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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 are 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.

Keywords: Atherosclerosis, Peroxisome Proliferator Activated Receptors, von Willebrand factor, Leukotriene B_4, Oxidized low density lipoprotein

1. Introduction

1.1 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. [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. [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]

1.2 Endothelium:

Vascular endothelium tissue is a versatile and multifunctional that had both synthetic and metabolic properties. As a semi-permeable membrane, endothelium controls transferring the both smaller and larger molecules into arterial wall and through walls of capillaries and vessels.[5]

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.[6,7]

1.2.1 *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. [8]

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:[9]

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

1.3 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. It has two main functions which act as a carrier and stabilizer of factor VIII pro-coagulent protein. [10]

1.4 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,

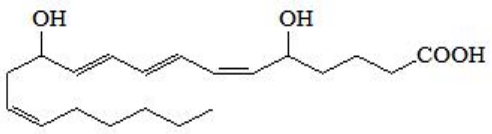
PPAR-gamma and PPAR-delta. PPAR-alpha which higherly expressed is in liver, muscle and kidney, also expressed in heart tissue,where it stimulates the beta-oxidative degradation of fatty acids. PPAR-gamma is predominately expression is in intestine and adipose tissue, triggering adipocyte differentiation and promoting lipid storage.[11]

1.5 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. [12]

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. [13]

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. [14]



LTB_4

1.6 Oxidized low- density lipoprotein (ox-LDL)

Low-density lipoprotein (LDL) Oxidation is the key process in atherogenesis, antioxidant supplements prevent the oxidation of LDL and accordingly, atherogenesis process.[15]

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). 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. [16]

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 LTB4 and ox-LDL respectively, and the endothelial dysfunction marker 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

3.1 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.

3.2 Methods

3.2.1 Clinical investigation

All atherosclerotic patients were clinically investigated as follows:

1- Standard 12- leads electrocardiogram.

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

3- Measurement of Intima-media thickness (IMT) of the carotid artery.[17]

4- Measurement of flow mediated and nitroglycerine induced dilation of the brachial artery.[18]

5- Measurement of ankle / brachial systolic pressure index (ABI). [19]

3.2.2 Laboratory Investigations

3.2.2.1 Determination of von Willebrand factor (vWF) [20]

3.2.2.1.1 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. Unbound conjugate is removed by washing. A chromogenic substance of tetramethyl benzidine (TMB) and hydrogen peroxide is added to develop a color reaction. The intensity color is measured in optical density (O.D) units with a spectrophotometer at 450 nm.

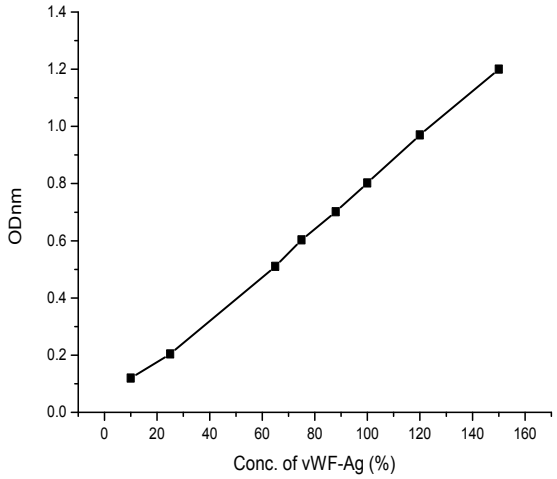


Figure 1: A standard curve for determination of vWF

3.2.2.1.2 Procedure

1-Micro well strips were removed.

2-Reference plasma dilution was done.

3-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

4-1: 25 dilution for sample and control was selected.

5-100ul dilutions was added to micro wells

6-100ul sample diluent to reagent well was added.

7-Washing with working wash solution was done.

8-Incubation 15 minutes at room temperature was done.

9-100ul HRP conjugated Antibody was added.

10-Incubation for 15 minutes at room temperature was made.

11-Washing 4 times with working wash solution was done.

12- 100ul substrate was added to each well.

13-100ul stopping solution was added to each well.

14-Optical density was measured at 450 nm

3.2.2.1.3 Calculations

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.

3.2.2.2 Determination of leukotriene B4 (LTB4) [21]

3.2.2.2.1 Principle

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 micro plate, following a wash to remove excess conjugate and unbound sample, a substrate solution is added to wells to determine the bound enzyme activity. The color development is stopped and absorbency is read at 405 nm.

3.2.2.2.2 Procedure

1-Reagents, Working standards and samples were prepared.

2-Excess micro plate wells strips were removed.

3-Wells and substrate blank were reserved.

4-150ul assay buffer were added to wells.

5-100ul assay buffer to wells were added.

6-100ul sample to remaining wells were added.

7-50ul LTB4 conjugate was added to wells.

8-50ul LTB4 Antibody solution was added to wells.

9-Washing was made.

10-5ul LTB4 conjugate was added to wells.

11-200ul substrate was added to wells.

12-50ul stop solution was added.

13-Optical density was measured at 405 nm.

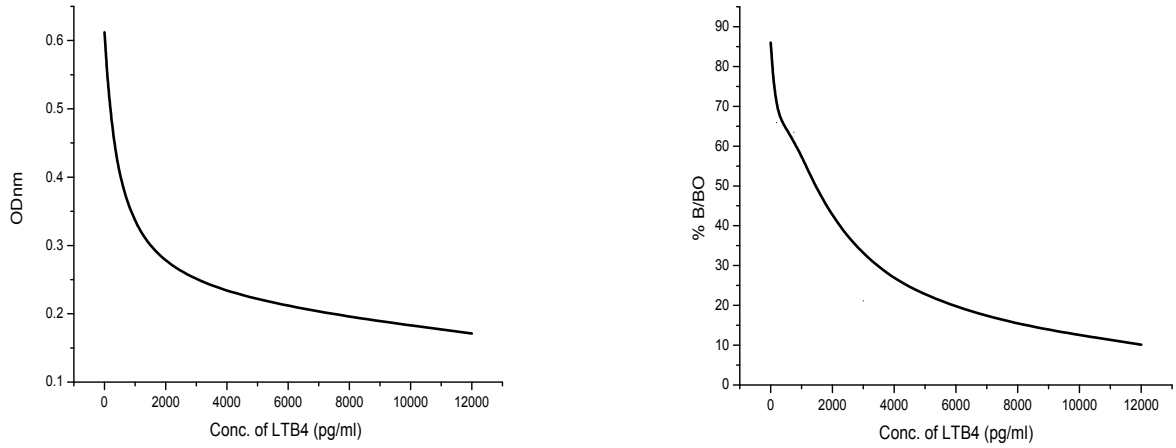
3.2.2.2.3 Calculations

1- A standard curve was created by plotting the mean absorbance for each standard against concentration.

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

3- Concentration of LTB4 was calculated corresponding to the mean absorbance or % B/Bo from standard curve.

(Where, **TA** = Total activity, **Bo** = 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

3.2.2.3 Determination of oxidized low density lipoprotein (ox-LDL) [22]

3.2.2.3.1 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.

3.2.2.3.2 Procedure

1-25ul standard, control and samples and 100ul assay buffer were added to anti-oxidized LDL wells.

2-Incubation for 2 hours at room temperature was made.

3-Washing with 350ul wash solution was done.

4-100ul conjugate solution was added.

5-Incubation for 1 hour at room temperature was made.

6-Washing was done.

7-100ul peroxidase substrate was added.

8-Incubation for 15 minutes was made.

9-50ul stop solution was added.

10-Shaking for 15 seconds was done.

11-Absorbance at 450 nm was measured.

3.2.2.3.3 Calculations

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 read from the standard curve.

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

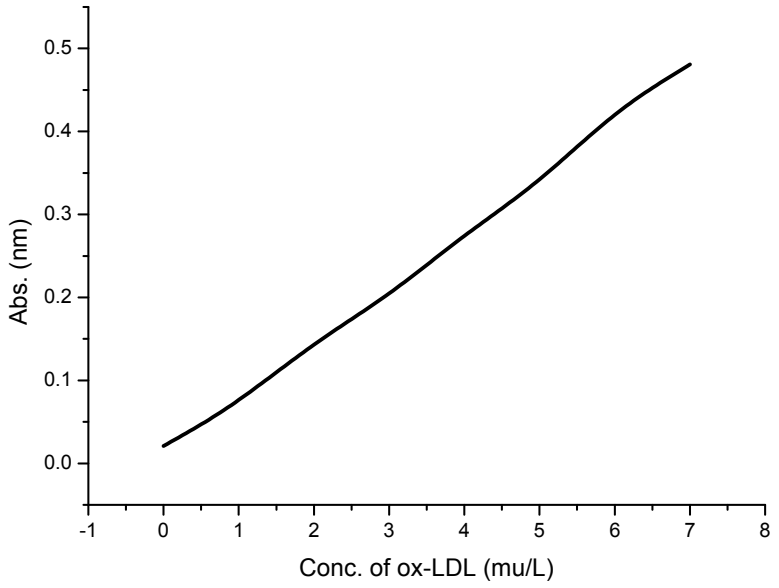


Figure 3: A standard curve of ox-LDL where absorbance is plotted against conc

3.2.2.4 Determination of serum total cholesterol concentration: [23]

3.2.2.4.1. Principle

Cholesterol is determined enzymatically using cholesterol esterase and cholesterol oxidase. 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.

3.2.2.4.2 Procedure

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

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

3.2.2.4.3. Calculations:

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

n = standard conc. = 200 mg/dl.

3.2.2.5 Determination of serum triglycerides concentrations: [24]

3.2.2.5.1 Principle

The enzymatic colorimetric test was done and quinonimine was formed, the colour of quinonimine was measured at 546 nm.

3.2.2.5.2 Procedure

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

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

3.2.2.5.3 Calculations

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

OD =optical density.

N =standard conc. =200 mg/dl

3.2.2.6 Determination of serum high density lipoprotein - cholesterol (HDL-C):[25]

3.2.2.6.1 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.

3.2.2.6.2 Procedure

i. Precipitation:

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

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

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 100ul 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.

3.2.2.6.3 Calculations

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

3.2.2.7 Estimation of serum low-density lipoprotein cholesterol (LDL-C): [26]

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.

3.2.2.8 Determination of lipoprotein (a) in serum: [27]

3.2.2.8.1 Principle

The wells of polystyrene microplate strips have been coated with mouse monoclonal anti-Lp (a). Incubation with enzyme substrate produced a blue color in the test well, which turned yellow when the reaction was stopped. The amount of color produced was directly proportional to amount of Lp (a) in the sample or standard solution.

3.2.2.8.2 Procedure

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

2- 100ul sample diluent was 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- 100ul 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.

3.2.2.8.3 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.

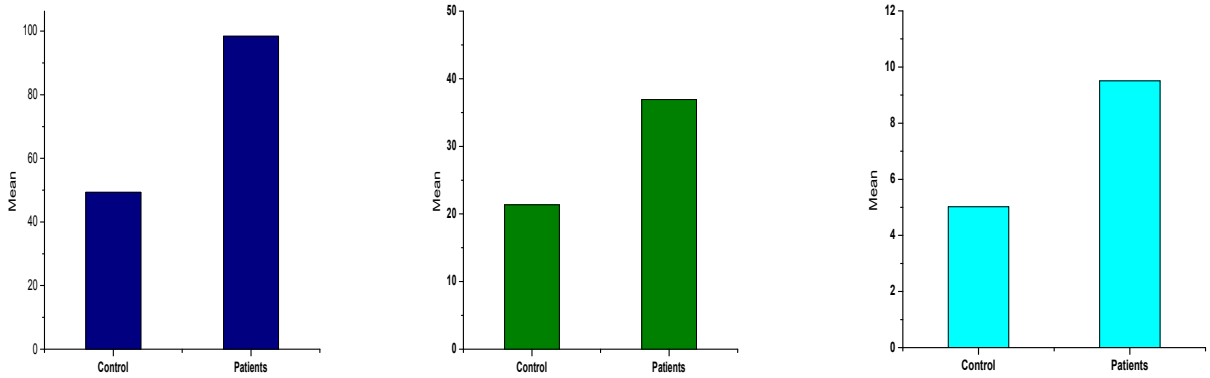
4. Results

The study included 30 patients with PAOD and 10 normal non-smoker subjects as controls. 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 (LTB4) in the control and patient groups are showed in table (2). 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 LTB4. Plasma level of oxidized low density lipoprotein (ox-LDL) in the control and patient groups are regarding in table (2). 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). 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.

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

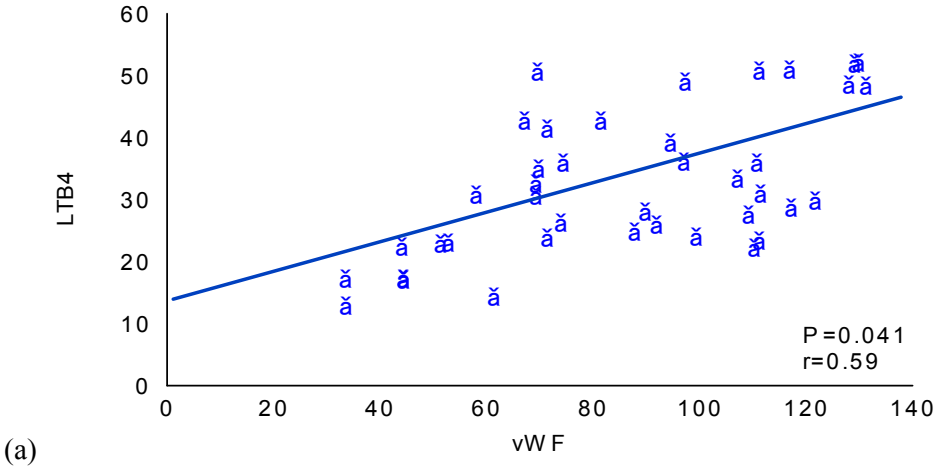
Parameter	vWF (%)		LTB4 (pg /ml)		ox-LDL (mU/L)	
No.	Control (n=10)	Patients (n=30)	Control (n=10)	Patients (n=30)	Control (n=10)	Patients (n=30)
Range	33.50-69.30	67.20-131.30	13.40-31.30	22.70-52.50	3.2-8.1	6.6-14.9
Mean	49.310	98.437	21.360	36.923	5.02	9.51
SD	11.599	20.985	6.261	10.171	1.45	1.91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

*p < 0.05 is considered significant.

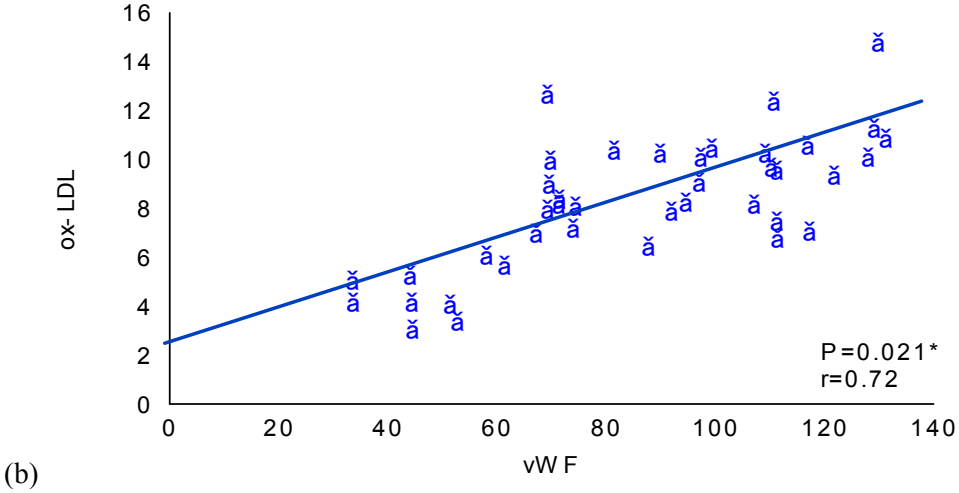


(a) **(b)** **(c)**

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)



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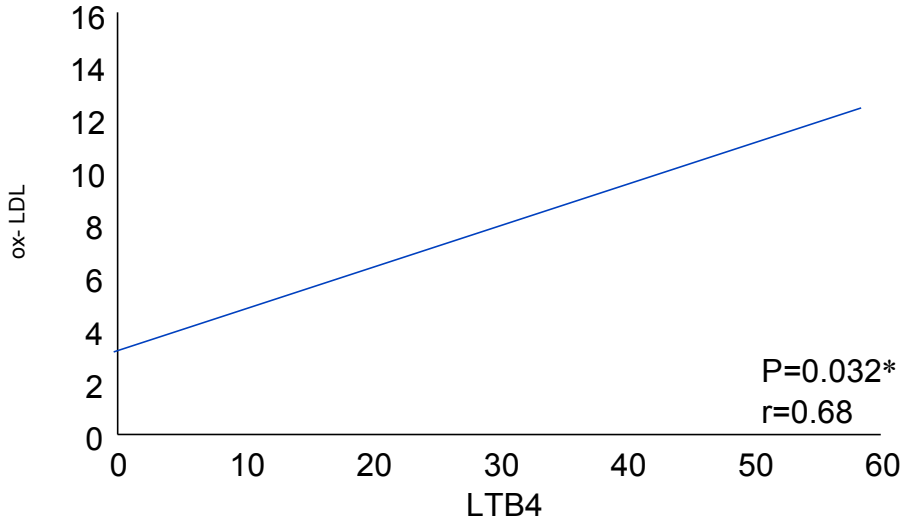

(c)

Figure 5: (a) Correlation between the vWF and LTB4, (b) Correlation between vWF and ox- LDL and (c) Correlation between LTB4 and 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)[23]	258.43	55.61	185.9	16.2	0.001*
TG (mg/dl)[24]	216.38	70.17	106.6	22.3	0.003*
LDL-C (mg/dl)[26]	170.11	76.90	123.3	32.3	0.021*
HDL-C (mg/dl)[25]	39.29	15.70	30.6	4.32	0.005*
Lp(a) (mg/dl)[27]	158.8	46.05	123.3	15.9	0.001*
ox-LDL (mu/L)[22]	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.

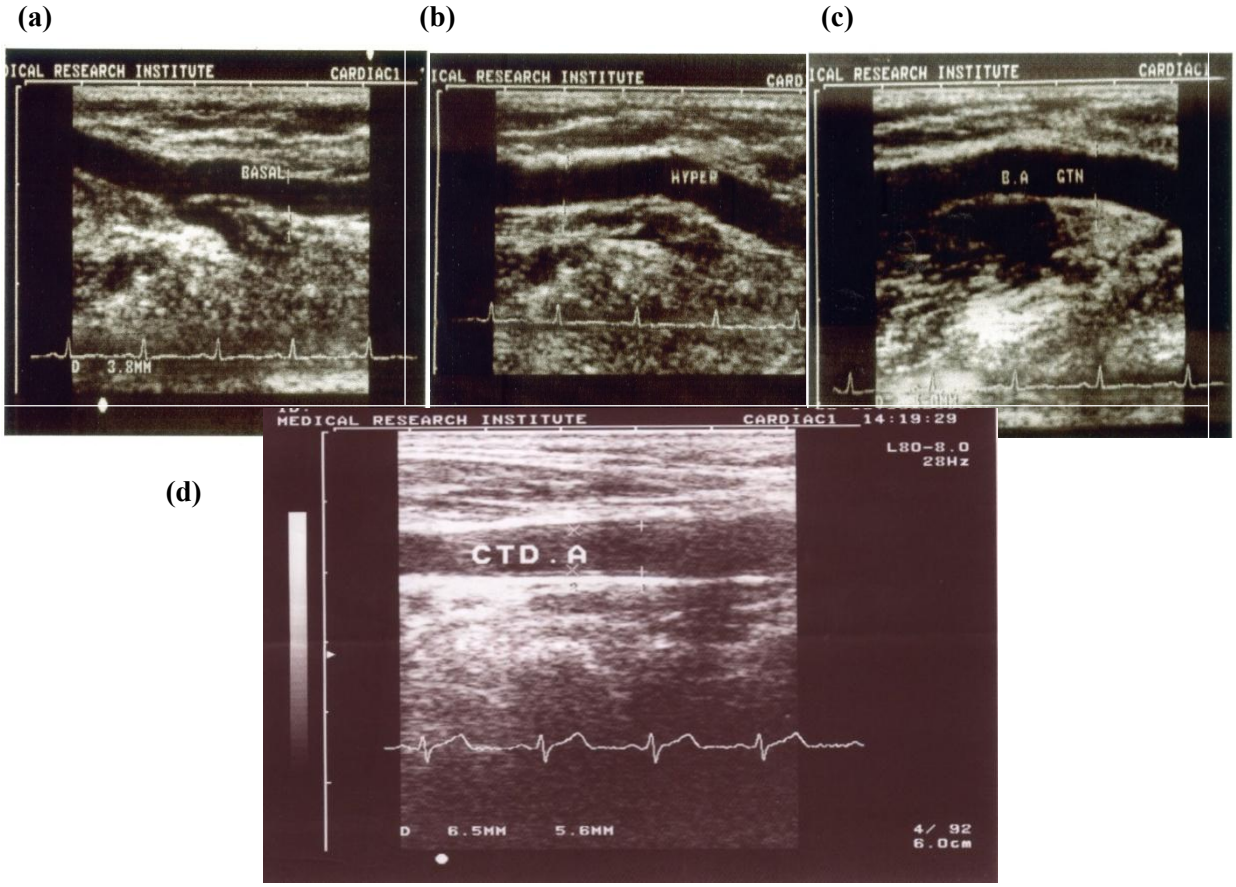

Figure 6: (a): Brachial artery diameter (basal), (b): Brachial artery diameter (flow mediated; FMD), (c): Brachial artery diameter (after glycerol trinitrate; GTN) and (d): Measurement of Intima-Media Thickness of carotid artery

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. [28] 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.

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. [29]

Raised plasma concentration of circulating vWF has an association with endothelial damage and represents the most sensitive marker of peripheral atherosclerosis. [30] The results showed that von Willebrand factor (vWF) showed significant higher values in CAD patients comparing to the control group ($p<0.05$).

LTB4 is the major product in the arachidonic acid metabolism which is found via 5-lipoxygenase pathway. LTB4 helps stimulating leukocytes function which includes release of lysosomal enzyme, adhesion and aggregation of poly-morpho nuclear leukocytes. [31] 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. [32] 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. [33]

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. [34] 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. [35]

6. Conclusions

Atherosclerosis is a systemic disease generated which involves arterial structure and function.
That can be detected both biophysically by Flow Mediated Dilation (FMD) and Glyceryl Nitrate (GTN %) and biochemically (vWF). Aspects of vascular endothelium deteriorate in parallel with disease progression and can be used as diagnostic markers of atherosclerosis. The standard accurate non-invasive methods to assess ankle-brachial index, compliance and distensibility proved to be mostly predictive for the presence of extent of PAOD severity. vWF, LTB4 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

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