Research Article

Silent hyperlipidaemia modulated vascular endothelial markers

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Abstract

The aetiology of ischemic heart diseases is mainly based on atherosclerosis of coronary artery. Inflammation and oxidative reactions are initiating and aggravating the illness resulting in pathological remodelling of vasculaturze at site of injury. Endothelium lining of blood vessels participated in the reaction biochemically through releasing some proteins into circulatory system which further complicate the condition. The aim of this study was to determine early diagnosed hyperlipidaemia-associated changes of the plasma level of some of these endothelial biomolecules. Compared to healthy control, hyperlipidaemic patients have significantly increased arginase, metalloendopeptidase, peroxidase, myeloperoxidase, and peroxynitrite with concomitant reduction in arylesterase and nitric oxide. The present study concluded that hyperlipidaemia play a great role in modulation of certain plasma protein markers which might be directly related to patient pathological condition or could be used as a tool for diagnosis or patient follow up indicating the stage of vasculature remodelling, healing, inflammation or resolution.

Keywords

Introduction

Coronary artery disease is one of the major cardiovascular illnesses associated with great morbidity and mortality (Song et al. 2015). Atherosclerosis is the hallmark of the underlying etiology of coronary artery diseases leading to stenosis of the vasculature system (Mohammad et al. 2021). At the site of stenosis, different pathophysiological reactions are continuously propagating culminating and coordinating together to tackle the pathological condition associated with stenosis (Joseph et al. 1992). These reactions include, inflammatory process and oxidation reaction resulting in exacerbation of the condition at site of injury or stenosis (Vaisman et al. 2012). Vascular endothelium has been shown to play a great role in the overall scenario of the coronary arterial disease. The contribution of endothelium has been rely on two important parameters (Almulathanon et al. 2021; Joseph et al. 1992); local reaction processing and systemic secretome released in response to the inflammation (Song et al. 2015).

The systemic biomolecules served as potential markers to shape the severity of the atherosclerotic plaques (Joseph et al. 1992). Some of these biomolecules are useful while others are harmful reflecting the situation of atherosclerotic plaque at site of injury or inflammation (Pacher et al. 2007). Moreover, the plasma level of these

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biomolecules clarifies the prognosis of the atherosclerotic plaque and could be used as a diagnostic tool predicting the future status of the patient (Zouki et al. 2001). However, some of these parameters are intrinsically derived from endothelium itself while other are inducible; in response to stress or injury, by extravascular cells (e.g., immune cells and to certain extents platelets) to secrete their components but still under control of endothelial signaling (Pacher et al. 2007; Song et al. 2015). For example, myeloperoxidase, arginase, nitric oxide, metalloproteinase, and peroxidase.

Materials and methods

Sample collection

A total of 88 subjects (controls and hyperlipidemic patients) were enrolled in the present study. List for every subject has been completed and recorded in the questionnaire form as shown in Table 1. Control group included (45) healthy subjects of both sexes (25 females, 20 males), with age matching to the patients group. Patients group included (43) patients of both sexes (18 females, 25 males) with newly diagnosed hyperlipidemia. Venous blood samples (10 mL) were drawn after an overnight fasting from patients and control subjects. The blood transferred immediately to a clean dry plain tube without anticoagulant and incubated in water bath at 37 °C for 10 minutes to allow the blood to clot. Centrifugation was then done at (4000 × g) for (10 mins). Serum samples were transferred immediately by micropipette to other plain tubes (Bacchus et al. 1980) and stored at -20 °C to be analyzed later on.

Table 1. Demographic parameters of subjects included in the study.

Demographic parameters	Control group (n=45)	Patient group (n=43)	p value
Age (years)	35–65	35–66	–
Sex (M, F)	(20, 25)	(25, 18)	–
BMI (kg/m^2)	26 ± 1.5	27 ± 1.7	0.001

Estimation of lipid profile

Biochemical analysis of blood parameters was conducted on 6ml of venous blood collected from overnight fasted subjects. Serum was collected and frozen down at -20 for future analysis. Fasting serum total cholesterol (TC), Triglycerides (TG), and high-density lipoprotein (HDL) were measured based on enzymatic colorimetric methods using BIOLABO kit (Ruotolo et al.). Cholesterol and its esters are released from lipoprotein by detergent cholesterol easterase which hydrolyzes the esters, H2O2 is formed by the subsequent enzymatic oxidation of cholesterol with cholesterol oxidase. The absorbance of the colored complex (Quinoneimine), which is proportional to the amount of TC in the specimen was measured at 500 nm.

Triglyceride are hydrolyzed by lipase producing glycerol and free fatty acids. Glycerol participates in a series of reactions that end with the formation of a pink quinoneimine. The absorbance of the colored complex (Quinoneimine) was measured at 500nm.

Serum HDL-C was estimated by the precipitation method (Connell 2012), Using BIOLABO kit (Ruotolo et al.). High-density lipoprotein obtained in supernatant after centrifugation was measured with total cholesterol reagent (Kroll 1999). LDL and VLDL were calculated using special questions and plotted against control group.

Estimation of aryleasterase activity

Aryleasterase activity was assayed following the method of (Tomas et al. 2001). The method depends on the enzymatic hydrolysis of phenyl acetate to phenol and acetic acid. The absorbance of phenol produced was estimated at 270 nm. Serum (5 μL) was added to (1.5 mL) of buffer solution, mixed well, and (0.5mL) of substrate was added; the absorbance was recorded after 5 min against blank at 270 nm.

Estimation of metalloendopeptidase activity

Metalloendopeptidase was assayed following the method of (Kanazawa and Johnston 1991). The method depends on the enzymatic hydrolysis of casein (substrate) which was followed spectrophotometrically to measure the product at 275 nm. Casein substrate solution was prepared by dissolving (0.6 g) Casein in (4 mL) of sodium hydroxide solution in water bath at (60 °C) with stirring the mixture was cooled at room temperature and (10 mL) sodium tetraborate and (80 mL) distilled water were added, pH was adjusted to 11 and at 30 °C by using (1M) sodium hydroxide, distilled water was added to complete the volume to (100 mL).

Estimation of nitric oxide activity

The concentration of nitric oxide determined by Griess method (Kalayci et al. 2014, Larsen et al. 2008), the principle of assay based on colorimetry using kit supplied by Cayman, USA and the optical density at 540nm measured by microplate reader BioTek-Synergy, USA. The concentration of the samples was then extrapolated from the standards supplied by manufacturer kits.

Estimation of peroxynitrite activity

The concentration of peroxynitrite determined by kit supplied Cell Technology, USA by the principle of assay based on fluorescence techniques measured by microplate reader BioTek-Synergy, USA at an excitation/emission of 488nm/515 nm (Pacher et al. 2007). The concentration of the samples was then extrapolated from the standards supplied by manufacturer kits.

Estimation of arginase activity

Arginase concentration quantified using enzymatic technique of conversion of arginine to ornithine through measuring rate of conversion. The principle of assay based on colorimetric techniques.

Estimation of myeloperoxidase activity

Myeloperoxidase activity were quantified using enzymatic method (Kumar et al. 2002), the principle of assay was based on oxidation of dianisidine to colored material measured at 40 nm catalyzed by hydrogen peroxide

Estimation of peroxidase activity

Peroxidase activity was assayed depends on enzymatic oxidation of hydrogen peroxide by peroxidase to produce colored material whose concentration can be measured at 470nm (Joseph et al. 1992). The reaction was initiated by the addition of (50 µL) serum to the working solution and all the assays were performed in water bath at 37 °C. The absorbance was measured at 470 nm. One unit of peroxidase activity (U) represents the amount of enzyme catalyzing the oxidation of (1µ mole) of guaiacol in (1 min).

Data analysis

Data were expressed as the mean ± standard deviation. Comparisons between the investigated parameters for control and patients groups were conducted using the t test. P < 0.05 was considered a statistically significant difference. Statistical results were obtained using GraphPad Prism 5.0 (GraphPad Software Inc., La Jolla, CA, USA). The histogram chart was designed using a Microsoft Office 2010 Excel program.

Results

## Clinical characteristics of hyperlipidemic and control subjects

Baseline characteristics of the study groups (age, sex and body mass index) are listed in Table 1. Except for BMI, no significant differences have been found between control and hyperlipidemic patients.

Validation of lipid profile

The results of lipid profile tests indicated that LDL and VLDL significantly elevated in patient group compared to health control group (Figure 1).

Validation of serum AE, PO, AG, MEP, MPO, PN and NO

The measured endothelial markers indicated that hyperlipidemia associated with significant (p<0.001) elevation of
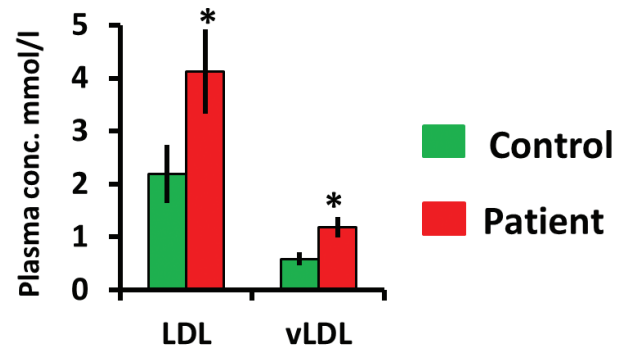
Figure 1. Lipid profile is higher in patient group compared to healthy individuals. LDL=low density lipoprotein, VLDL= very low-density lipoprotein. Data expressed as mean±SD, *p<0.001.

vascular endothelial markers
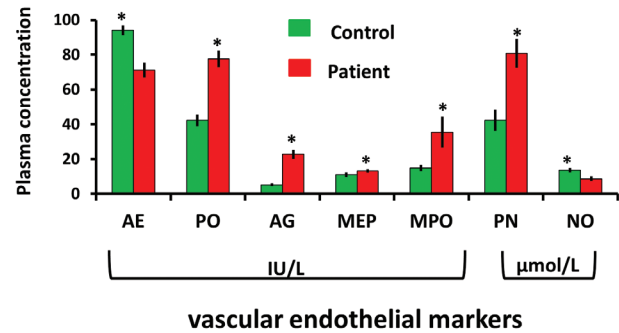
Figure 2. Hyperlipidaemia associated with modulation of vascular endothelial markers. Comparison between result analysed from serum of healthy and patient group indicated that Hyperlipidaemia reduced arylesterase (AE) and nitric oxide (NO) together with increase peroxidae (PO), arginase (AG), metalloendopeptidase (MEP), myeloperoxidase (MPO), and peroxynitrite (PN). Data expressed as mean±SD, *p<0.001.

plasma PO, AG, MEP, MPO, and PN together with significant (p<0.001) reduction of AE and NO.

Discussion

The endothelium play a great role in cardiovascular homeostasis in health and diseases (Pacher et al. 2007). This role has been dramatically linked to vasoactive biomolecules production (Vaisman et al. 2012). Hyperlipidemia is the initial step in atherosclerotic plaque formation which is primarily started by few successive steps including expression of endothelial adhesion molecules, trapping leucocytes, accumulating LDL together with oxidative stress and endothelial dysfunction (Pacher et al. 2007, Zouki et al. 2001). Some of these steps are partly inhibited by free radical nitric oxide NO˙ formation from arginine aided by nitric oxide synthase enzyme. Upregulation of arginase inhibit this metabolic pathway and encourage arginine conversion to threonine and urea (Hwang et al. 2015). This study has proved that newly diagnosed hyperlipidemia was associated with elevated arginase expression and subsequently reduced NO˙. The results have been confirmed

by other studies conducted on experimental animal and hyperlipidemic patients. A study conducted by Hwang et al. (2015), reported that elevated arginase and reduced NO levels has been associated with hyperlipidemia. Moreover, the condition has improved with lipid lowering agents or using plant-derived arginase inhibitor (Akinyemi et al. 2016). Arginase inhibition has been associated with restoration of hyperlipidemia-associated endothelial dysfunction in laboratory animals (Minozzo et al. 2018). Moreover, treatment of hyperlipidemic patients with atorvastatin (hypolipidemic agents) induced significant increase in NO level and reduced arginase activity (Khaleel et al. 2018). However, Ibrahim Suhad et al. (2019), reported that when hyperlipidemia coexist with type 2 diabetes mellitus the arginase overexpression is gender linked with greater expression in female than male patients.

Both NO and superoxide ($O_2\bullet-$) are considered as an important parameters in vascular endothelial physiology (Libby et al. 2011). In pathological condition, these two important physiologically active biomolecules interact to form tissue destructive biogenic compound; peroxynitrite (ONOO−) (Li et al. 2013, Pacher et al. 2007). The key point in ONOO− pathology is stimulation leukocyte activation and subsequent endothelial adherence and translocation resulting in loss of endothelial integrity (Pacher et al. 2007, Zouki et al. 2001). The present study demonstrated that early stages of hyperlipidemia were associated with elevated plasma ONOO− levels; which could be a marker for early endothelial injury. When hyperlipidemia coincide with peroxynitrite overproduction; due to pathological events, this will ensue LDL oxidation forming ox-LDL; a molecule with high affinity for vascular endothelial deposition (Lai and Yen 2002). In vitro induced hyperlipidemia model using laboratory animals revealed that there are a coexistence of hyperlipidemia with peroxynitrite overproduction, the study has explained that this effect might be primarily linked with elevated plasma lipid levels resulting in cholesterol deposition at subcellular level and increased ox-LDL levels (Kupai et al. 2009, Onody et al. 2003), alongside increasing susceptibility of cardiac defects (Li et al. 2013). Furthermore, subsequent study has demonstrated that inhibition of peroxynitrite formation using plant-derived isoflavones; has been associated with reducing of lipid-derangement associated with peroxynitrite; imparting cardiac protection (Csont et al. 2007, Lai and Yen 2002).

Once endothelial injury initiated; lipid deposition enhanced within vascular layer through LDL oxidation step which eventually lead to increase in intima media thickness (Odawara et al. 1997, Pati and Pati 1998). Oxidation of lipoproteins; especially LDL, has been antagonized by a family of protein called paraoxonase-1 (Antikainen et al. 1996). Arylesterase is a member of paraoxonase-1 family responsible about hydrolysis of lactone ring of these biomolecules and hence modulating the oxidation step (Pati and Pati 1998). The present study confirmed that hyperlipidemia reduced the plasma level of arylesterase encouraging lipid oxidation and subsequently its deposi-

tion in vascular spaces. The results are in agreement with studies conducted on hyperlipidemic patients versus control group; the results showed association between different paraoxonase isoforms expression and hyperlipidemia (Odawara et al. 1997, Pati and Pati 1998). However, results from other studies confirmed contradictory outcomes and suggested that genotype polymorphism and advanced cases might be linked to such variation in the results (Antikainen et al. 1996, Aubo et al. 2000, Suehiro et al. 1996).

Leukocyte activation were associated with increased myeloperoxidase activity, especially macrophages and neutrophils (Fathi et al. 2020). Myeloperoxidase associated with beneficial microbial killing, these actions were inevitably associated with tissue damage at the battle zone (Askari et al. 2003, Brennan and Hazen 2003). Correspondingly, the macrophages dealing with lipid digestion converted to foam cells in atherosclerotic plaque, during processing they release myeloperoxidase resulting in further endothelial damage and dysfunction. The vascular dysfunction were the result of increased NO consumption and LDL and HDL oxidation (Brennan and Hazen 2003). HOCl produced by myeloperoxidase associated with tissue destruction and plaque instability and increased vulnerability (Askari et al. 2003, Vasilyev et al. 2005). The present study demonstrated that early stages of hyperlipidemia were associated with increased plasma peroxidase and myeloperoxidase concentration and this might be associated with endothelial dysfunction and damage. A study conducted by Song et al. (2015) demonstrated that peroxidase and myeloperoxidase associated with atherosclerosis and promote cardiovascular disease progression (Brennan and Hazen 2003). Furthermore, knockout of MPO gene from laboratory animal after induction of myocardial infarction revealed reduction in leucocyte recruitment at subendothelial spaces (Askari et al. 2003) and reduced generation of tissue destructive aldehyde species (Vasilyev et al. 2005), confirming the great role of MPO in these inevitable actions.

Atherosclerotic plaque formation is associated with hyperlipidemia. Stable plaque by itself will not lead to acute and urgent effect. Stability is correlated to extracellular matrix intactness. Metalloendopeptidase enzyme degrade the extracellular matrix and increase plaque vulnerability leading to urgent conditions (thrombus or thromboembolic status). The endothelial damage associated with increased vulnerability to leukocyte infiltration and subsequent inflammatory pathway stimulation. The present study confirmed that early stages of hyperlipidemia might be associated with slight increase in plasma concentration of metalloendopeptidase enzyme and this could be a representative of progression of the hyperlipidemia to lipid deposition in sub-vascular spaces and also indicate leukocyte infiltration. Discrepant results have been reported by various studies regarding correlation between hyperlipidemia and increased plasma endopeptidases level; a study conducted by Weiß et al. (2020), confirmed that the metalloendopeptidases elevated in hyperlipidemic patients.

Conclusion

The present study confirmed that newly diagnosed hyperlipidemia is associated with a microscopic vascular lesion which are correlated with an elevation of vascular biomolecules.

Acknowledgements

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