherosclerosis. In Cu-oxLDL, 7-hydroperoxycholesterol (7OOH) is an important intermediate in the early stages of oxidation. In heavily oxidized LDL up to 50% of the cholesterol is converted to 7-ketocholesterol (7 K), 7-hydroxycholesterol (7OH), and 5,6-epoxycholesterol are also formed from oxidation of the B-ring of cholesterol. 7-ketocholesterol (7KC) inhibits cholesterol efflux from macrophage foam cells induced by apolipoprotein A-I (apoA-I) treatment. Such oxysterols may promote foam cell formation in atherosclerotic lesions by preventing effective clearance of excess cholesterol.

Esterified fatty acids in oxLDL are extensively oxidized, with up to 90% of the steryl ester acyl groups modified. Linoleic acid is a predominant polyunsaturated fatty acid present in LDL cholesteryl esters. In mildly oxidized LDL, cholesteryl hydroperoxy-octadecadienoate (Chol-HPODE) and cholesteryl hydroxyoctadecadienoate (Chol-PODE), were detected as the main oxidation products. Chol-HPODE was reported to inactivate platelet-derived growth factor. Lipid peroxidation products that remain esterified to the parent lipid are termed core-aldehydes, and one of the major core-aldehydes found in Cu-oxLDL is 9-oxo-nonanoyl cholesterol, which is derived from cholesteryl linoleate.

4. β2GPI and its atherogenic antigen complexes

β2GPI is a major target antigen for aPL present in patients with APS. β2GPI is a 50 kDa single-chain polypeptide composed of 326 amino acid residues, arranged in 5 homologous repeats known as complement control protein domains. The crystal structure of β2GPI
has been determined and its fifth domain contains a patch of positively charged amino acids that likely represents the binding region for phospholipids [19]. β2GPI binds to negatively charged molecules including phospholipids, heparin, and certain lipoproteins, as well as to activated platelets and apoptotic cell membranes. The aforementioned interactions of β2GPI are calcium independent. This binding may aid the clearance of apoptotic cells from circulation but its entire physiological role is not completely explained. Furthermore, β2GPI has anticoagulant properties, as it has been shown in vitro to inhibit the intrinsic coagulation pathway, prothrombinase activity, and ADP-dependent platelet aggregation.

β2GPI specifically binds to oxLDL [5,8]. Lipids extracted from oxLDL contained the β2GPI ligands, such as 7-ketocholesterol-9-carboxynonanoate (9-oxo-9-(7-ketocholest-5-en-3β-yloxy) nonanoic acid) (oxLig-1) (Fig. 1B) and 7-ketocholesterol-12-carboxy (keto) dodecanoate (oxLig-2) (Fig. 1C) [6,8,20]. These ligands are assumed to be oxidized products of cholesterol linolate, the major cholesteryl ester of LDL (Fig. 1A). Both of them had 7-ketocholesterol and a carboxyl function at the ω-
position of acyl chain (Fig. 1D), as a common structural feature. Our recent data indicate that ω-carboxyl function of the acyl chain and the ketone function at position 7 of the cholesterol backbone are necessary for high-affinity interaction of these ligands with β2GPI.

Since β2GPI specifically binds to oxLDL via lipid ligands that have the potential to react chemically with the protein, the resulting oxLDL/β2GPI complex was further characterized. The ligand-mediated non-covalent interaction of β2GPI and oxLDL converted, in a temperature- and time-dependent manner, to much more stable complexes (Fig. 2A). The stable complexes are possibly formed by both electrostatic interaction and some labile covalent interaction, such as Schiff-base formation between ε-amines of lysine residues of β2GPI and oxidatively generated aldehydes on the Cu-oxLDL particles. These stable oxLDL/β2GPI complexes frequently are detected in sera from antoimmune and/or atherosclerotic patients with APS, SLE, DM, etc.

5. CRP and its complexes with oxLDL/β2GPI

CRP was originally discovered during the course of studies of patients with Streptococcus pneumoniae infection. It binds to specific molecular configurations that are typically exposed during cell death or found on the surfaces of pathogens [21]. Synthesis of CRP increases within hours after tissue injury by infection, and its regulation by IL-6 suggests that the protein contributes to host defense and belongs to the innate immune response.

CRP is a 118 kDa acute-phase reactant that belongs to the highly conserved pentraxin family of plasma proteins. The protein is a pattern recognition molecule, that consists of five identical, noncovalently associated ~23 kDa protomers arranged symmetrically around a central pore. Each protomer has a recognition face with a phosphocholine binding site consisting of two coordinated calcium ions adjacent to a hydrophobic pocket. The opposite face of the pentamer is the effector face, where complement C1q binds and Fcγ receptors are presumed to bind.

An association between minor CRP elevation in plasma, between 3 and 10 μg/ml, and future major cardiovascular events has been recognized, leading to the recommendation that patients at intermediate risk of coronary heart disease might benefit from CRP measurement [22]. It is suggested that many of these conditions involve a low level of underlying chronic inflammation that could be reflected by these minor CRP increases.

![Diagram of complex formation](image)

Fig. 2. Complex formation of oxLDL with β2GPI and CRP. (A) β2GPI Ca-independently interacts with oxLDL in a two-step reaction. Initially, β2GPI binds to oxLDL via the specific and high-affinity ligands, namely, oxLig-1 and -2. Stable complexes between apoB100 and oxidized lipids are then formed at neutral pH. (B) CRP Ca-dependently binds to oxLDL via phosphorylcholine moiety on oxidized phosphatidylcholine. At the physiological Ca concentration, such as 1.25 mM, both β2GPI and CRP simultaneously form the complexes with oxLDL.
Furthermore, CRP up-regulates the expression of adhesion molecules in endothelial cells [23] and binds to oxLDL via oxidized phosphatidylcholines, but not to native LDL. CRP enhances binding of oxLDL to macrophages via Fcγ receptors [24]. CRP also interacts with enzymatically degraded LDL in a Ca2+-dependent manner and the resulting complex enhances complement activation in human serum. During inflammation, the clearance of CRP-opsonized particles would be predicted to enhance the clearance of modified LDL via phagocytic complement receptors as well.

Several recent reports suggested that CRP also is produced by endothelial cells, macrophages, and smooth muscle cells in atherosclerotic lesions [25–27]. We demonstrated that CRP forms complexes with oxLDL as well as with β2GPI at physiological calcium concentrations (Fig. 2B). This probably occurs in atherosclerotic lesions and the CRP/oxLDL/β2GPI complexes are released into the circulation [12]. Such CRP/oxLDL/β2GPI complexes were detected in DM patients with atherosclerosis (Fig. 3). In contrast, non-complexed CRP was only detected in pyrogenic patients.

Annexin V is well known to bind to negatively charged phospholipids and is one of the major candidates for the target of Abs appeared in APS. It has been demonstrated that annexin V also can bind to oxLDL at physiological concentrations and does not interfere with the binding of CRP to oxLDL [24]. Thus, annexin V/oxLDL and/or annexin V/oxLDL/CRP complexes may be candidates for the target of aPL and may also be associated with atherogenesis.

6. Clinical significance of oxLDL complexes

Lipid peroxidation resulting in oxLDL production is a common occurrence in patients with systemic autoimmune diseases as well as in chronic inflammatory (non-autoimmune) disorders and certain systemic infections. OxLDL binds to β2GPI to form oxLDL/β2GPI complexes in the intima [5,7,8] and oxLDL/β2GPI complexes circulate in patients with SLE, APS, and SSc with generalized vascular complications [28,29]. The formation of these complexes might be related to atherosclerosis, i.e., chronic inflammation of the vasculature (and oxidative stress) that occurs in those patients. In contrast, these complexes are not significantly elevated in patients with rheumatoid arthritis (RA), one of the major rheumatic diseases [28].

OxLDL/β2GPI complexes have also been detected in patients with DM [12], chronic renal diseases (i.e., IgA nephropathy) [30], and infectious endocarditis. Oxidative stress from chronic hyperglycemia may promote oxLDL/β2GPI complex formation and premature CVD in DM [12]. Dyslipidemias have been implicated in the pathogenesis of chronic renal disorders [31] and oxLDL is known to contribute to progressive glomerulosclerosis [32]. These findings suggest that oxLDL/β2GPI complexes represent a common metabolic product relevant to atherogenesis. oxLDL/β2GPI complexes also have been implicated as pro-atherogenic autoantigens [7]. Thus, their presence may represent a risk factor or an indirect but significant contributor for atherothrombotic complications in autoimmune patients.

We have demonstrated that CRP/oxLDL/β2GPI complexes were predominantly found in sera of DM patients with atherosclerosis [12]. In contrast, non-complexed CRP isoforms were present in sera of patients with acute/chronic inflammation, i.e., various pyrogenic diseases, RA and DM. Immunohistochemistry staining co-localized oxLDL, β2GPI, and CRP in carotid artery plaques but not in synovial tissue from RA patients, suggesting that complex formation occurs during the development of atherosclerosis. Serum levels of CRP correlated with soluble forms of ICAM-1 and VCAM-1, and oxLDL/β2GPI complexes correlated with total cholesterol and hemoglobin A1c. Overall, the generation of CRP/oxLDL/β2GPI complexes seems to be associated with arterial inflammation, hyperglycemia, and hypercholesterolemia. CRP/oxLDL/β2GPI complexes can be distinguished from pyrogenic non-complexed CRP isoforms and may represent a more specific and predictive marker for atherosclerosis.

7. Antibody-mediated regulation of atherosclerosis

The causal relationship between cholesterol metabolism and atherosclerosis is well established. In particular, the oxidation of LDL has been identified as an early proatherogenic event. The oxidation products of LDL are cytotoxic, chemotactic, and proinflammatory, and promote the formation of macrophage-derived foam cells. The accelerated development of atherosclerosis also has been observed in patients with not only CVD but also systemic autoimmune diseases, such as SLE and/or APS.

IgG anti-oxLDL/β2GPI Abs also have been measured in SLE, SSc and RA patients. SLE and SSc patients had significantly higher anti-oxLDL/β2GPI Ab levels as compared to the controls. RA patients showed higher antibody levels than the controls but this difference was not statistically significant. IgG anti-oxLDL/β2GPI Abs were significantly higher in SLE patients with APS compared to SLE controls without APS. IgG anti-oxLDL/β2GPI Abs were evaluated for their association with the major clinical manifestations
A stronger association with arterial thrombosis compared to venous thrombosis and pregnancy morbidity was observed. These observations may provide concrete explanation for the accelerated (premature) development of atherosclerosis in autoimmune patients. Thus, IgG anti-oxLDL/β2GPI antibodies appear to be useful serologic markers for atherothrombotic risk in autoimmune patients with high specificity for APS.

The in vitro macrophage uptake of oxLDL/β2GPI complexes was significantly enhanced in the presence of IgG anti-β2GPI Ab, i.e., WB-CAL-1, which is derived from nonimmunized NZW × BXSB F1 male mice as an animal model of APS [5,34]. This observation supports the idea that IgG anti-β2GPI auto-Abs may be proatherogenic because the in vivo oxLDL uptake is likely mediated by Fcγ receptors rather than by scavenger receptors.

Oxidation of LDL generates a variety of oxidatively modified lipids and proteins that present highly immunogenic neoepitopes. In atherosclerosis-prone ApoE−/− mice, the autoimmune response to epitopes of oxLDL is vigorous. Palinski and colleagues isolated a panel of B-cell hybridomas with specificity for oxLDL neoepitopes from the spleens of diseased ApoE−/− mice that had not received experimental immunization or in vitro stimulation [35]. The monoclonal IgM natural Abs were termed “EO” antibodies, which bound strongly to MDA-LDL and Cu-oxLDL.

These Abs recognize the phosphorylcholine of oxidized phospholipids, such as 1-palmitoyl-2-(5-oxovalerol)-...
phosphatidylcholine (POVPC), an oxidation product derived from 1-palmitoyl-2-arachidonyl-phosphatidylcholine (PAPC). The genes encoding the antigen binding site of EO6 antibody were found to be genetically and structurally identical to those of T15 Abs [36]. T15 is a classic anti-EO6 antibody were found to be genetically and structurally line (PAPC). The genes encoding the antigen binding site of derived from 1-palmitoyl-2-arachidonyl-phosphatidylcholine (POVPC), an oxidation product.

Recent, high titers of anti-oxLDL Abs have been documented in blood and atherosclerotic lesions of humans and animals [10,38-40]. Epidemiologic surveys indicate that anti-oxLDL titers often correlate with classical risk factors of CVD as well as with clinical markers of disease severity.

Collectively, these data suggest that IgM Abs that are related to “natural Abs” can bind oxLDL and interfere with its uptake by macrophages. This interference may prevent both scavenger receptor mediated uptake and Fcγ receptor mediated uptake of IgG immune complexes containing β2-GPI/oxLDL. The interference by natural Abs may also serve to suppress the uptake and processing of β2-GPI/oxLDL complexes that are required by antigen producing cells to elicit the production of the IgG Abs.

Take-home messages

• Oxidized LDL (oxLDL) represents a variety of modification of lipid and apolipoprotein B components by lipid peroxidation
• β2-glycoprotein I (β2-GPI), a major autoantigen for antiphospholipid syndrome, can complex with oxLDL and with oxLDL/C-reactive protein (CRP) complexes and these complexes may be novel markers for predicting and monitoring atherosclerosis/cardiovascular diseases.
• Antibodies against oxLDL and its complexes may also be markers of cardiovascular diseases.

References

Anticardiolipin, anti-oxLDL in periodontitis in essential hypertension

Oxidation of lipoproteins is a process deeply studied in the context of atherosclerosis and it was recently reviewed by Gounopoulos et al. (Minerva Cardioangiol 2007; 55: 821-37). After LDL enters in subendothelial region, it is oxidized and generates foam cells. Oxidized LDL (oxLDL) is immunogenic and results in generation of autoantibodies against oxLDL. It is known that periodontitis is a chronic inflammatory process associated with atherosclerosis. Antibodies against oxLDL and also cardiopin have been described in atherosclerotic patients. In a recent paper, Turkgoglu et al. (J Periodontol 2008; 79:332-40), have studied seventy-two individuals separated in four groups as follow: healthy, hypertensive with good health mouth, hypertensive with periodontitis, and hypertensive with gingivitis. They found that the frequency of IgM anticardiolipin was higher in hypertensive and periodontitis subjects than to the other three groups. Additionally, a positive correlation was seen between IgM anticardiolipin levels and supragingival plaque, as well as, bleeding on probing and probing depth. This study suggests a link among inflammation (gingivitis), autoimmunity and hypertensive disorder, however has a limited number of enrolled people and this finding has to be taking account when its results are analyzed.