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Pathological Mechanism of Atherosclerosis

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Abstract

Atherosclerosis is a multifactorial, smoldering, focal (intima of bifurcated blood arteries), chronic, progressive asymptotically, immune-inflammatory, disorder driven by lipid imbalance, in the large to medium sized (upto3mm external diameter) arteries with many cardiovascular clinical manifestations. Atherosclerosis developmentinvolves many cells, organs and even disturbed blood flow. The progression of atherosclerotic disease depends on the presence, degree, and persistence of risk factors like high-fat diet, smoking, hypertension, history of heart diseases, or diabetes. Endothelial dysfunction, ROS, accumulation of LDL, recruitment of Monocytes and T cells, differentiation of monocytes into macrophages and foam cells, formation of plaque and rupturing of plaque are key steps behind the clinical manifestation of atherosclerosis in cardiovascular diseases. This article describes the pathogenesis of atherosclerosis, possibility of therapeutically targeting mechanism and interventions which can be helpful to reverse or slower the atherosclerosis.

Keywords: Endothelial dysfunction; ROS; LDL; oxLDL; Macrophages; Foam cells; Plaque; NOS; hypercholesterolemia; microRNAs (miRNAs); proprotein convertase subtilisin/kexin type 9 (PCSK9) gene; telomere length (LTL)**.**

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1. Introduction

According to WHO the Cardiovascular diseases (CVDs) cause nearly 17.9 million deaths per year. Atherosclerotic related vascular disease continues to be the leading cause of death in the Western world. The frequency of CVD in specific geographical populations provides evidence of inheritance. This also shows the prevalence and frequency of different risk factors among different populations. [1]In the lower and middle income countries the prevalence is also alarming. CVD is cause of 1/3 deaths in the world [2]. The underlying cause of the CVD process in coronary heart disease, stroke, and heart attack is atherosclerosis. Heart attack caused 17.3million death is 2008 and stroke cause 6,2million death. [3]

The atherosclerosis causes coronary artery disease (CAD), cerebrovascular disease (Stroke), aorta and related arteries diseases including hypertension and peripheral vascular disease (PVD). The economic cost of the CVD is very devastating. [4] The risk factors fatty diet, smoking, genetic history, diabetes and hypertension and their frequency of persistence determine the appearance and progression of the atherosclerosis [5-6]. The atherosclerosis is fueled by low density lipoproteins is inflammatory process which harden the blood arteries even block them, and stop the oxygen supply to the tissues, due to disturbed blood supply to the area. [7]

The modifiable risk factors are distributed with different frequency level in different regions [8] there is need to provide accessible interventions on the regional need basis to control the CVD [9]. There is need to deeply understand the atherosclerosis mechanism to develop interventions and therapeutics [10]. Many efforts from last decades made to understand the atherosclerosis but it is still considered difficult to treat. The basic mechanism which elucidated in literature starts with high level of LDL in the plasma, accumulation of LDL in vascular intima, oxidation of LDL by ROS in intima, endothelial expression of adhesive molecules, recruitment of macrophages to engulf the oxidized LDL (oxLDL), formation of foam cells, fatty streak appearance and plaque formation and rupture [11].

Many genes, molecules, cells, enzymes are involved in the whole process. Inflammation also play important role [12]. In order to prevent atherosclerosis from initiation to its progress we need to understand many underlying mechanisms to identify the possible intervention opportunities. Many years ago appearance of fatty streak in the blood vessels was considered as initiation point of atherosclerosis can be seen grossly or histologically, this can appear in any age [13]. Cellular and molecular research proved that fatty streak is only visible effect there are many processes behind it [14].

Now it is considered endothelial dysfunction is starting point of atherosclerosis which can be induced by atherosclerosis related risk factors [15].Many industrial and traffic pollutants such as carbon monoxide (CO), Carbon disulfide (CS_2) and toxic metals Lead (Pb), Cadmium (Cd), Arsenic (As) and Mercury (Hg) can play role in atherosclerosis. The CO cause hypoxia which cause oxidative stress and atherosclerosis. The CS_2increase atherosclerosis by interfering with lipid metabolism and serum cholesterol, the toxic metals cause atherosclerosis by ROS and lipid peroxidation [80-81-82-83-84-85].

The diagram below shows the linear flow of cellular events happening in the atherosclerosis.

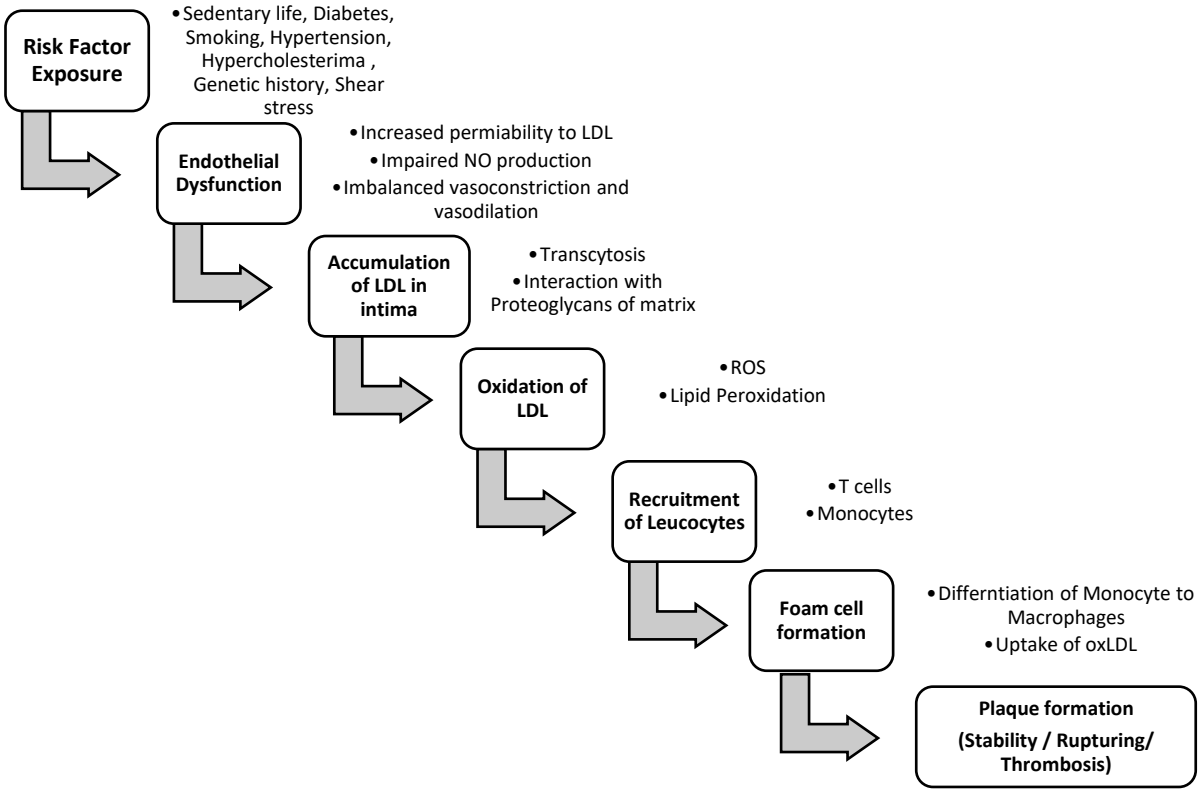

Figure 1: Linear Flow of Cellular Events in Atherosclerosis

Risk factors external, genetic or internal can initiate endothelial dysfunction. Ds- Functioned endothelial has impaired Nitric oxide (NO) production with increased permeability to low density lipids (LDL) and imbalanced vasoconstriction and dilation, LDL transcytosis the endothelium in intima and binds wit extracellular proteoglycans. Reactive oxygen species (ROS) in intima oxidized the components of LDL to oxLDL. These oxLDL components induce the production of adhesion molecules, T cells and Monocytes adhere with dysfunctional endothelium diapedesis into intima, here monocytes differentiate into macrophages which engulf oxLDL and form the foam cells. Foam cells progress into fatty streak and calcified plaque narrowing the artery which ruptures to form clot through thrombosis. Artery Narrowing and blockage interrupt oxygenated blood to the tissue which cause different clinical manifestations in the form of cardiovascular disease.

2. Endothelial dysfunction

Normal functional endothelium does not allow adhesion and penetration of blood cells (leucocytes and platelets) on its surface, balanced vasoconstriction and vasodilation, inhibition of vascular smooth cells proliferation and migration [16]. Some adhesive molecules are required for adhesion of blood cells with endothelium. These molecules are expressed on the time of demand under different circumstances. Atherosclerosis starts when endothelium becomes dysfunctional due to local or external factors. The atherosclerosis event occurs at the point of artery branch where endothelial cells face disturbed blood flow [17]. Natural vasoconstrictor angiotensin-II can produce reactive oxygen species (ROS) which can induce endothelial cells to express adhesive molecules [18].

Defective production of Nitric Oxide (NO) plays important role in dysfunction of endothelial cells [19]. Endothelial dysfunction can be induced by many of the atherosclerotic risk factors [20].Laminar blood flow keeps endothelium healthy, by maintaining its glycocalyx layer and coaxial alignment [21], oscillatory shear stress with MEK5 signaling induce expression of eNOS its anti-atherogenic role is through NO [22].

A dysfunctional endothelium should have defective NO machinery and it can produce molecules which can help cells to adhere and penetrate inside the intima to initiate the atherosclerotic process. The morphological changes which occur in dysfunction endothelial cells increased permeability of the plasma membrane to LDL and VLDL, ApoB containing chylomicrons through transcytosis into the sub endothelial space [23-24].Endothelial cells produce NO from L-Arginine with the help of Nitric Oxide Synthase (NOS) enzyme. Shear stress increase eNOS production, caveolin-1 protein inhibit activity of eNOS, calcium ions activate eNOS. Asymmetric dimethylarginine (ADMA) inhibits NO. Similarly, isoprenoid geranylgeranyl pyrophosphates, intermediate of cholesterol synthesis pathway also inhibit eNOS [25-26]. Mitochondrial oxidative stress reduces the NO production and enhances the inflammation and atherosclerosis [61]. In the vascular wall endothelial cells mitochondrial dysfunction is very important it puts its contribution through oxidative stress, impaired mitophagy and metabolic breakdown can be therapeutically targeted, it also need research to elaborate clear mechanism [62-63].

3. Intake and Oxidation of LDL

Fatty diet can increase level of LDL in plasma when reached the site of endothelial dysfunction it transcytosis to the intima, if cross a threshold level can be pathological [27] the ApoB100 component of the LDL remained bind with the proteoglycan matrix of the intima [28]. Under ROS, these LDL are oxidized by the lipid peroxidation process [29] these oxidized components of the LDL can serve as epitopes for the immune system [23] lipoxygenase and phospholipase A-2 enzymes alter LDL to oxLDL [31].These modifications make LDL higher in density, degrade ApoB, amend lysine residue of ApoB and hydrolyzing phosphatidylcholine [32] Polyunsaturated fatty acids are more oxidized than monounsaturated fatty acids. [33]

4. Expression of adhesion molecules

The oxLDL components now can induce expression of different molecules by the endothelial cells via LOX-1 receptor. LOX-1 is endothelial receptor for oxidized LDL [34] it is over expressed in atherosclerotic condition; this is lectin like receptor. The oxidized components of the LDL like lyso phosphatidyl choline (PC), oxidized phospholipids, 9-hydroxyeicosatetraenoic acid, Modified Apo B, cholesterol, oxysterols and other oxidized lipids of LDL each can exert Proatherogenic Effects in different ways by interacting with endothelial cells, monocytes and T cells [35]. The endothelial cells are induced by oxLDL to produce adhesion molecules such as intracellular adhesion molecule -1 (ICAM-1) and vascular cell adhesion molecule (VCAM-1) [36] these molecules allow leucocytes to attach on the surface of endothelium for future infiltration into the intima.

5. Recruitment and Differentiation of Leucocytes

Normal endothelial cells have NO which stops monocytes to attach with endothelial cells. OxLDL reduce the

NO production in the endothelial cells, it encourages monocytes to attach with endothelium and ICAM-1 and VCAM-1 adhere them with endothelial cell [37]. Endothelial cells also express E-Selectin and P-Selectin molecules on its surface which helps in rolling of leucocytes on endothelium for infiltration into the intima [38] in response to Fractkalkine, MCP-1 and RANTES (Ccl5) [39]. This migration is in sequence chemoattractant MCP-1 (monocyte chemotactic protein--1) attract leucocytes, CCR-2 is receptor of MCP-1, VCAM-1 firmly adheres leucocytes, P and E Selectins helps in rolling. By this way Monocytes and T cells diapedesis into intima. Inside the intima migrated monocytes differentiate into macrophage. The cytokine Macrophage-colony stimulating factor (M-CSF) produced by endothelial cells and also stromal cells (fibroblast cells) [40] differentiate monocytes to macrophages. Now macrophages are highly efficient for taking oxLDL through pattern recognition receptor, i.e. Scavenger receptors (SR-A), toll-like receptors and CD36 [41-42].

These differentiated macrophages either pro-inflammatory with M-1 phenotype or anti-inflammatory M-2 phenotype, depending upon the signals, M-1 predominates in atheroma progression phase and M-2 is abundant at the time of atheroma regression [43]. Th1 cells produce LPS (lipopolysaccharide), Interferon-g (IFN)-g and Tissue necrosis factor alpha (TNF-a) are pro-inflammatory and induce M-1. Interleukin-4 (IL-4), Interleukin-10, Interleukin-13 and tumor growth factor beta (TFG)-b are anti-inflammatory and are produced by Th-2 cells [44]. Other pro-inflammatory biomarkers associated with atherosclerosis are C reactive protein (CRP) of Hepatocyte, Tumor necrosis factor alpha and Interleuken-1 (IL-1), Interleuken-12 (IL-12), Interleuken-15 (IL-15), Interleuken-8 (IL-8) by Macrophage and Interleuken-6 (IL-6) by macrophages and endothelial cells act as acute phase reactant, cytokines, and chemokines [45].

6. Uptake of oxLDL and Formation of Foam cells

The scavenger receptors (SR)-A, −BI on the surface of the macrophage intake oxLDL these modified derivatives inside the macrophage stops the expression of LDL receptors but not the scavenger receptors [46]. The Liver X receptors (LXRs) and peroxisome proliferator-activated receptors (PPARs) are transcription factors, efficiently work for cholesterol and fatty acids homeostasis inside the macrophages, and prevent foam cell formation and development of atherosclerosis [47], the athero-protective mechanism works as cholesterol efflux. Smooth muscle cells of the vascular walls can also express the receptors for the LDL and can form foam cells [48]. Many micro RNAs (miRNAs) regulate the genes involved in the lipid up taking in the macrophages and conversion to foam cells [49]. ATP binding cassette transporter A1 (ABCA1) expressed in liver and macrophages is plasma membrane lipid pump, when its level is decreased lipids are trapped inside the macrophage and convert to foam cell [50]. Smooth muscle cells form a fibrous capsule around these foam cells and fatty streak is formed, which can be seen histologically. The lipid loaded macrophages cells undergoes apoptosis mediated by P^{53} and FAS [51], however in endothelial dysfunctional cells miR-590 inhibits the oxLDL induced expression of apoptotic machinery factors P^{53} and BAX. Apoptotic machinery disturbance enables now macrophages engorged with loaded lipids and endothelial cells to progress towards plaque formation.

7. Plaque formation and rupturing

This fatty streak can be a physiological process to protect the tissue from cytotoxic effects of the oxLDL [52], the

progression of streak depends upon the persistence of the risk factors exposure [53], and regression also depends on the balance between level of LDL in plasma and intima. T cells which produce the IFN-gamma plays role in plaque destabilization by reducing fibrous cap [54], Macrophages, Th-3 cells and smooth muscle cells can express TGF-beta which is involved in plaque stabilization by stimulating the collagen synthesis and cap formation [55]with time the focal calcification of plaque occurs. Large number of macrophages along with pro-inflammatory cytokines accumulate in the vascular wall late in the atherosclerosis secreting the matrix metalloproteinase (MMPs), degradation of the extracellular matrix occurs, rupture the plaque, cause bleeding and thrombosis [56]. This disruption and thrombotic event can lead to the clinical manifestations in the form of cardiovascular diseases.

8. Genes, Enzymes, Cytokines, Chemokines of atherosclerosis

Proprotein convertase subtilisin/kexin type 9 (PCSK9) gene located on the short arm of chromosome 1 near the familial hypercholesterolemia, mainly responsible for plasma level of LDL especially cholesterol in LDL [57-58] by controlling LDL receptors (LDLr). NOS genes has three isoforms, located on 12, 17 and 7, isoform NOS3 express membrane bound endothelial NOS enzymes [59], in macrophages isoform NOS2 is expressed to form soluble NOS enzyme [60]. ADAMTS-16 is quantitative trait gene has been investigated for its role in the regulation of blood pressure [64-65], which is risk factor in the form of hypertension, however ADAMTS4 can reduce the monocyte infiltration in intima [66]. Telomere on the end of chromosomes have repetitive sequence of (TTAGGG)n, telomere length (LTL) differs in tissues. In leucocytes in the peripheral tissues LTL is associated with the CVD risk [67] LTL shortening plays role in atherosclerosis and age related CVD [68-69]. Other atherosclerotic genes are 12/15-LO, M-CSF, MCP-1, CCR2, P- and E-selectin, CXCR-2, SR-A, CD36, IFNg receptor, CD154 and IL-10, however Paraoxinase, Apo A-I, PPARg and SR-B1 are antiatherogenic genes [70].

9. Diagnosis and clinical interventions

Initiating step in atherosclerosis i.e. Endothelial dysfunction can be detected by fluid mediated dilation (FMD) and acetylcholine test, the level of CRP test also relates with the endothelial dysfunction and cardiac event [71-72].Other noninvasive method such as Doppler ultrasound studies are in use to detect the endothelial dysfunction. Lipid lowering therapy (HMG-CoA), reductase inhibitors as in case of mostly used statins can correct endothelial function, Atorvastatin and pravastatin treatment with time can reduce the level of CRP [73-74-75-76]. PCSK9 inhibitor armamentarium clinically is in use and can reduce the cholesterol level in plasma [77]. The telomere based therapies in the experimental mice showed improved cardiac functioning [78], this approach can be a prospective therapy for non-modifiable factor such as age.

10. Risk management interventions

In the effort to manage the risks for the atherosclerosis there is need to lessen the intensity of exposure to the atherogenic risk factors i.e. elevated LDL, Smoking, Sedentary life, Alcohol use, diabetes, hypertension, genetic background. Among these age and genetic back ground are non-modifiable. But other factors can be managed by the social and clinical interventions. Among these are adaptation to healthy life style to reduce the chances of

endothelial dysfunction and initiation of atherosclerosis. Second option is usage or exposure to the athero-protective factors which are exercise and use of HDL and others which can contribute to the "reverse cholesterol transport" mechanism

11. Conclusion

Consistent exposure to the atherosclerotic risk factors either external or inside the body intimate endothelial dysfunction. This first step of atherosclerosis is detectable by invasive and noninvasive methods and correlate with the risk factors presence. Early detection along with risk factor management and adaptation to the athero-protective clinical interventions can restore the endothelial dysfunction. Once dysfunctional endothelial inhibits NO production and starts expression of adhesive molecules, now leucocytes adhere and diapedesis in intima, differentiate into foam cells, fatty steak, calcified, rupture and thrombotic event occurs with clinical manifestations in the form of cardiovascular diseases.

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