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Relationship between Natural Activators of Peroxisome Proliferator Activated Receptors (PPARs) and Endothelial Dysfunction in Patients with Peripheral and Coronary Artery Disease

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Abstract

Atherosclerosis is considered as a systemic disease which leads to functional and structural changes in several segments of the arterial system. Morbidity and mortality is mostly caused by Peripheral arterial occlusive disease (PAOD). Vascular endothelium, which is a versatile multifunctional tissue, had synthetic and metabolic properties. Endothelial injury may be responsible, for the initiation of atherosclerosis and vascular lesions which are followed by monocyte infiltration, macrophage differentiation, and migration of smooth muscle cells. von Willebrand factor (vWF), acts as a glycoprotein synthesized mainly by endothelial cells, and is an indicator to endothelial damage. It represents the most sensitive marker of peripheral atherosclerosis. Moreover, intima-media thickness (IMT) increasing is used as a non-invasive of early arterial wall alteration marker and is one method of assessing the development of early atherosclerosis. The Peroxisome proliferator-activated receptors (PPARs) regulate both lipid and lipoprotein metabolism and glucose homeostasis so they influence cellular's proliferation, differentiation and cell apoptotic process. PPAR-alpha activity occurs by leukotriene B4, while, PPAR-gamma activator is the oxidized low-density lipoprotein (ox-LDL). PPAR activation decreases the incidence of cardiovascular disease. Leukotriene B4 (LTB4) causes vascular permeability and attraction and activation of leukocytes. So, Oxidized low-density lipoprotein (ox-LDL) plays a crucial role in the inflammatory process genesis occurring in the atherosclerotic lesion. Increased ox-LDL levels have a direct relation to the acute coronary syndromes severity. The levels of vWF, LTB4 and ox-LDL were measured to find the relationship between these parameters and the severe effects of the disease.

Key words: Atherosclerosis, Peripheral arterial occlusive disease, Coronary artery disease, Flow mediated dilation, Intima media thickness, Peroxisome Proliferator Activated Receptors, von Willebrand factor, Leukotriene B_4, Oxidized low density lipoprotein.

1-Introduction

I- Peripheral and Coronary artery diseases:

Atherosclerosis is the systemic disease which leads to functional and structural changes in several segments of the arterial system. [1] The Peripheral arterial occlusive disease (PAOD) is considered as the major cause of morbidity and mortality. It is characterized by atherosclerotic lesions in large vessels and disturbances on the micro-circulator level, including local regulation on tone of vascular system also on micro-vascular perfusion. [2]

Its reaction is inflammatory at the wall of vessels in response to dyslipidemia along with endothelial distress including, the inflammatory recruitment of leukocytes with the activation of local vascular cells. Atherosclerotic plaques are asymptomatic, having obstruction affect which causes stable angina; few become prone to thrombosis considered to be vulnerable leading to athero-thrombosis which includes the acute myocardial infarction (AMI), and the ischemia of lower limb. [3]

The Artery diseases, markedly atherosclerosis and arteriolosclerosis, are the most lethal diseases in the industrial countries leading to sudden death, myocardial infarction, stroke, kidney failure, and limbs ischemia. [4]

One should realize that PAOD patients and atherosclerotic narrowing are likely to be present in other territories such as, the coronary and cerebral circulation.[5] PAOD may be accompanied by intermittent claudication, rest pain or it may be asymptomatic necessitating the use of new diagnostic modalities(e.g. ankle-brachial systolic pressure index).[6]

Myocardial ischemia takes place as a markedly imbalance occurring between oxygen supply (and other essential myocardial nutrients) and the myocardial demand for these substances. Coronary artery disease gives rise to a wide variety of clinical presentations. These range from relatively stable angina to the acutely coronary diseases of unstable angina and myocardial infarction. [7]

Myocardial infarction causes the majority of morbidity and mortality in both developed and developing countries. Acute myocardial infarction (AMI) definition is the myocardial necrosis occurring due to cessation of its blood flow. [7]

Approximately 900,000 persons in United States experiences acute myocardial infarction annually, of these about 225,000 die, of those who die, approximately one half dies within one hour of the onset of symptoms before reaching a hospital.[8,9] By far coronary atherosclerosis commonly causes obstructive coronary disease, resulting in myocardial ischemia.[9]

Coronary artery blood flow to the myocardial region is reduced by the mechanical obstruction which is due to atheroma, thrombosis, spasm, embolus, coronary ostial stenosis or coronary arteritis. [10]

I.A.Pathogenesis of coronary atherosclerosis

Atherosclerosis of coronary is a process which is characterized by lipid and macrophages accumulation also smooth muscle cells migration to the intimal plaques in both large and medium sizes of epicardial coronary arteries. The vascular endothelium has an important effect on maintaining vascular integrity and homeostasis. [11]

Mechanical shear stresses, biochemical abnormalities and immunological factors may contribute to initial endothelial injury which is believed to trigger atherogenesis, the resultant

endothelial dysfunction allows accumulation of oxidized lipoproteins which are engulfed by macrophagic cells to produce lipid laden foam cells. [11-13]

Release of host cytokines, like the platelet-derived growth factor, also the transforming growth factor β can promote further accumulation of macrophages as well as smooth muscle cells migration and proliferation. [14]

Atherosclerosis of the coronary artery is considered as a progressive disease. Disease progression occurs by two basic mechanisms, gradual increase in plaque size by incorporation of lipids and the more unpredictable changes in lumen encroachment resulting in plaque rupture. Plaque rupture results from fissuring in fibrous cap leading to exposing extracellular matrix (in particular tissue factor) which triggers platelet adhesion, aggregation and activation. Plaques that are vulnerable to rupture are those having large lipid cores, thinner fibrous caps (with high densities of activated macrophages and loss density of smooth muscle cells). [15]

The resultant platelet activation leads to release of active mediators (including thromboxane A_2, 5-hydroxytryptamine and adenosine diphosphate (ADP) which triggers activation of platelet, also, activation of coagulation cascade. As a result the thrombus formed reducing the lumen further. [16]

In addition, vasoactive substances such as thromboxane A_2 and 5-hydroxytryptamine, released from aggregated platelets cause transient local vasoconstriction, and dysfunctional endothelium produces less vasodilator substances such as nitric oxide and prostacyclin.[17] Myocardial infarction always occurs within the patients with atheroma in coronary artery resulting in plaque rupture and super added thrombus. This occlusive thrombus consists of platelet rich core (white clot) and bulkier fibrin rich (red) clot, about six hours after the onset of infarction, the myocardium is swollen and pale, and at 24 hours, the necrotic tissue appear deep red owing to hemorrhage.[18]

Within few weeks, the inflammatory reaction developing occur leading to grey infracted tissue and formation of a thin, fibrous scar gradually. Remodeling refers to the alteration in size, shape and thickness of both the infracted myocardium (which thins and expands) and hypertrophy occurring in other areas of the myocardium. The resultant global ventricular dilation may help in maintaining the stroke volume of the heart. [18]

I.B. Pathophysiological considerations

Under normal circumstances, coronary blood flow closely parallels myocardial demands despite fluctuation in oxygen consumption by heart. [19]

Precise evaluation of the sufficiency of myocardial oxygen supply must be made at the level of the mitochondria. While oxygen diffusion into myocytes from the capillaries takes place, ischemia occurring at partial pressure of oxygen (PO_2) becomes available at the mitochondria falls below the level required to rephosphorylate ATP being utilized for myocardial work. [20]

A fundamental property of the normal circulation in coronary artery is the coronary auto-regulation by which coronary arterial blood flow remains remarkably constant despite changes in the perfusion pressure. [20]

The mechanism of auto-regulation is likely identical to the mechanism that adjust the tone of coronary vessels compared to changing of metabolic needs of the myocardium, both are hyper-polarization in the vascular smooth muscle membrane of the cell caused by opening of ATP potassium channels (K^+–ATP) which are sensitive, thus progressively more K^+– ATP channels become activated during ischaemia.[21]

II-Endothelium:

Vascular endothelium tissue is a versatile and multifunctional that had both synthetic and metabolic properties. As a semi-permeable membrane, endothelium controls transfering the both

smaller and larger molecules into arterial wall and through walls of capillaries and vessels. Moreover, endothelial cells have role in the maintainance of a non-thrombogenic blood-tissue interface, the modulation of blood flow and vascular resistance.[22]

In addition to contributing in formation of thrombi, endothelial injury is involved in initiation of atherosclerosis and vascular lesions which are followed by monocyte infiltration, macrophages differentiation and in smooth muscle cells migration.[23,24]

The arterial endothelial dysfunction is detected in patients pocess insulin-dependent diabetes mellitus, contributes to high levels of atherosclerosis of patients.[25]

Coenzyme Q(10) has an improving effect on endothelial function in arteries of the peripheral circulation for patients with dyslipidaemia having diabetes type II disease. The mechanism involving increasing release from the endothelium also the nitric oxide (NO) release leads to improving the oxidative stress of vascular system, this is not affected by changing the plasma F(2)-isoprostane concentrations levels.[26]

The endothelium regulates vascular tone through the release of vasoactive agents that act on the underlying the vascular smooth muscle cells. This endothelial function is impaired in certain cardiovascular conditions which is atherosclerosis, and hypercholesterolemia. In the patients with hypertension it is unclear whether endothelium-dependent vasodilation is impaired.[27]

Coronary spasm induction is caused by acetyl choline, serotonin, or histamine , and can be relieved by nitroglycerin, causing vasodilation directly in smooth muscle cells. Dysfunction of endothelium is involved in coronary artery spasm pathogenesis. Dependent endothelial brachial arteries vasodilation impairment occurs for those with coronary spastic angina. Thus endothelial vasomotor dysregulation may be present in systemic arteries as well as coronary arteries in patients with coronary spastic angina.[28]

The association between endothelial constitutive gene polymorphism of nitric oxides synthase (ecNos) with vascular endothelial function occurs. The ecNos4a allele doesn't depend on determination for reduced flow-mediated dilation (FMD) in those whom smoke and not in non smokers. so ecNos4a allele is considered as genetically risk factor of endothelial dysfunction in diabetic patients, especially in smokers.[29]

Shear stress is considered as main mechanical stimuli of endothelial cells. Hypertension-endothelium dependent vasodilation is mainly due to a chronic decrease in shear stress. In diabetics, the lower endothelium-dependent vasodilation showed no result of an alteration in shear stress.[30]

The endothelium is the mostly organ involved by many cardiovascular risk factors, including hypercholesterolemia, hypertension inflammation, aging smoking-changes in the endothelium function which lead the coronary artery circulation to be unable to cope with increasing metabolism of myocardial muscle independently on a reduced coronary artery diameter. The function of endothelium is impaired in those with diabetes and is fully unclear, but less of normal endothelial function could be involved in the pathogenesis of diabetic angiopathy, as endothelial dysfunction is associated with diabetic microangiopathy and macroangiopathy as well.[31]

Impairment of the release of the endothelium-dependent relaxing factor occur which is found to be nitric oxide or its related substances, from endothelial cells. Induction of Flow mediated dilation (FMD) by reactive hyperemia was known to be endothelial dependent, which can be detected during reactive hyperemia byultrasound resolution of superficial arteries. Several coronary risk factors as hypercholesterolemia, smoking and hyperhomocysteinemia have been reported to be significantly related with decreased FMD.[32]

Nitric oxide (NO) inhalation reaches the vasculature and inhibits endothelium where "NO" undergoes S-nitrosation with protein-bound thiol groups, forming stable S-nitroso proteins including

S-nitroso albumin, that could function as a NO delivery system. S-nitroso albumin possesses endothelium-derived relaxing factors-like properties in vitro including vasodilation and inhibition of platelet aggregation.[33]

Hemoglobin was also identified as a potential carrier of NO in the form of S-nitrosohemoglobin and also possessed vasodilatory properties. It was demonstrated that hemoglobin can be S-nitrosated in the lung and that the NO group could dissociate in the capillaries[32] so continuous delivery of NO to the blood in the form of NO inhalation could produce a variety of NO adducts that could impact on the peripheral microvasculature. Inhalation of the NO reaches the endothelium at the distal microvasculature leading to alteration in the response of oxidative and non-oxidative activator of endothelium.[33]

II.A.Intima Media Thickness (IMT)

Measuring of intima media thickness is a non-involvement marker in detection of early alteration in arterial wall. Both IMT and flow associated dilation (FAD%) are established markers of early atherosclerosis. Early change in morphology in atherosclerotic process shows an increasing of IMT, which can be detected by high resolution ultrasound B mode imaging.Increased IMT for common carotid artery is associated with both coronary artery disease and also with peripheral vascular disease.[34]

Standardization of Carotid intima-media thickness (CIMT) has become a surrogate marker of early vascular changes, and has been widely used in adults, also associated with the cardiovascular risk factors, which are a family history of hypercholesterolemia, obesity, hypertension, and diabetes. The measurement of CIMT should be introduced as a tool for detection and monitoring of target-organ damage in children with cardiovascular risk factors. [35]

The carotid artery is scanned in bilateral form and six different blinded measurements in longitudinal projections (2cm) below bifurcation at the start of the bulbus are performed.[36] Patients having coronary artery disease show continuous linear correlation between IMT and flow mediated dilation.[36]

No significant influencing was found of current drug treatment on flow associated dilation (FAD%) and intima-media thickness(IMT) as published by Enderle et al (1998) in the following table:[37]

Table (1): influence of current drug treatment on flow associated dilation (FAD%) and intima-media thickness(IMT)

Variable	FAD% (p value)	IMT(p value)
Aspirin	0.765	0.594
Nitrites	0.131	0.166
Ca antagonists	0.608	0.955
β-Blockers	0.077	0.495
Diuretics	0.948	0.997
Statins	0.273	0.777

The determination of increased IMT is not effective in discriminating presence or absence of CAD, in patients having CAD, an appearance of association in extent of disease severity occurs. Ultrasound procedures in patients with chest pain in comparison with non-invasive diagnostic tools (such as, electrocardiography, stress electrocardiography, nuclear myocardial scintigraphy) appears to be evaluated.[37]

III. von Willebrand factor (vWF):

vWF is a large, adhesive, multimeric glycoprotein presents in plasma, platelets and sub-endothelium, which can be synthesized as a precursor consisting of signal peptide, a propeptide (von Willebrand antigen II) and the subunit of vWF. [38]

It has two main functions which acts as a carrier and stabilizer of factor VIII pro-coagulent protein and acts as a bridge in platelet adhesion to the exposed sub-endothelium of damaged vessels. [38]

The storage of von Willebrand factor (vWF) as single pre pro-protein in endothelial cells (as Weibel-Palade bodies) occurs mainly and in lesser extent in the megakaryocytes (in the α-granules). vWF protein secreted is made of many subunits which are covalently linked and number of subunits (2–40) is important in determination of aggregation of the platelets by using vWF. Reductions of the level or in activity of vWF protein is leading to bleeding diathesis to occur, so increased levels of vWF increases in von Willebrand's disease (vWD), and are reported in patients with acutely coronary syndromes, hypertension of pulmonary artery and in scleroderma [39]

In circulating blood, vWF is part of a non-covalent bimolecular complex with the factor VIII. This complex stabilizes factor VIII and protects it from rapid removal from circulation. vWF in circulation makes proteolytic cleavage in physiological conditions, thus distinguishing it from platelet vWF which is not proteolized can take place. [38]

Vascular endothelium is the primary source of the synthesis and release of plasma vWF, the other type of cell that synthesizes vWF is the megakaryocyte (approximately 15% of the circulating vWF is produced in the megakaryocyte). vWF circulates in platelets storage primarily in the alpha granules. Releasing of platelets vWF from the alpha granules takes place by various agonists and subsequently rebinds to the glycoprotein IIb/IIIa complex. [38]

Immunological activation of endothelial cells is accompanied by the appearance at the cell surface of increased numbers of the leucocyte adhesion molecules E-selectin,which is also specific for the endothelium. Soluble E-selectin is found in tissue culture supernatants coming from endothelial cells activation also, in plasma and rising of plasma levels occurs for diabetes, in septic shock and in hypertension.[40]

Since hyperlipidaemia is linked with the development of atherosclerosis, increased levels of vWF and E-selectin are predictive of future cardiovascular events.[40] patients having majorly atherosclerotic risk factors "e.g. hypercholesterolaemia[41], hypertension[42], smoking[41] and diabetes[43], all having high levels for vWF which links the risk factors with developing of actual disease.[44] However, increased vWF has no specificity for the cardiovascular disease and is also found in the connecting tissues and in cancer.[45] vWF is found to be associating with the ABO blood group.[46]

von Willebrand factor becomes increasingly regarded as an effective marker in endothelial cells injury while in vivo data support the hypothesis that hyper cholesterolaemia damage the endothelium, one of several potential mechanisms by which may contributing to the atherosclerotic process.[47]

Classification of congenital von Willebrand disease (vWD) detected by recommendations of the von Willebrand factor (vWF) Scientific Standardization Committee (vWF- SSC) of the

International Society on Thrombosis and Haemostasis (ISTH) takes place. The classification of ISTH of those with vWD type 1 depends on measurement of factor VIII - Coagulant activity (FVIII: C) quantitatively and vWF antigen (vWF: Ag) and also on 3 insensitive laboratory tests of vWF ristocetine cofactor (vWF: RCo) activity, ristocetine-induced platelet aggregation (RIPA), and vWF multimers.[48]

Gynecological bleeding is frequently reported in women with von Willebrand disease (VWD). Low von Willebrand factor (VWF) levels are associated with significant bleeding phenotype is only mild plasma VWF reductions. Contributing of gynecological bleeding to that phenotype was not clear. Clinical bleeding assessment tool (BAT) to evaluate bleeding remains also unclearly till now. [49]

Although it may still be possible that increased vWF may be only a marker of existing atherosclerosis rather than a consequence of it, we believe that there is now little doubt that high levels predict an increase in the risk of coronary artery disease, peripheral vascular disease and cerebrovascular disease.[47]

The precise initiating events in development of atherosclerosis are unclear but are believed to involve injury to the endothelium. Circulating levels of a special endothelial product (vWF) are shown to be raised indicating that there is damage in vasculature, example in inflammatory vasculitides, so higher levels indicates poor prognosis in patients suffering of a cardiovascular event. von Willebrand factor Plasma levels are raised in atherosclerosis, indicating damage to endothelium. [50]

Elevated levels of circulating vWF are important not only as an effective marker of damage to the endothelium, but also in promoting the formation of thrombus and in atheroma growth. [41] Increased vWF in vivo has an association with adversely clinical outcome (fatal and non-fatal re-infarction) in ischaemic heart disease and increased risk of thrombo-embolism in inflammatory vascular disease. [51]

In megakaryocytes demonstration of vWF and also in platelets takes place, which suggesting that no change in the two compartments of platelet and plasma vWF occurs. Furthermore, levels of vWF show no correlation with those of beta-thromboglobulins an established marker of platelet activity and low levels in serum than in plasma is seen. So that higher circulating vWF levels reflects an increasing release only from the endothelium, while the possibility of vWF derived in a direct form from the megakaryocytes does not occur. Raised Von Willebrand Factor (vWF) occurs in patients having atherosclerotic risk factors, including hypercholesterolaemia, where it correlates with total and LDL cholesterol and falls with reductions in total cholesterol. [52]

A reduced level of two measures of antioxidant capacity is seen together with evidence of endothelial cell dysfunction (i.e., raised vWF), no differences in patients with hypercholesterolaemia in antioxidants or vWF levels observed in patients with familial hypercholesterolaemia(FH) or non-FH. It has been concluded that hypercholesterolaemia patients possessing a reduction in antioxidant capacity and severely in patients with apparently vascular disease. This is linked to increasing levels of vWF in hypercholesterolaemia with highest levels observed in patients with vascular disease is due to the loss of antioxidant capacity exposing the vascular endothelium to excessive oxidative damage. Higher relation between hypercholesterolaemia, inability to resist the free radical attack, and atherosclerosis developing process occurs. [53]

IV. Peroxisome Proliferator Activated Receptors (PPARS)

Peroxisome proliferator activated receptors (PPARs) which are called ligand–activated transcription factors, considered as a regulatory substance for lipid and lipoprotein metabolism and for glucose homeostasis, and in influencing the cellular proliferation, differentiation and also apoptosis. Its types are PPAR-alpha, PPAR-beta, PPAR-gamma and PPAR-delta. PPAR-alpha which higherly expression is in liver, muscle and kidney, also expressed in in heart tissue,where it stimulates the beta-oxidative degradation of fatty acids. PPAR-gamma is predominately expression is in intestine and adipose tissue, triggering adipocyte differentiation and promoting lipid storage.[54]

The expression of PPAR-alpha and PPAR-gamma was also reported in vascular wall cells, such as monocyte,macrophage, endothelial and cells of smooth muscles. Hypolipidemic fibrates and antidiabetic glitazones are considered as synthetic ligands for PPAR-alpha and PPAR-gamma, respectively. Furthermore, fatty acids-derivatives and eicosanoids are PPAR ligands. PPAR-alpha activation is by leukotriene B_4, while the prostaglandin J_2 is called PPAR-gamma ligand, also, certain oxidized LDL components as well. These have suggestion as an important role to PPARs which not in metabolism only but also in inflammation control and accordingly, in relating diseases as in atherosclerosis.[54]

PPAR activators inhibit the activity of response genes of inflammation mainly IL-2, IL-6, IL-8, TNF alpha and also metalloproteases through interference negatively with the NF-kappa B, signaling pathway in vascular wall cells. Furthermore, PPARs, may also control lipids metabolism in the atherosclerotic plaque cells.[54]

So PPAR-alpha and PPAR-gamma directly modulate vessels wall functions, and its consequences in the control of cardiovascular disease.[54]

PPAR-alpha and PPAR-gamma expression occuring in cells of endothelium which are involved in transcriptional regulation of a large number of genes that affect lipoprotein and metabolism of fatty acids and also inflammation, therefore, the effects of PPAR-alpha and PPAR-gamma ligands on expression of LOX-1 was explored.[55]

Cultured treatment of bovine aortic endothelial cells (BAECs) with PPAR-alpha ligands remarkably up regulates expression of LOX-1, detected with western and northern blot analysis. LOX-1 promotor-reporter gene assay indicates that PPAR-alpha ligands increase transcription activity of LOX-1 gene, PPAR-gamma ligands, in contrast no significant effect of LOX-1 expressing on BAECs.[55]

PPAR-alpha has a key regulation role in promoting LOX-1 gene expression in BAECs up regulated of LOX-1 expression modulates the catabolism of oxidized LDL, also, atherosclerotic progression.[55]

PPAR-alpha is a part in the nuclear receptors family. Activation of PPAR-alpha will stimulates expression of gene involving in the metabolism of fatty acid and lipoprotein. PPAR-alpha activators, mainly, normolipidaemic fibric acids which leads to decrease in concentrations of triglyceride and inhancing lipoprotein lipase expression and lowering concentration of apo C-III. So that, they elevate HDL-cholesterol by raising expression of apo A-I and apo A-II.[56]

Activation of PPAR-alpha with fibric acids improving the insulin sensibility and lowers thrombosis and vascular inflammation process.[56]

PPAR-alpha activators (gemfibrozil) lowers coronary heart disease risk in those patients pocessing normal LDL-cholesterol and lower HDL-cholesterol, accordingly slowing premature coronary atherosclerosis progression.[56]

So PPARs, which are ligand-activated nuclear receptors which shows expression at endothelial cells mainly, are an important pathway influencing the vascular response both directly and indirectly by alternating gene expression. PPAR-ligands mainly fibrates (PPAR-alpha) as well as insulin-sensitizing thiazolidine diones (PPAR-gamma) are used clinically and altering the atherosclerotic process.[57]

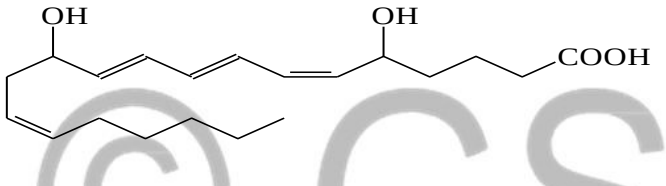
Finally, PPARs regulates adhesion of both leukocytes and monocytes modulatation to atherosclerotic lesion. Also, PPARs are controlling homeostasis of macrophage-lipid t scavenging receptors, making regulation of genes involving in the the beginning steps in reverse cholesterol transport pathway.[58]

V. Leukotriene B_4 (LTB_4)

Compared with plants, animal tissues have limited ability in de-saturating fatty acids. This necessities dietary intake of certain polyunsaturated fatty acids derived ultimately from a plant source. Fatty acids (essential) gives the eicosanoic (C20) fatty acids, such families derives compounds termed eicosanoids. These making prostaglandins (PG), thromboxanes (TX), leukotrienes (LT) and the lipoxins (LX) compounds. [59]

The prefix leuko is refering to leukocytes, these cells mainly produce Leukotrienes, and the term triene is referring to the three conjugated double bonds in Leukotrienes chemical structures. LTs are generated enzymatically from arachidonic acid (AA) and are released from the enzymes by phospho- lipase A (PLA) of membrane phospholipids. LTs production is by immune cells, which include mast cells, neutrophils, macrophages and dendritic cells (DCs), due to these cells express 5-lipoxygenase (5-LO) and 5-LO-activating protein (FLAP), all of these required for the biosynthesis of Leukotriene.[60]

The Arachidonate and C20 fatty acids have methylene-interrupted bonds giving rise to eicosanoids. Arachidonate, usually derived from the 2-position of phospholipids in the plasma membrane, so that phospholipase A2 activity, will be the substrate for the synthesis of PG_2, TX_2, LTB_4 and LX_4 compounds. [61]



LTB_4

The pathways of metabolism are divergent, the synthesis of the PG_2 and TX_2 series (prostanoids) competing with the synthesis of LTB_4 and LX_4 for the arachidonate substrate. These two Pathways are known as the cyclooxygenase and lipoxygenase pathways, respectively.[61]

Leukotrienes are conjugated trienes synthesized from eicosanoic acids in leukocytes, mastcytoma cells, platelets and macrophagic cells by the action of lipoxygenase pathway, by both immunologic and non-immunologic stimuli. The different three lipoxygenases (dioxygenases) inserting oxygen into the positions of 5,12 and 15 of arachidonic acid giving rise to hydroperoxides, Hydro peroxy eicosa tetraenoic acid(HPETE),only 5 – lipoxygenase forms leukotrienes. The first formed is leukotriene A4 which in turn is metabolized to either leukotriene B4 or leukotriene C4.[62]

The slow– reacting anaphylaxis substance is a mixture of leukotrienes C4, D4 & E4. This mixture of leukotrienes is more potent about 100-1000 times than that in histamine or prostaglandins constricts the bronchial airway musculature. These leukotrienes together with leukotriene B4 also cause vascular permeability and attraction and activating leukocytes and seem to be important regulators in many diseases involving inflammatory or immediate hypersensitivity reactions such as asthma. Leukotrienes are vasoactive and 5-lipoxygenase was found in the walls of arteries. [63]

Leukotrienes regulate the permeability of the capillaries, in the migration of leukocytes, the formation of inflammatory processes and in the evocation of asthma bronchiale. [64]

Leukotriene B4 (LTB_4) induces the human neutrophils aggregation with thromboxane B2, prostaglandin E2 formation and lysozyme release, enhanced platelet aggregation and / or serotonin release caused by threshold concentrations of calcimycin or ADP, and increased neutrophil which

adhere to endothelial cells of human umbilical vein . Some of its action was inhibited by cobra venom. These results indicate that LTB_4 possesses pharmacological effects on cells blood as well as endothelial cells, and would be useful for searching new type of anti-inflammatory drugs.(64) LTB_4 of vascular endothelium has an important role for atherosclerotic lesions genesis. The ability of this tissue to synthesize the LTB_4 is unclear. [65]

Lymphocytes Pretreatment decreases slightly binding and the pretreatment with LTB_4 for lymphocytes and endothelium prior to adherence assay has no effect on the binding. These results suggest that LTB_4 is regulating the vascular side, not the white cell side of that interaction process. LTB_4 treatment of cultured endothelium increases the binding of lymphocytes to endothelial cell mono-layers within minutes. This effect is dose and reversible upon removal of leukotriene. [66] LTB_4 is a potent chemo-attractant for neutrophils both in vivo and in vitro. LTB_4 has been found to stimulates monocyte migration in an agarose micro-droplet assay.[67]

Neutrophils promote early responses against pathogens, and their activation occurs during endothelial transmigration toward the site of inflammation. Human neutrophils activation in vitro is with immobilization of proteins of extracellular matrix, such as fibronectin (FN), laminin, and collagen. Activation of neutrophil by FN, not by other extracellular matrix proteins, has a role in induction of the granules' contents release, which is measured as matrix metalloproteinase 9 and activity of neutrophil elastase in cultured supernatant, and also reactive oxygen species production. The granule proteases release, like matrix metalloproteinase 9, myeloperoxidase, neutrophil and elastase, leads to activation of macrophages and inflammatory mediators' production, TNF-alpha and leukotriene B4 (LTB4), which are involved in parasite killing by infected macrophages. [68]

The neutrophil granulocyte is the first of the leukocytes to reach an inflammatory site in response to noxious stimuli. Microcirculation studies have revealed several stages in this procedure: the cell first adheres to the vascular endothelium and subsequently migration through the vessel wall occurs to the surrounding tissue. During this process, the vascular permeability to macromolecule increases which is often seen and various studies have confirmed the inherent ability of the neutrophil to induce edema in different inflammatory models. [69]

The particular mechanisms behind neutrophil-induced vascular leakage have remained controversial.In vitro studies utilizing phorbol esters, platelet-activating factor or the chemotactic peptide formyl-methionyl-leucyl-phenyl alanine(fMLP)have suggested actual damage of the endothelial mono-layer caused by the activated neutrophil leading to loss of integrity.[70]

In general, more neutrophils than monocytes initially accumulate at inflammatory lesions. However, within 24-48 hrs, mononuclear cells usually predominates.The kinetics of this cellular transition and the factors that regulate this progression are poorly understood. The white blood cell accumulation process involves their migration across vascular endothelium, its underlying basement membrane, and connective tissue surrounding.[71]

LTB_4 is a strong chemo-attractant and the vascular leakage seen in vivo is always accompanied by neutrophil emigration. Possibly the emigration through the vascular wall might lead to leakage of macromolecules. Endothelial cells were grown on amnion membranes and no increase in permeability of ions or macromolecules could be detected, even though fMLP-or LTB_4-stimulated neutrophil chemotaxis across the endothelium occurred. [72]

Some studies suggested that cells migrate through the endothelium, and other studies suggested that cells traverse the intercellular space between endothelial cells. LTB_4 stimulates the trans-endothelial human neutrophils migration in vivo and in vitro. However the effect of this mediator on monocyte remains to be elucidated. [73]

LTB_4 can also stimulate neutrophil emigration through endothelial monolayers grown on polycarbonate filters, such filters are advantageous over amnion models for studies of endothelial permeability, because the amnion itself might constitute a barrier against diffusion.[74]

If LTB4 is added together with the neutrophil on the luminal side of the endothelium in such a system, no leakage of protein can be detected. This finding is possibly due to the lack of neutrophil migration across the endothelium, because no concentration gradient of chemotaxin is present. [75]

LTB_4 was shown to increase albumin clearance over human endothelial cells grown on polycarbonate filters but only in presence of neutrophil.[76]

Historically, neutrophil induced permeability has been conceived as the result of damage to endothelial layer, such occuring damage can takes place as the stimulated neutrophil are in vicinity of the endothelial layer. Phorbol esters and formylated peptides are able to stimulate neutrophil degranulation, and hence, liberation of toxic products.[77]

So LTB_4 was shown to induce endothelial leakage of FITC-albumin, but only in presence of neutrophils. Additionally, chemotactic gradient establishment over endothelium was necessary for the leakage to occur.[78]

LTB_4 induces transient state of hyperadhesiveness in vitro in cultured umbilical vein endothelial cells of human (HUVEC), which leads to a 2.2 fold increasing in the neutrophil granulocytes bindings which showed less than that conferred by platelet activating factor (PAF) which was more than that thrombin showed (3.4 or 2.0 fold increase, respectively).[79]

Since neutrophils endothelial cells adhesion influences neutrophils activation responses, so examined adhesion of neutrophils to TNF alpha – activation of umbilical vein endothelial cells of human (HUVEC) stimulates LTB_4 takes place and the endothelial adhesivity towards neutrophil increase after HUVEC pretreatment with TNF alpha for 4h.[80]

The amnion connective tissue provides a natural autologous substrate for endothelial cell growth. The cells adhere well to the connective tissue and production of basement membrane occurs and development of a trans-endothelial electrical resistance takes place. Monocytes readily adhere to the endothelium, indented its surface and were stimulated to traverse the endothelial monolayer by LTB_4 and F–Met– Leu–Phe.[81]

There is a strong attraction between monocytes and endothelial cells. Monocytes adherence to endothelium is greater than that observed with either neutrophils or lymphocytes. While monocytes adherence to endothelial monolayers is greater than that of neutrophils, monocyte accumulation in connective tissue is slower.[82]

Monocytes exhibiting this behaviour increased with time of incubation and was not affected by the method of isolation, carrier medium or presence or location of the chemo attractant (LTB_4).[83]

Monocytes, like neutrophils, initially tend to wedge themselves or send psendopods between the endothelium and the basal lamina and then squeeze through perforations in the basal lamina. [84]

Existance of exogenous chemo- attractants (LTB_4 and F–Met–leu–Phe) may stimulate an inflammatory state in which one sees increased numbers of migrating monocytes [85]

Once monocytes have increased in the endothelial monolayer, they accumulate at the connective tissue, and may differentiate into macrophages. [86]

VI. Oxidized low- density lipoprotein (ox-LDL)

Low-density lipoprotein (LDL) Oxidation is the key process in atherogenesis, and vitamin E (alpha-tocopherol, TOH) has received attention for its potential to attenuate the disease. Two oxidants

electrons have the responsiblity for TOH oxidation at arterial wall, and TOH oxidation extent is limited. So antioxidant supplements prevent the oxidation of LDL and accordingly, atherogenesis process.[87]

Increasing in the oxidative stress in relation of an oxidized LDL (ox-LDL) as a proxy, is associated with oxidant and antioxidant imbalance in biological metabolic syndrome (MS), whether ox-LDL mediates the agents and resulting in damage to bio molecules which include association between central obesity and MS, and either proteins, nucleic acids, or lipids, which can damage insulin resistance mediates the association between biological molecules are used as oxidative stress bio- ox-LDL and MS. Ox-LDL reflects core mechanisms. Concentrations of glucose induce LDL oxidation by MS components development and progression which occurs within insulin resistance process. Also, obesity causes MS origin and is involved in the induction of oxidative stress. [88]

Oxidative modification of lipoproteins plays important roles in the initiation and progression of atherosclerosis. Oxidized low-density lipoprotein (ox-LDL) generation is through enzymatic and non-enzymatic oxidation reactions on lipid molecules and apo lipoprotein B (apo B) together with subsequent protein modifications with oxidized phosphatidyl Choline (ox-PC) and reactive lipid aldehydes, including malondialdehyde (MDA) and acrolein. LDL oxidative modifying increases the net negative charge of LDL particles, allowing them to gain high affinity for scavenger receptors while losing their affinity for LDL receptor. Uptake of ox-LDL via scavenging receptors has leading to atherosclerotic lesion development through foam cell formation. [89]

Antibodies to oxidized LDL predict myocardial infarction and progression of atherosclerosis in carotid artery. Oxidized LDL antibodies are cross reactive with antiphospholipid. The occurrence of these antibodies and are associating with arterial thrombosis in patients with antiphospholipid syndrome show an involving effect in developing and accelerating atherosclerosis. Certain studies show that oxidized LDL antibodies have an atherogenic effect by enhancement of lipid accumulation into macrophages in the atherosclerotic vessels, these antibodies are considered as markers of the pathogenic determinants of atherosclerosis, as in enhanced lipid oxidation, the proinflammatory stage and impaired vasodilation.[90]

HDL and the related major apolipoprotein, apo A-I are known to remove cholesterol and phospholipids from cell. Cholesteryl ester hydroperoxides can be transferred from LDL to HDL, in part, mediation is by transtering cholesteryl ester protein. there was a selective uptake of HDL oxidized cholesteryl esters by liver parenchyma cells. Both apo A-I and apo A-II can reduce cholesteryl ester hyperoxides via mechanism that involve oxidation of specific methionine residues.[91]

LDL must be “seeded” with reactive oxygen species before it can be oxidized. Albumin was capable of removing biologically active lipids from mildly oxidized LDL. Consequently, lipid-binding properties for apolipoprotein A-I (apo A-I), we reasoned that apo A-I was likely to be more effective than defatted albumin in binding and removing lipid therefore, we use apo A-I and apo A-II mimetic peptides to treat LDL.[92]

The seeding molecules present in freshly isolated LDL are derived from the cellular metabolism linoleic acid [hydro peroxy octa decadienoic acid (HPODE)] and arachidonic acid [hydro peroxy eicosa tetraenoic acid (HPETE)].[93]

Seeding molecules in freshly isolated LDL can be removed and/or inactivated by normal HDL and its components (i.e., apo A-I, and paraoxonase). Biologically active lipids in mildly oxidized LDL are formed in a series of three steps.The seeding of LDL is the first step occuring with the metabolic products for linoleic acid and arachidonic acid also with cholesteryl hydroperoxides compounds. Trapping of LDL and accumulation of reactive oxygen species which are derived from near by arterial wall cell is the second step. The non-enzymatic oxidation of LDL phospholipids is the third step that takes place when a critical threshold for reactive oxygen species (ROS) is reached which result in

specific oxidized lipids formation inducing binding of monocyte, chemotaxis and differentiation into macrophages.[94]

In conclusion, there is HDL and its components, opo A-I and PON in regulating the first step in a three step process that the forms mildly oxidized LDL.The LDL must be "seeded" with reactive oxygen species before it can be oxidized when hydrogen peroxide or its lipid hydroperoxide equivalent may be an important in seeding LDL.[95]

Apo A-I was not only capable of favorably altering the susceptibility of LDL to oxidation by artery wall cells but also HDL specifically with HDL-associated enzyme PON.The PON has peroxidase activity, which may explain the role of PON in protecting against atherosclerosis.[96]

Metabolism of both arachidonic acids and linoleic can also acts as LDL seeding molecule, also there is critical threshold for the seeding molecules that is necessary for oxidation.[97]

Apo A-I and apo A-II can not reduce cholesteryl ester hyperoxidase via mechanism that involves oxidation of specific methionine residues (i.e. apo A-I and not apo A-II was able to reduce the oxidation of LDL). HDL has been demonstrated to be a strong inverse prediction of risk for atherosclerosis; it has been shown to reduce atherosclerosis in animal models. However, over express of apo A-II has been demonstrated to enhance atherosclerosis. Apo A-I but not apo A-II is capable of removing seeding molecules from freshly isolated LDL also normal HDL its components can also inhibit the second and third steps in forming mildly oxidized LDL.[98]

12-lipoxygenase (12-LO) protein is present in arteral wall cells of humans and is required for producing mild oxidized LDL by the artery wall cells. Lipoxygenase mediate the peroxidation of cholesteryl linoleate largely by a non enzymatic process. The non enzymatic oxidation of cholesteryl linoleate is greatly enhanced by 13-hydro peroxy octa deca dienoic acid [13 - HPODE] present.[99]

The third step in the mildly oxidized LDL formation is the non-enzymatically oxidation of LDL phospholipids occur when a certain critical threshold for "seeding molecule" occurs for the LDL.As seeding molecule reach a critical value, a nonenzymatic oxidation exists of the major LDL phospholipid, 1-palmitoyl-2-arachidonyl-sn-glycero-3-phosphocholine (PAPC). This result in forming active biologically oxidized phospholipids.1- palmitoyl - 2- (5-oxovaleryl) –sn - glycero- 3- phosphocholine (POVPC, m/z 594), 1-pamitoyl-2-glutaryl-sn-glycero-3-phosphocholine (PGPC, m/z 610), and 1-palmitoyl-2-(5,6-epoxyisoprostane E2)-snglycero-3 –phosphocholine (PEIPC, m/z 82).[100]

HDL taken from those having coronary artery disease, who show normal blood lipid levels and were neither diabetic nor taking hypolipidemic medications, did not protect LDL against oxidation in arterial wall cells of Humans and failed to inhibit the biological activity of oxidized PAPC.[101]

HDL and the enzymes associated inhibit arterial wall cells of human from contributing the reactive oxygen species which are additional and necessary for circulation LDL to reach the critical threshold required for oxidation of PAPC into biologically active phospholipids. Several mechanisms explain that "lipooxygenase-derived hydroperoxides or secondary reactive lipid species may be transferred across the cell membrane to seed the extra cellular LDL, which would then be more susceptible to a variety mechanisms that could promote lipid peroxidation".[102]

12 LO protein is present in arterial cells wall of human and is required for human arterial wall cells to modify LDL to a form that induces the artery wall cells to produce monocyte chemotactic activity.[103]

The non enzymatically oxidation of PAPC in formation of the active three biologically phospholipids (POVPC, PGPC and PEIPC) could be enhanced by 13-HPODE and 15-HPETE. The ability of 13-HPODE and 15-HPETE for oxidation of PAPC to the active biologically phospholipids showed two orders more in magnitude to be effective than hydrogen peroxide shows. The amount of

H_2O_2 necessary to produce oxidation of LDL shows greater two orders of magnitude than that produced by endothelial cells that oxidized LDL.[103]

The inhibition of biological activity of ox-PAPC and its components (POVPC, PGPC and PEIPC) by PON and by normal HDL but not HDL from patients with angiographically proven atherosclerosis despite normal plasma HDL-cholesterol levels, suggests that an abnormality in HDL is responsible in atherosclerosis in this relatively rare subset of patients.[104]

ox-LDL has an association with apoptosis of human smooth muscle cells of vascular system atherosclerotic plaques. Cell loss and apoptotic cell death have a role in the pathophysiology of atherosclerosis, and many studies have showed the presence of apoptosis in human and in vitro atherosclerotic plaques formed. Plaques that were exposed to ruptures possessing thinner fibrous cap with few vascular smooth muscle cells (VSMCs) and a denser infiltration of the macrophages.[105]

VSMCs are important in maintaining tensile strength in fibrous cap, because these are the only cells in the cap that can synthesize collagen fibers type I and III.[106]

Oxidative modifying process of LDL has involvement in the atherogenesis and the uptake of ox-LDL by macrophagic cells and VSMCs which leads to formating the foam cells and lipid droplets accumulation occurs. ox-LDL shows cytotoxicity for and to promote apoptosis of cultured VSMCs.[107]

ox-LDL in early atherosclerotic lesion is localized not only in the intima, where it is associated with macrophage infiltration but also in medial VSMCs, where it colocalizes with B cell lymphoma 2-associated X protein(BAX),aproapoptotic protein. In plaques of atherosclerosis, VSMCs is maintaining tensile strength of fibrous cap, so plaques proning to rupture possess a thinner fibrous cap with few VSMCs and a denser infiltration of macrophagic cells. Infiltration Macrophags are associated with apoptosis, whereas lesions consist only of VSMCs can undergo very little apoptosis.[108]

ox-LDL localization is associated with decreased actin immuno reactivity and increased TUNEL-positive VSMCs as well as macrophage infiltration. Infiltration macrophages may destabilize plaque by secreting or activity metallo proteinases that digest matrix. Alternatively VSMCs could be programmed to die because of the effect of ox-LDL or macrophage-derived factors such as TNF.[109]

Highly oxidized LDL unlike tightly ox-LDL stimulates VSMC apoptosis. Apoptosis can be induced by lipid peroxides, a component of ox-LDL in cultured VSMCs through caspase activation[110]

In summary, ox-LDL is localized not only in macrophage-rich intimal area in early atherosclerotic lesions but also in medial VSMCs that co-express BAX a proapoptotic protein. The co-localization of ox-LDL and BAX in these medial VSMCs in early lesions predisposes these to under go apoptosis and most likely contribution to the lipid core formation, which is characterized by ox-LDL accumulation, BAX expression and TUNEL-positive VSMCs.[111]

In advanced plaques, TUNEL-positive VSMCs and higher BAX immuno reactivity in the intima positive for ox-LDL may be involved in plaque instability and rupture.[112]

The association between HDL cholesterol / total cholesterol (HDL-C/TC) ratio and arterial elasticity was studied in healthy men also relationships between LDL oxidation and elasticity.[113]

Arterial atherosclerotic fatty streak involvement is inversely related to HDL-C level and directly to TC level. HDL-C/TC ratio has been a better marker of disease risk for coronary artery than either TC or HDL-C levels.[113]

Oxidation of LDL may play a part on developing the atherosclerotic process. ox-LDL interacts with normal arterial vasodilatory function. ox-LDL may influence vessel wall elasticity by enhancing vasoconstriction via increasing the intracellular calcium concentration in smooth cells, by inducing endothelial dysfunction, or by promoting smooth muscle cell proliferation.[113]

Association between arterial elasticity and total cholesterol concentration and a direct association between elasticity and HDL-concentration occurs. The increased elasticity in the early phases of atherosclerosis is due to enhanced intake of lipids into vessel wall and formation of foam cells.[113]

There is a response increase that acute coronary diseases related to activating the immune mediated inflammatory process which is associated with atherosclerotic plaques formation.[114]

ox-LDL plays an important role in genesis of the process of inflammation in the atherosclerotic lesions. A previous study measuring levels of ox-LDL in plasma indicated that levels of ox-LDL were correlated with coronary artery disease extent in patients with heart transplantion.[115]

Plasma levels of malonaldehyde – modified LDL is highly significant in those with acute coronary syndromes more than that patients with stable coronary artery disease.[116]

ox-LDL correlates positively with the severly acute coronary disease using an anti-ox-LDL monoclonal antibody and an apo B polyclonal antibody for measuring ox-LDL in the circulatory fractions of LDL in blood plasma.[117]

Increased number of inflammatory cells in coronary altherosclerotic plaques is related to increasing the severe effect for acute coronary diseases. Oxidation modification of lipoproteins is a key in developing of atherosclerotic process. ox-LDL may plays a role thrombosis triggering by induction of platelet adhesion and reduction of the fibrolytic capacities of endothelial cells.[118]

Macrophages and lymphocytes have capablity of LDL oxidation. The lesions in patients with AMI have abundant macrophages and T-lymphocytes.[119]

So, ox-LDL in macrophage which are derived foam cells can be enhanced within unstable plaques in association with progressing the inflammation of plaque. One could hypothesize that ox-LDLis located with unstable plaques is released into blood stream in patients with severe endothelial injury as in plaque erosion or its rupture.[119]

Neutrophiles can oxidatively modify LDL into the form that is rapidly incorporated by macrophages. Neutrophiles are known to accumulate at sites of plaque rupture or erosion occuring in patients with AMI. Neutrophiles which may accumulate at inflammatory reactions sites is unstable, eroded or ruptured, especially at early stages after injuries could contribute to increasing in ox-LDL levels in the blood. There is a pivotal role for ox-LDL in of coronary plaque genesis and instability, and developing of acute coronary syndromes. In conclusion, ox-LDL levels has a direct relation to the severe effect of coronary syndromes.[119]

2-Aim of the work

This study was planned to find the following relationships between:

a- Levels of natural activators of (PPAR-alpha, PPAR-gamma) namely Leukotriene B_4 (LTB_4) and oxidized low-density lipoprotein (ox-LDL) respectively, and the endothelial dysfunction marker von Willebrand factor (vWF).

b- Intima-media thickness (IMT) and the flow mediated dilation (FMD) response in brachial artery in patients with peripheral and coronary artery disease.

3-Materials and Methods

Materials

The study includes thirty (30) patients aged from (55-65) years suffering from atherosclerosis. Ten age and sex matched normal healthy persons, were chosen as controls. The patients were taken from those admitted to the clinical cardiology unit of the Medical Research Institute. Atherosclerosis were confined by coronary angiography and/or documented by historical features of myocardial infarction within five years. Exclusion criteria include clinical manifestation of cerebro-vascular disease, venous thrombo-embotism, cancer, liver or renal failure.

Methods

I- Clinical investigation

All atherosclerotic patients were clinically investigated as follows:

i- Standard 12- leads electrocardiogram.

ii- M. Mode 2D and Doppler – electrocardiographic study.

iii- Measurement of Intima-media thickness (IMT) of the carotid artery.[120]

iv- Measurement of flow mediated and nitroglycerine induced dilation of the brachial artery.[121]

v- Measurement of ankle / brachial systolic pressure index (ABI). [122]

II. Laboratory Investigations:

II.A. Determination of von Willebrand factor (vWF) [123]

II. A. a. Principle

REAADs vWF-Ag is an assay for sandwich ELISA. Antibody capture occur specific for human vWF where coating to micro well polystyrene plates takes place. Dilution of patient plasma is incubated into wells, showing any available vWF: Ag to bind to the anti-human vWF antibody on the microwell surface. The plates are washed removing unbound proteins or other plasma molecules, bound vWF: Ag is quantitated using horse radish peroxidase (HRP) conjugated antihuman vWF detection antibody. Following incubation, unbound conjugate is removed by washing. A chromogenic substance of tetramethyl benzidine (TMB) and hydrogen peroxide is added to develop a colour reaction.The intensity colour is measured in optical density (O.D) units with a spectrophotometer at 450 nm. Patient vWF: Ag in relative percent concentration is determined against a curve made from reference plasma provided.

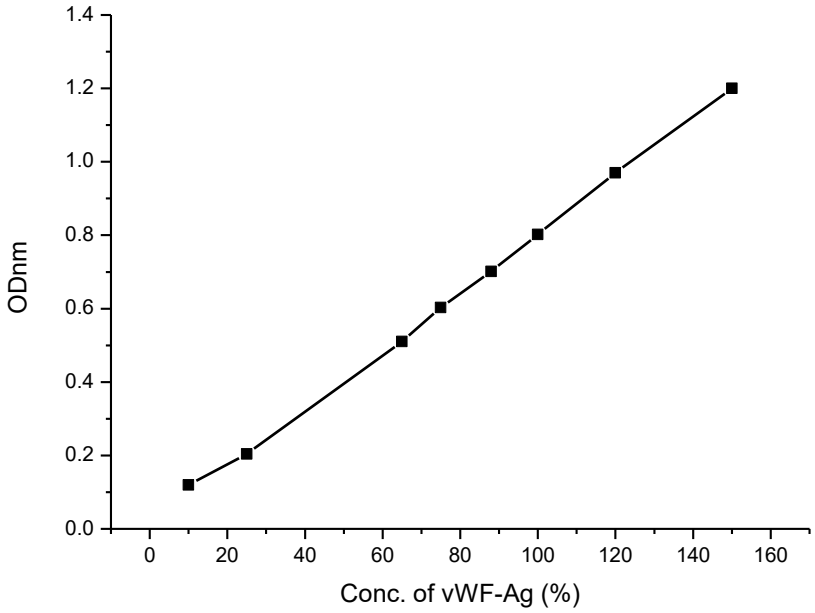


Figure 1: A standard curve for determination of vWF.

II. A. b. Reagents

1- Ninety six stabilized antibody coated micro wells, with frame holder. Wells are coated with antihuman vWF antibody.

2- One bottle (60 ml) sample dilute (blue-green solution).

3- Three vials (0.5 ml) trophilized reference plasma for preparation of reference curve.

4- One bottle (12 ml) HRP conjugated antihuman vWF antibody solution (red solution).

5- One bottle (13 ml) one component substrate (TMB and H2O2).

6- One bottle (15 ml) stopping solution (0.36 N Sulfuric acid).

7- One bottle (30 ml) wash concentration {33% phosphate buffered saline (PBS)}.

Procedure was done exactly as explained by the kit, [123] and the following was made:

Volume reference plasma		Volume sample diluent		Reference level
30 µL	+	500 µL	=	150.00
20 µL	+	500 µL	=	100.00
15 µL	+	500 µL	=	75.00
10 µL	+	600 µL	=	50.00
10 µL	+	1000 µL	=	25.00
10 µL	+	2000 µL	=	52.50
10 µL	+	4000 µL	=	6.25

II. A. c. Calculation

1- Mean O.D. values were calculated duplicate of reference plasma dilutions, control and patient samples.

2- The O. D. was plotted on Y-axis against the corresponding value of reference level (X-axis). The curve was linear or plotted. A line was drawn to connect the points.

3- The mean O. D. was used to determine the control and patients relative values from the graph.

4- vWF: Ag percent (%) was calculated:

The % actual vWF:Ag value=relative value X reference value.

Ex: Patient relative value = 40.

Reference plasma value = 105% of normal.

Actual patient vWF: Ag value (as % of normal).

Was 40 x 1.05 = 42%.

II. B. Determination of leukotriene B4 (LTB4) [124]

II. B. a. Principle

The competitive binding technique in which LTB4 present in a sample competes with a fixed amount of alkaline phosphatase-labeled LTB4 for sites on a rabbit polyclonal antibody. During the incubation, the polyclonal antibody becomes bound to the gout anti-rabbit antibody coated on to the microplate, following a wash to remove excess conjugate and unbound sample, a substrate solution is added to the wells to determine the bound enzyme activity. The colour development is stopped and absorbency is read at 405 nm. The intensity of the colour is inversely proportional to the concentration of LTB4 in the sample.

II. B. b. Reagents

1- Microplate - ninety six well microplate coated with a goat anti-rabbit polyclonal antibody.

2- LTB4 conjugate-5ml of LTB4 conjugated to alkaline phosphatase, with blue dye and preservative.

3- LTB4 standard 0.5 ml of LTB4 (120,000 pg) ml in buffer with preservative.

4- LTB4 antibody solution 5ml of rabbit polyclonal to LTB4 with yellow dye and preservative.

5- Assay buffer 1-30 ml of a buffered protein base with preservative.

6- Wash buffer concentrate-30 ml of a 10-fold concentrated solution of a buffered surfactant with preservative.

7- PNPP substrate-20 ml of p-nitrophenyl phosphate in a buffered solution.

8- Stop solution-6 ml of a trisodium phosphate (TSP) solution.

9- Plate cover-one adhesive strip.

Procedure was done exactly as explained by the kit. [124]

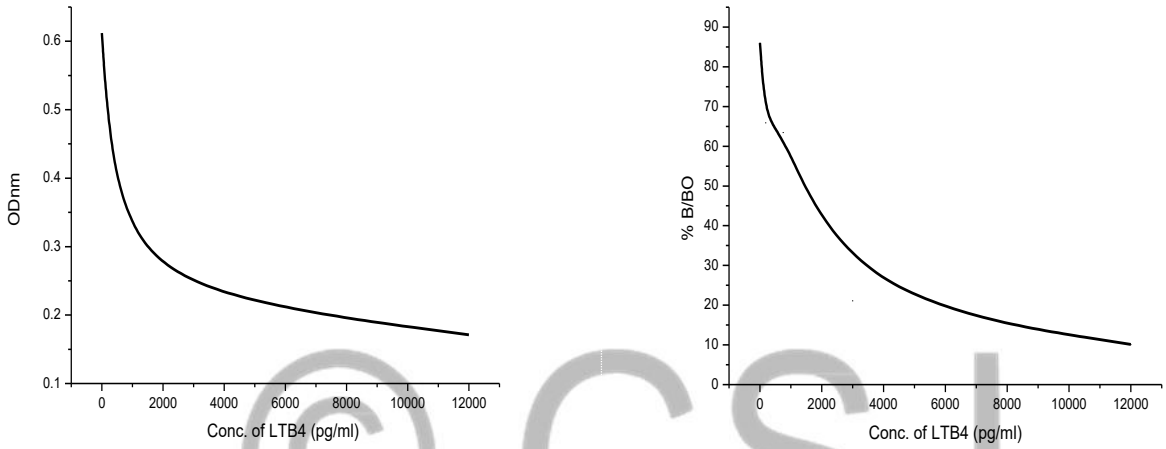
II. B. c. Calculation

1- A standard curve was created by plotting the mean absorbance for each standard on a linear Y-axis against concentration on logarithmic X-axis.

2- % B/B_o was calculated by dividing the correction O. D. for each standard or sample by corrected B_o O. D. and multiplying by 100.

3- The concentration of LTB4 was calculated corresponding to the mean absorbance or % B/B_o from the standard curve.

(Where, **TA** = Total activity, $\mathbf{B_o}$ = Maximum binding, and **NSB =** Non-specific binding).



(a) **(b)**

Figure 2 : (a) A standard curve between optical density and LTB_4 concentration , (b) : A curve between % B/B_0 and LTB_4 concentration.

II. C. Determination of oxidized low density lipoprotein (ox-LDL) [125]

II. C. a. Principle

ox- LDL ELISA is a solid phase two site enzyme immunoassay. It is based on the direct sandwich technique in which two monoclonal antibodies are directed against separate antigens determinants on the oxidized apo-lipoprotein molecule. During incubation ox-LDL in the sample reacts with anti-oxidized LDL antibodies bound to titration well. After washing, that removes non-reacted plasma components, a peroxidase conjugated anti- apo-lipoprotein B antibody recognizes the oxidized LDL, bound to the solid phase. After a second incubation and a simple washing step that removes unbound enzyme labeled antibody, the bound conjugate is detected by reaction with 3, 3', 5, 5'-tetra methyl benzidine (TMB). The reaction is stopped by adding acid to give a calorimetric end point that is read spectrophotometically at 450 nm.

II. C. b. Reagents

1- Antioxidized LDL wells.

2- Standards/control (25 µL).

3- Buffer (100 µL).

4- Washing solution.

5- Absorbent paper.

6- Conjugate solution (100 µL).

7- Peroxidase substrate (100 µL).

8- Stop solution (50 µL).

Procedure as mentioned by the kit. [125]

II. C. c. Calculation

1- The absorbance values were plotted against the ox-LDL concentrations on lin-log paper and a standard curve is constructed.

2- The concentration of controls and unknown samples were readed from the standard curve.

3- The concentration on controls and unknown samples were multiplied by dilution factor (e.g. X6561).

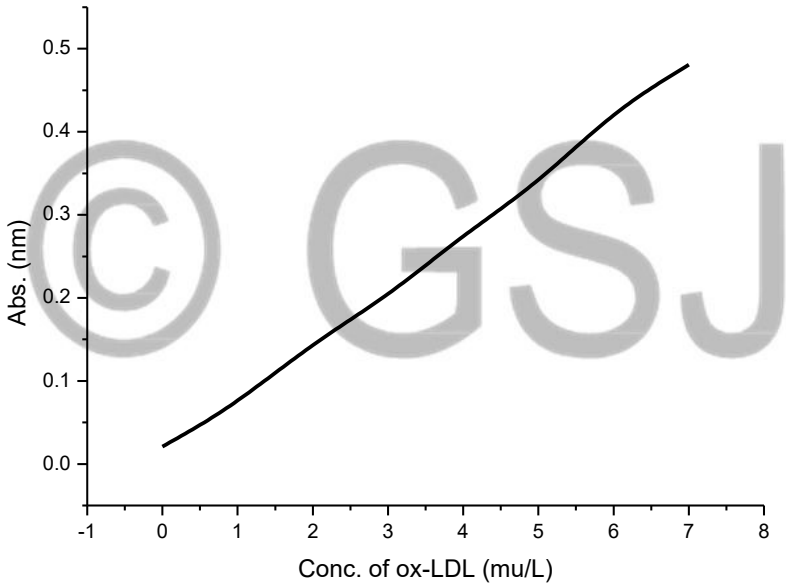


Figure 3: A standard curve of ox-LDL where absorbance is plotted against conc.

III. A. Determination of serum total cholesterol concentration: [126]

III. A. a. Principle:

Cholesterol is determined enzymatically using cholesterol esterase and cholesterol oxidase.

Cholesterol ester + H_2O —cholesterol esterase→ cholesterol+fatty acids

Cholesterol esters are cleaved by the action of cholesterol esterase to yield free cholesterol and fatty acids.

Cholesterol+O_2 —cholesterol oxidase→ cholestene-3-one + H_2O_2

$2H_2O_2$+ phenol + 4-amino antipyrene $\xrightarrow{\text{peroxidase}}$ quinoneimine dye + $4H_2O$

Cholesterol is converted by oxygen with the aid of cholesterol oxidase to

cholest-4-en 3-one and H_2O_2.

The created H2O2 forms a red dyestuff by reacting with 4-amino antipyrene and phenol under the catalytic action of peroxidase.The red quinoneimine dye was measured at 546 nm by spectrophotometer.

III. A. b. Reagents:

Reagent(1):

Pipes buffer pH=6.9	90 mmol/L
Phenol	26mmol/L

Reagent(2):

Cholesterol oxidase	200U/L
Cholesterol esterase	300U/L
Peroxidase	1250U/L
4-amino antipyrine	0.4 mmol/L

Reagent(3):

Cholesterol	200 mg/dl

III. A. c. Procedure:

1-Prepare three test tubes for blank, standard, and sample, 1 ml of working reagent was put in each one.

2-10 ul of dist. H_2O were added to blank tube, 10 ul of standard solution were added to standard tube, and 10 ul of serum were added to sample tube.

3-All were mixed and incubated for 5 minutes at 37 C.

4-The optical density of sample and standard were read against blank at 546 nm.

Preparation of working solution:

Dissolve reagent 2 in a suitable volume (20 ml) of reagent (1) and shake.

III. A. d. Calculations:

Conc. of cholesterol (mg/dl) = OD sample/OD standard X n

n = standard conc. = 200 mg/dl.

III. B. Determination of serum triglycerides concentrations: [127]

III. B. a. Principle:

The enzymatic colorimetric test:

$$\text{Triglycerides} + 3\,H_2O \xrightarrow{\text{LPL}} \text{Glycerol} + 3\text{RCOOH}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow[Mg2+]{\text{GK}} \text{glycerol-3-phosphate} + \text{ADP}$$

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow{\text{GPO}} \text{dihydroxy acetone phosphate} + H_2O_2$$

$$H_2O_2 + \text{4-amino antipyrene} + \text{p-chlorophenol} \xrightarrow{\text{POD}} \text{quinonimine} + \text{Hcl} + 4H_2O$$

The colour of quinonimine was measured at 546 nm.

III. B. b. Reagents:

Reagent (1):

Pipes buffer, pH =7.2	50 mmol/L
p- chlorophenol	2 mmol/L

Reagent (2):

-lipoprotein lipase	150000 U/L
-glycerol kinase	800 U/L
-glycerol-3-phosphate oxidase	4000 U/L
-peroxidase	440 U/L
4-amino antipyrene	0.7 mmol/L
ATP	0.3 mmol/L

Reagent (3):

-triglyceride	200 mg/dl

III. B. c. Procedure:

1-Prepare three test tubes for blank, standard and sample.1 ml working solution was put in each one.

2-10 ul distilled H2O were added to blank tube, 10 ul of standard solution were added to standard tube, and 10 ul serum were added to sample tube.

3-All tubes were mixed and incubated for 5 minutes at 37 C.

4-The OD of sample and standard was read against blank at 546 nm.

III. B. d. calculations:

conc. of serum triglyceride (mg/dl) =OD sample/OD standard X n.

OD =optical density.

N =standard conc. =200 mg/dl

III.C. Determination of serum high density lipoprotein - cholesterol (HDL-C): [128]

III. C. a. Principle:

The chylomicron, VlDL and LDL are precipitated by addition of phospho tungestic acid and magnesium chloride. After centrifugation, the supernatant fluid contains HDL fraction which was assayed.

III. C. b. Reagents:

preccipitant reagent:

phospho tungestic acid	0.55mmol/L
Magnesium chloride	25 mmol/L

III. C. c. Procedure:

i. Precipitation:

1-500 ul diluted reagent were pipette into centrifuge tube and then 200 ul serum were added.

2-The tubes were mixed well, let to stand for 10 minutes at room temperature and centrifugated at 4000 g.

3-After centrifugation, the supernatant (HDL- supernatant),was separated within 1 hr, and the cholesterol concentration was determined.

ii. Determination of cholesterol concentration:

1- In two test tubes for blank and sample, 1 ml cholesterol reagent was put in each one.

2-100 ml dist. H2O was added to blank tube and 100 ul HDL-supernatant were added to sample tube.

3-All tubes were mixed and incubated for 10 minutes at 20-25 C.

4-The absorbance of sample was read against blank at 500 nm, the absorbance of sample = A.

III. C. d. Calculations:

Conc. of HDL-C = A X 210 mg/dl.

III.D. Estimation of serum low-density lipoprotein cholesterol (LDL-C):[129]

LDL-C concentration is calculated from total cholesterol conc. (TC), HDL cholesterol conc. (HDL-C) and triglycerides conc.(TG) according to the following equation:

Conc. of LDL-C = (TC)-(HDL-C) - (TG)/5 mg/dl.

III.E. Determination of lipoprotein (a) in serum: [130]

III. E. a. Principle:

The wells of polystyrene microplate strips have been coated with mouse monoclonal anti-Lp(a).The test sample is incubated in such well ; Lp(a),if present in the sample or standard solution ,will bind to the solid- phase antibody. Unbound substances are removed by washing the plate. A sheep anti-apo B polyclonal antibody is labeled with horse-radish peroxidase (HRP) was added. The labeled antibody binds to any solid-phase antibody/Lp(a) complex. Incubation with enzyme substrate produced a blue color in the test well, which turned yellow when the reaction was stopped with sulphuric acid. The amount of color produced in the well was directly proportional to the amount of Lp (a) in the sample or standard solution.

III. E. b. Reagents:

1- Coated test wells with anti Lp(a).

2- Lp(a) standard (phosphate buffer) 100mg/dl.

3- A 0.25 ml lyophilized control serum. (LEVEL 1)

4- A 0.25 ml lyophilized control serum. (LEVEL 2)

5- Sample diluent (phosphate buffer with stabilizing protein) (25 ml).

6- A 0.4 ml conjugate (sheep anti-apo B polyclonal antibody labelled with HRP).

7- A 20 ml conjugate diluent (phosphate buffer with stabilizing protein).

8- TMB substrate solution.

9- Substrate buffer (phosphate citrate buffer containing 0.006% H_2O_2).

10- Wash solution (Phosphate buffer containing detergent).

III. E. c. Procedure:

1- 10 ul specimen, standard or control was added to each well.

2- 100 ul sample diluent were added for specimen, standard and controls.

3-Incubation 120 minutes at 37 C was made.

4-Washing 4 times was made.

5-100 ul conjugate solution was added to each well.

6-Incubation 60 minutes at 37 C was made.

7-Washing 4 times was made.

8- 100 ul substrate solution was added to each well.

9- Incubation 30 minutes at 20-25 C was made.

10-100 ml sulphuric acid was added to each well.

11-Absorbance was read at 450 nm.

III.E.d. calculations:

1-A standard curve was constructed by plotting mean absorbance values of Lp(a) standard solution on Y-axis versus corresponding Lp(a) conc. on X-axis and a curve was made.

2-Using mean absorbance value for each sample,the conc. of Lp(a) was obtained.

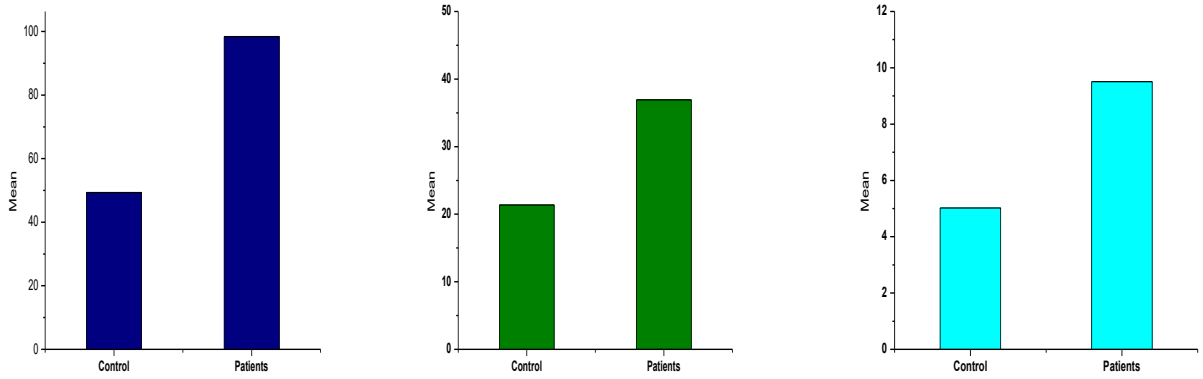
4-Results

The study included 30 patients with PAOD and 10 normal non-smoker subjects as controls. The mean age of patients was 59.7±14, while that of controls was 57.3±8. There was 26 male (86.6%) and 4 female patients (13.3%), 11 patients (36.6%) had symptoms of PAOD, 8 patients (26.7%) had intermittent claudication, 3 patients (10%) had rest pain associated with absent peripheral pulsation and traplic changes, 16 patients (53.3%) had associated coronary artery disease, of them 4 had coronary artery bypass graft (CABG). Ten patients (33.3%) had diabetes mellitus, 13 (43.3%) patients had hypertension and 16 patients (53.3%>) were smokers. Plasma level of vWF (%) in the control and patient groups are showed in table (2). It showed that, the plasma levels of vWF ranged between 33.50-69.30 with a mean of 49.310±11.599 for control group, while it ranged between 67.20-131.30 with a mean of 98.437±20.985 for patients group respectively. This indicating a statistical significant difference between the two studied groups regarding vWF. Plasma level of leukotriene B4 (LTB_4) in the control and patient groups are showed in table (2). It demonstrated that, levels of LTB4 are ranged between 13.40-31.30 with a mean of 21.360±6.261 for the control group, while it ranged between 22.70-52.50 with a mean of 36.923±10.171 for patients group. This indicating a statistical significant difference between the two studied groups regarding LTB_4. Plasma level of oxidized low density lipoprotein (ox-LDL) in the control and patient groups are regarding in table (2). It illustrated that, the plasma level of ox-LDL ranged between 3.2-8.1 with a mean of 5.02±1.45 for control group, while it ranged between 6.6-14.9 with a mean of 9.51±1.91 for patients group. This indicating a statistical significant increase between them regarding ox-LDL.The correlation between different studied parameters was significant and positive between vWF & LTB4 (r=0.59, P=0.041) and vWF & ox-LDL (r=0.72, P=0.021) and between LTB4 & ox-LDL (r = 0.68, p = 0.032). (Table 3) and Figures (5a, 5b and 5c). Functional arterial properties are shown in table (4). The mean of Aortic compliance, aortic distensibility, carotid compliance, carotid distensibility, femoral compliance, femoral distensibility were 0.69±0.12, 19.33±5.87, 0.56±0.18, 17.87±4.95, 0.46±0.15 and 7.5±2.24 for patients group respectively. While it was 0.91±0.1, 27.5±4.21, 0.71±0.31, 25.3±5.62, 0.71±0.31 and 14.0±4.25 for control group respectively.This indicating a statistical significant difference between the two studied groups regarding all functional arterial properties.Table (5) demonstrated the lipid profile and lipoproteins in the study groups. It showed that, the mean of T.C (mg/dl) was 258.43±55.61 and 185.9±16.2 for patients and control groups respectively. The mean of TG (mg/dl) was 216.38±70.17 and 106.6±22.3 for patients and control group respectively, LDL-C (mg/dl) was 170.11±76.90 and 123.3±32.3 for patients and control groups respectively, HDL-C (mg/dl) was 39.29±15.70 and 30.6±4.32 for patients and control groups respectively , Lp(a) (mg/dl) was 158.8±46.05 and 123.3±15.9 for patients and control groups respectively, ox-LDL (mu/L) was 9.51±1.91 and 5.02±1.45 for patients and control groups respectively. This indicating a statistical significant difference between the two study groups regarding lipid profile and lipoproteins. Figure (6a) showed the brachial artery diameter (basal), while figure (6b) showed brachial artery diameter (flow mediated dilation, FMD), finally figure (6c) showed brachial artery diameter (after glyceryl trinitrate, GTN). Figure (6d) showed the intima-media thickness of the carotid artery.

Table (2): Plasma levels of von Willbrand factor (vWF)(%), Leukotriene B_4 (LTB_4) (pg/ml) and Oxidized low density lipoprotein (ox-LDL) (mU/L) in the control and patients groups.

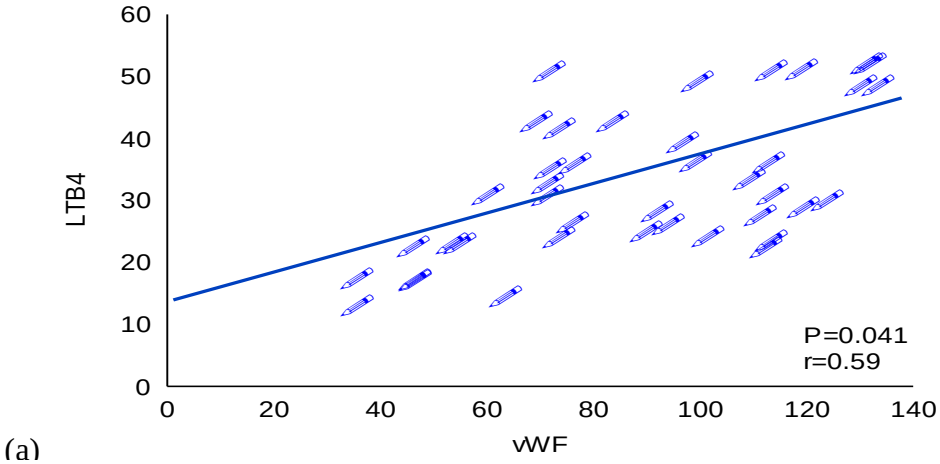
Parameter	vWF (%)		LTB4 (pg /ml)		ox-LDL (mU/L)	
No.	**Control (n=10)**	**Patients (n=30)**	**Control (n=10)**	**Patients (n=30)**	**Control (n=10)**	**Patients (n=30)**
Range	33.50-69.30	67.20-131.30	13.40-31.30	22.70-52.50	3.2-8.1	6.6-14.9
Mean	49.310	98.437	21.360	36.923	5.02	9.51
SD	11.599	20.985	6.261	10.171	1.45	1.91
t	7.014		4.537		6.796	
P	0.0001*		0.000*		0.0001*	

* $p < 0.05$ is considered significant.

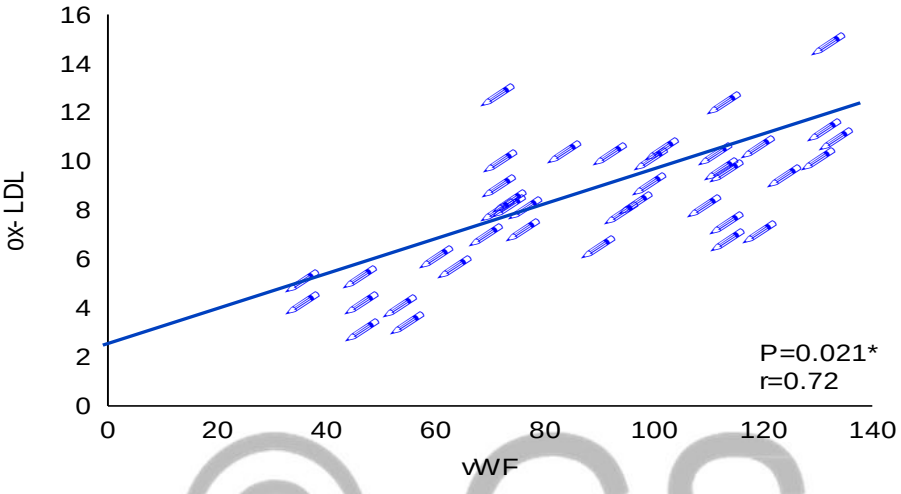


(a) **(b)** **(c)**

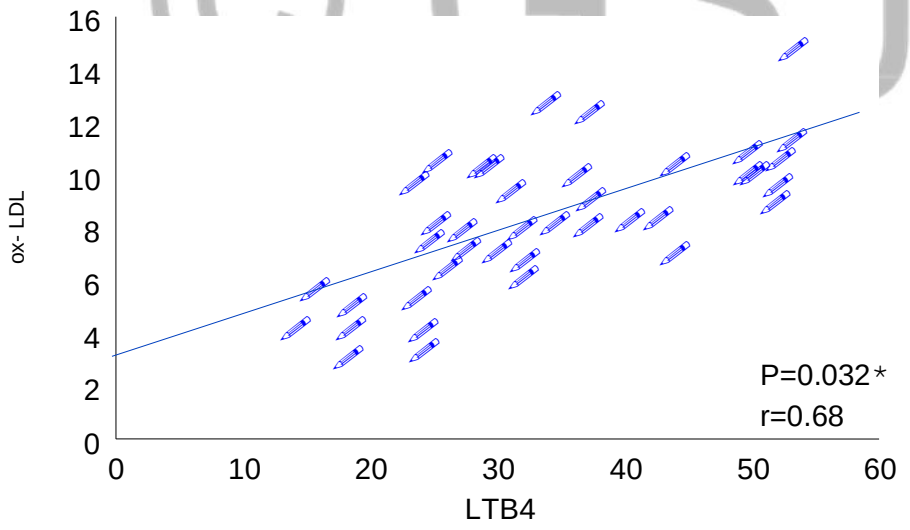
Figure (4): (a) Plasma level of vWF (%), (b) Plasma level of LTB4 (pg/ml), and (c) Plasma level of ox-LDL (mU/L), in both the control and patient groups



(a)



(b)



(c)

Figure 5 : (a) Correlation between the von Willebrand factor (vWF) and LTB4, (b) Correlation between the von Willebrand factor (vWF) and ox- LDL and (c) Correlation between the Leukotriene B_4 (LTB4) and Oxidized low density lipoprotein (ox-LDL).

Table (3): Correlation between different studied parameters

Parameter	vWF		LTB_4	
	r	p	r	p
LTB_4	0.59	0.041*		
Ox-LDL	0.72	0.021*	0.68	0.032*

Table (4): Functional arterial properties

Properties	Patients "n=30"	Control "n=10"	P
Aortic compliance	0.69±0.12	0.91±0.1	0.0001*
Aortic distensibility	19.33±5.87	27.5±4.21	0.0021*
Carotid compliance	0.56±0.18	0.71±0.31	0.032*
Carotid distensibility	17.84±4.95	25.3±5.62	0.0029*
Femoral compliance	0.46±0.15	0.71±0.31	0.0012*
Femoral distensibility	7.5±2.24	14.0±4.25	0.0001*

Table (5): Lipid profile and lipoproteins in the study groups

Variable	Patients "n=30"		Control "n=10"		P
	Mean	S.D.	Mean	S.D.	
T.C (mg/dl)[126]	258.43	55.61	185.9	16.2	0.001*
TG (mg/dl)[127]	216.38	70.17	106.6	22.3	0.003*
LDL-C (mg/dl)[129]	170.11	76.90	123.3	32.3	0.021*
HDL-C (mg/dl)[128]	39.29	15.70	30.6	4.32	0.005*
Lp(a) (mg/dl)[130]	158.8	46.05	123.3	15.9	0.001*
ox-LDL (mu/L)[125]	9.51	1.91	5.02	1.45	0.0001*

Where, **T.C**: Total cholesterol, **T.G**: Triglycerides, **LDL-C**: Low density lipoprotein cholesterol, **HDL-C** : High density lipoprotein cholesterol, **Lp (a)**: Lipoprotein (a) and **ox-LDL**: oxidized Low density lipoprotein.

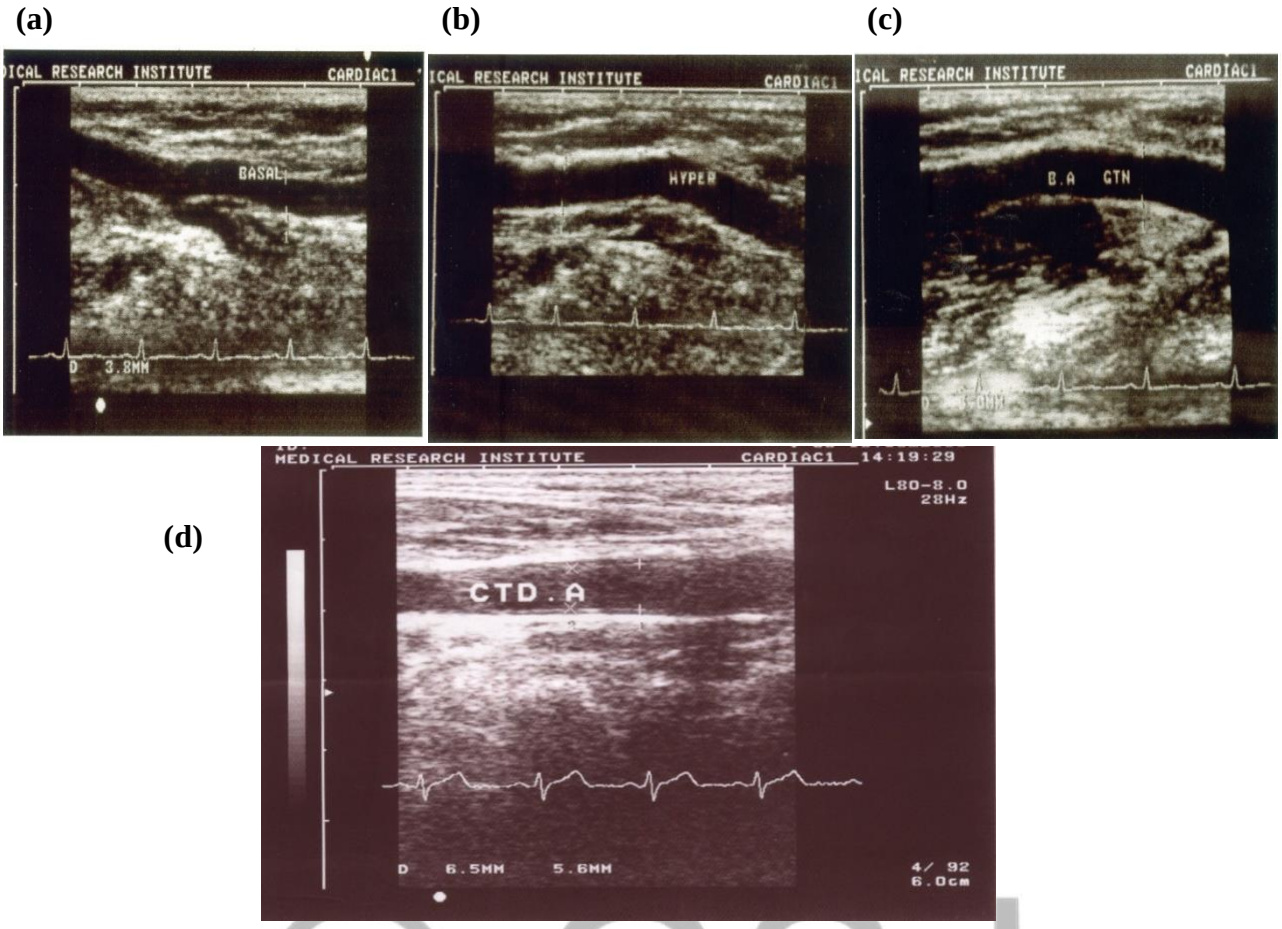


Figure 6: (a): Brachial artery diameter (basal), (b): Brachial artery diameter (flow mediated; FMD), (c): Brachial artery diameter (after glycerol trinitrate; GTN) and (d): Measurement of Intima-Media Thickness of carotid artery.

5-Discussion

Atherosclerosis is systemic disease. Peripheral arterial occlusive disease is considered as the major cause of mortality and morbidity, endothelial function measures the vascular health which shows impairment in patients with PAOD. [131]

Cardiovascular disease (CVD) causes death and disability in developed countries and is increased rapidly in developing world. Atherosclerotic vascular disease (ASVD), encompassing coronary heart disease, cerebro-vascular disease and peripheral arterial disease, is responsible for the majority of cases of CVD in both developing and developed counties. [132]

Major risk factors occurring for coronary atherosclerosis are including elevation in blood pressure, dyslipidemia, smoking, and diabetes mellitus and reducing these factors can reduce morbidity and mortality associated with ASVD. A number of few markers are significant predictors of atherosclerotic process and complications. Among important emerging risk predictors are homocysteine, C - reactive protein, and fibrinogen. [132]

In the present study, the risk factors of CAD were detected in CAD patients and controls, these risk factors include total cholesterol and triglyceride level, as well as, LDL-C and HDL-C levels.

New insights for endothelium, a dynamic, interactive organ have generated increased interest in the endothelial cell over the last few years. (51) Endothelial injury has been reported to be the first initially step in the developing and progression of atherosclerosis, moreover the endothelium regulates vascular tone through releasing of the vasoactive agents. Endothelial damage can be quantified by

specific endothelial cell markers such as von Willebrand factor (vWF) which is considered as an endothelial derived protein that is involved in hemostasis regulation. [133]

Endothelial function can be measured by biophysical and biochemical methods. Simon, *et al* (1999) found that non-invasive high resolution ultrasound is utilized to assess the dilation changes of brachial arteries during reactive hyperemia, in flow mediated dilation (FMD) and after sublingual uptake of glyceryl trinitrate (GTN) have confirmed as surrogate test for endothelial function. [134]

Raised plasma concentration of circulating vWF has an association with endothelial damage and represents the most sensitive marker of peripheral atherosclerosis. [135]

The results showed that von Willebrand factor (vWF) showed significant higher values in CAD patients comparing to the control group ($p<0.05$).

LTB_4 is the major product in the arachidonic acid metabolism which is found via 5-lipoxygenase pathway. LTB_4 helps stimulating leukocytes function which includes release of lysosomal enzyme, adhesion and aggregation of poly-morphonuclear leukocytes. LTB_4 shows an implication as a potent mediator in the inflammatory disease and immuno regulation. [136]

Our results showed that LTB4 was significantly higher in CAD patients as compared to control group ($p<0.05$).

Oxidized low density lipoprotein (ox-LDL) has an important role in atherosclerotic plaques genesis. The first stage of the formation of atherosclerotic plaque is involving oxidation of LDL and the subsequent uptake by macrophage. Ox-LDL increase cell adhesion between endothelial cells and monocytes, and accumulates estrified cholesterol in the macrophages leading to lipid rich plaque formation. [137]

Ox-LDL may influence vessel wall elasticity by enhancing vasoconstriction via increasing intracellular calcium concentration in smooth muscle cells, by inducing endothelial dysfunction or by promoting smooth muscle cells proliferation. [137]

Our results showed that ox-LDL had a significant higher values in CAD patients as compared to control group $p<0.05$.

Wilinink, *et al* (2000) found that lipoprotein (a) causes endothelial dysfunction and also elastic arterial properties alteration. [138]

A previous study demonstrated that parameters of arterial elasticity were associated with age, systolic blood pressure and the present coronary artery disease. [139] also a significant correlation between arterial distensibility and stiffness index into age, systolic pressure in presence of coronary artery disease was observed. [140]

PPAR-alpha activators decrease the inflammatory response in vivo and in vitro. PPAR-alpha activators undergo their anti-inflammatory action, at least in part by negatively regulating NF-KB transcriptional activity. This anti-inflammatory activity may apply generally to PPAR-family members as PPAR-γ has been found to inhibit macrophage activation in vitro. [141]

The clinical investigation showed that PAOD majorly is causing morbidity and mortality. A previous study aimed to study three important properties of the wall of artery using a conventional ultrasound scanner:

(I) ankle/brachial index (ABI), (II) wall elasticity by pulsatile diameter changes in the aorta, carotid and femoral arteries and (III) endothelial function expressed as flow-mediated dilation (FMD) and also glyceryl trinitrate dilation (GTN) of the brachial artery, in addition to biochemical parameters of endothelial function and dyslipidemia. PAOD was detected and graded according to the ABI level

which is the most cost effective non-invasive method to screen PAOD that proved to be most predictive for the angiographic severity of the disease. [142]

It is well established that endothelial function and elastic properties of arteries are impaired in atherosclerosis. The results of a previous study showed that flow mediated dilation (FMD) and glyceryl trinitrate (GTN) of the brachial arteries were impaired in patients with PAOD, this impaired dilation response was significantly related to disease severity where ABI is significantly correlated with FMD $r=0.74$, $p<0.01$. It was reported that FMD for the brachial artery was significantly lower in patients with PAOD than control, and the dilation capability of the brachial artery was linearly related to the ABI. Our values for FMD and GTN of brachial arteries were further impaired in occurring of diabetes mellitus and coronary artery disease. [143]

Abnormalities in endothelial function in diabetes could represent the impact disturbance of metabolism, or presence of common cellular defect, that influences both nitric oxide bio-availability and insulin mediated glucose disposal. The coronary artery disease present and endothelial dysfunction is due to decreased generation of nitric oxide by the endothelium. [144]

A previous study showed that increased total cholesterol, LDL-C, LDL/HDL-C ratio, high systolic blood pressure and smoking were independent predictors of percent change in brachial artery diameters.[145] It was found that the impairment of FMD was associated with increased LDL-cholesterol and hypertension patients with PAOD. Also hypercholesterolemia and smoking were associated with decreased FMD. [146]

The lipoprotein (a) levels were highly recorded in patients with PAOD than in controls, and the level of lipoprotein (a), were inversely correlated with ABI and FMD. Similar results showed that systemic atherosclerosis measured as the degree of PAOD is associated strongly with serum lipoprotein (a). [147] Also it was found a significantly correlation between lipoprotein (a) and impaired endothelial vasodilation, lipoprotein (a) is suggested to cause endothelial dysfunction and alteration of elastic arterial properties. [148]

In hypertension, impaired endothelial function is mainly due to altered shear stress.[148] Smoking injuries the endothelium by producing free radicals aromatic compounds, which diminish the endothelial synthesis of nitric oxide.[149] vWF is the most frequently used marker for endothelial damage. The data showed that vWF increased significantly in patients having PAOD relative to the controls; there was an additional significant increase in patients with atherosclerosis at multiple loci (any combination of coronary and femoral artery disease). [150]

There was a significantly association between the level of vWF and ABI. Also it was found that there was a strong association between vWF and angiographic extent of PAOD. [151]

Brevetti, *et al* (2001) found that patients with FMD $\leq 5.5\%$ had higher concentration of vWF in plasma than those with FMD $> 5.5\%$ detect that vWF could be used as sensitive marker for presence of PAOD and atherosclerosis and both biophysical (FMD) and biochemical vWF aspects of vascular endothelium deteriorated in parallel in presence of PAOD.[152]

Hulthe, *et al* (2002) fit into the concept that oxidative modifying LDL may enhance the expression of cell adhesion molecules for endothelial cells while elevated levels of cell adhesion molecules and markers of endothelial activation occurring in concert with an ongoing atherosclerotic process. [153]

Rosanov, *et al* (2005), demonstrated the parameters of arterial elasticity of femoral arteries, carotid arteries and aorta was significantly reduced in patients with PAOD than in controls denoting increased stiffness. [154]

Mickel, *et al* (2006), explain the reduced parameters of arterial elasticity and increased stiffness are caused by medial and intimal thickening, accumulation of collagen fibers, deposition of calcium and the degeneration of elastic lamina. [155]

Uday, *et al* (2005), found that, parameters of arterial elasticity were correlated with FMD and GTN of brachial artery. It was found that there was a positive correlation between FMD of brachial arteries and parameters of arterial elasticity. Increased arterial stiffness may further reduce blood already curtailed by stenosis and may also accompanied by accelerated atherosclerosis is due to increase in traumatic effect of intravascular pressure on vessel wall which lead to rupture of vessel wall. [156]

6-Conclusions

1- Atherosclerosis is a systemic disease generated which involves arterial structure and function.
2- Both biophysical {Flow Mediated Dilation (FMD) and Glyceryl Nitrate (GTN %) and biochemical (vWF) aspects of vascular endothelium deteriorates in parallel with disease progression and can be used as diagnostic markers of atherosclerosis.
3- The standard accurate non-invasive methods to assess ankle-brachial index, compliance and distensibility proved to be the most predictive for the presence of extent of PAOD severity.
4- vWF, LTB_4 and ox-LDL are elevated in patients with peripheral and coronary artery diseases, so they can be used as marker of the disease.

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Conflict of Interest

This research did not receive any specific grant from funding agencies in the public, commercial, or not – for profit sectors.

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