Lipoprotein (a) and Inflammatory Markers in Hypertensive Patients

Hind Shakir Ahmed
PhD

Abstract:

Background: Hypertension is the most important public health problem in developing countries and one of the major risk factors for cardiovascular diseases. Lipoprotein (a) is a known risk factor for cardiovascular diseases and systemic inflammation such as interleukin-6 and high sensitive C-reactive proteinareimplicated in the development of hypertension.

Objective: The aim of the present study wasto detectthe associations between inflammatory markers as interleukin-6 and high sensitive C-reactive protein with atherogenic indices in hypertensive patients and compared with the control.

Patients and Methods: This study was conducted in Medical City Hospital during the period from November2013 until the end of June2014. Ninetyhypertensive patients were enrolledin this study(45 male and 45 female); their age range was (40-55) years and compared with 90 healthy subjects as control group. They studied for their serum lipoprotein (a) and lipid profile including total cholesterol, triacylglycerols, high density lipoprotein cholesterol, low density lipoprotein cholesterol, very low density lipoprotein, and non high density lipoprotein cholesterol in fasting state. Also inflammatory marker such as interleukin-6 and high sensitive C-reactive protein were measured in those patients and compared with the control group.

Results: There was a significant increase in lipoprotein (a) levels, total cholesterol, low density lipoprotein cholesterol, non high density lipoprotein cholesterol, interleukin-6, and high sensitive C-reactive protein in hypertensive patients as compared to the control, (P=0.0001).Also, there wasa significant increase in lipoprotein (a) levels, total cholesterol, low density lipoprotein cholesterol, non high density lipoprotein cholesterol, interleukin-6, and high sensitive C-reactive protein in grade 3 hypertensive as compared to grade 1 and 2 hypertensive patients.

Conclusions: It can be concluded thatlipoprotein (a) level was significantly higher in hypertensive patients as compared with the control group. So lowering its concentration would help prevention of cardiovascular diseases.Serum interleukin-6 and high sensitive C-reactive protein estimation can be used as potential tools for early identification of individuals at the risk for development of hypertension and eventually cardiovascular diseases.

Keywords: Hypertension, lipoprotein (a), interleukin-6, high sensitive C-reactive protein.

Introduction:

Hypertension results from a complex interaction of genes and environmental factors. Numerous common genetic variants with small effects on blood pressure have been well-known[1]. Many interconnected factors may becontributed to the development of hypertension differently in different individuals. Among the factors that have been carefully evaluated are salt intake, obesity, insulin resistance, renin angiotension system, and the sympathetic nervous system [2].

Lipoprotein (a) is a lipoprotein subclass. It is a low density lipoprotein (LDL)-like particle with a lipid core encircled by a large glycoprotein, the apoprotein (a) is tightly linked to apolipoprotein B100 molecule [3]. The two molecules are most likely complexed in the hepatocyte cellular membrane and are connected biochemically byadisulfide bridge through cysteine residues within apo (a) (Cys 4057) and apo B100 (Cys 4326) [4]. Evidence suggests that Lp (a) catabolized primarily by hepatic and renal pathways. Elevated Lp (a) levels can potentially raise the risk of cardiovascular diseases (CVD) (i) via prothrombotic/anti-fibrinolytic effects as apo (a) possesses structural homology with plasminogen and plasmin but has no fibrinolytic activity and (ii) via accelerated atherogenesis as a result of intimal deposition of Lp (a) cholesterol, or both [5].Lipoprotein (a) accumulates in the vessel wall and inhibits binding of plasminogen to cell surface. This inhibition of Lp (a) promotes proliferates of smooth muscle cells. These unique features of Lp (a) propose that it causes generation of clots and atherosclerosis[6].

Biochemical markers like some inflammatory markers as interleukin-6(IL-6) and high sensitive C-reactive protein (hs-CRP) as risk factors of developing hypertension are less studied parameters. Both endothelial activation and chronic inflammation are pathophysiologic processes potentially concerned in the development of hypertension [7].

Interleukin-6 is acts as together a pro-inflammatory cytokine and an anti-inflammatory myokine. In humans, it is encoded by the *IL6* gene[8].

High sensitive C-reactive protein is a very sensitive marker of inflammation, which is synthesized in the liver, and this process is regulated predominately by IL-6[9]. It is positively associated with abdominal fat and closely

associated with increased risk of cardiovascular (CV) events [10].

In current strategies of global risk evaluation, lipids testing are generally the blood tests that are routinely recommended. However, hs-CRP and Lp (a) evaluation may have the potential to improve CV risk prediction models when used in addition to usual lipid profiles [11].

The aim of this study was to detectthe associations between inflammatory markers such as IL-6 and hs-CRP with both classical and atherogenic indices in hypertensive patients and compared to the control.

Patients and Methods:

This study was conducted in Medical City Hospital during the period from November 2013 until the end of June2014. Ninety hypertensive patients were enrolled in this study (30 grade 1, 30 grade 2, and 30 grade 3); their age range was (40-55) years and they were compared with 90 healthy controls.About5 ml of blood sample was obtained from every hypertensive patient and control. The separated serum was used for measurements of Lp (a), IL-6, hs-CRP, and lipid profile.

Classification of Blood Pressure and Hypertension:

The classification of blood pressure and hypertension was recommended by the Latin American Consensus[12].

Blood pressure	Value (mmHg)
Optimal	<120/80
Normal	120/80–129/84
High normal	130/85–139/89
Grade 1 hypertension	140/90–159/99
Grade 2 hypertension	160/100–179/109
Grade 3 hypertension	≥ 180/110

Exclusion Criteria:

All subjects who were using any medications that interfere in Lp (a) analysis (lipids lowering drugs,steroid), and subjects with renal diseases, chronic liver diseases, malignant disorders, diabetes mellitus, and thyroid gland were not considered to be in this investigation.

Measurements:-Anthropometric Measurements:

Blood pressures were recorded according to the guidelines adopted by WHO[13].Body mass index (BMI) was calculated by dividing subjects weight (Kg) by their height (m^2). BMI calculated as:BMI= mass (kg)/(height (m))^2 [13].

-Lipids and Lipoproteins Assessments:

Lipoprotein (a) in sample or standard cause agglutination of the latex particles coated with anti-Lp (a) antibodies. The agglutination is proportional to the Lp (a) concentration in the sample and can be measured by turbidimetry [14].Serum total cholesterol (TC), triacylglycerol (TAG), and high density lipoprotein cholesterol (HDL-C) were measured using an enzymatic method [15-17].Serum low density lipoprotein cholesterol (LDL-C)was calculated indirectly by using the Friedewald's equation [18]LDL-C = TC – [HDL-C + TAG/5]. This equation is only accurate when: TAG levels are below 400 mg/dl. Serum non high density lipoprotein cholesterol(non HDL-C)was calculated directly from the difference between serum total and high density lipoprotein cholesterol [19].Serum non HDL-C= (S.TC–S.HDL-C).

-Estimation of serum of IL-6 and hs-CRP:

Interleukin-6 was determined in serum using ELISA Kit[20]. High sensitive CRP assay employs the quantitative sandwich enzyme immunoassay technique [21].

Statistical Analysis:

Comparisons between groups were performed using ANOVA and student's *t*-tests.Data were expressed as means (±SD) and P values less than 0.05 was considered statistically significant. Simple and partial correlation coefficients between the

variables were performed to determine the relationships between the variables of interest.

Results:

Demographic and clinical characteristicsof hypertensive patients and the control group were shown in table (1). Based on analysis of variance, age, systolic blood pressure (SBP), diastolic blood pressure (DBP), BMI, Lp (a), TC, LDL-C, non HDL-C IL-6, and hs-CRP were significantly higher in hypertensive patients than in the control group, (P=0.0001), while there was a significant decrease in HDL-C in hypertensive patients as compared to the control, (P=0.0001).

Table (2) showed clinical and laboratory characteristics of the patients according to the grades of hypertension. Lipoprotein (a) levels more than 30 mg/dl is generally considered as the threshold value of high risk for its pathological effect. In the present study taking 30 mg/dl as the cut off value, grade 2 and grade 3 of hypertensive patients had Lp (a) levels more than 30 mg/dl. It was observed that means of age SBP, DBP, BMI, Lp (a), TC, LDL-C, non HDL-C, IL-6, and hs-CRP were significantly higher in grade 3 hypertensive patients than in grade 1 and 2, (P=0.0001), while there was a significant decrease in HDL-C in grade 3 hypertensive patients as compared to grade 1 and 2, (P=0.0001). Levels of TAG and VLDL were elevated in grade 3 hypertensive patients than ingrade 1 and 2, but they were not significant.

Correlations coefficient of the entire population showed that there wasa significant positive correlation between Lp (a) versus age, BMI, TC,LDL-C, non HDL-C, IL-6, and hs-CRP. While there was a significant negative correlation between Lp (a)and HDL-C, table (3).

Table (1): The Demographic and clinical characteristicsof the study group

Clinical Data	Hypertensive (n= 90)	Control (n=90)	P Value
Age (years)	43.62±2.71	40.27 ± 0.72	0.0001
(Male/Female) n.	45/45	45/45	-
SBP (mm Hg)	163.87±1.34	118.80 ±0.45	0.0001
DBP (mm Hg)	103.60±1.25	78.07 ± 4.71	0.0001
Duration (years)	2.24±0.95	-	-
BMI (Kg/m^2)	30.89±4.46	21.53 ± 0.31	0.0001
Lp (a) (mg/dl)	30.54±6.31	18.98 ± 1.07	0.0001
TC (mg/dl)	226.15±7.88	147.33 ± 2.93	0.0001
TAG (mg/dl)	106.25±8.15	91.93 ± 8.98	0.50 NS
HDL-C (mg/dl)	37.47±2.07	65.60 ± 4.32	0.0001
LDL-C (mg/dl)	167.43±5.38	63.35 ± 4.32	0.0001
VLDL-C (mg/dl)	21.25±1.43	18.39 ± 1.79	0.50 NS
Non HDL-C (mg/dl)	188.68±6.81	81.73 ± 4.17	0.0001
IL-6 (ng/ml)	146.47±0.22	5.75±0.22	0.0001
hs-CRP (mg/dl)	15.3 ± 2.5	0.5 ± 2.1	0.0001

Table (2): Clinical and laboratory characteristics of hypertensivepatients according to thegrades of hypertension

Clinical Data	Hypertensive Patients/Grade 1 (n= 30)	Hypertensive Patients/Grade 2 (n= 30)	Hypertensive Patients/ Grade 3 (n=30)
Age (years)	41.20 ± 0.64*	44.33 ± 0.68*	45.50 ± 0.73*
(Male/Female) n.	15/15	15/15	15/15
SBP (mm Hg)	142.87 ± 1.34*	162.87 ± 1.34**	185.87 ± 1.34***
DBP (mm Hg)	94.60 ± 1.25*	103.60 ± 1.25**	112.60 ± 1.25***
Duration (years)	1.60 ± 0.19NS	2.08 ± 0.21NS	3.03 ± 0.18 NS
BMI (Kg/m^2)	27.87 ± 3.24***	31.07 ± 0.28***	33.73 ± 3.24***
Lp (a) (mg/dl)	27.98 ± 3.75***	30.68 ± 0.42***	32.95 ± 3.90***
TC (mg/dl)	191.93 ± 5.83***	220.0 ± 1.73***	232.47 ± 1.56***
TAG (mg/dl)	98.0 ± 0.10 NS	109.87 ± 1.50 NS	110.20 ± 4.2 NS
HDL-C (mg/dl)	39.0 ± 0.54***	38.0 ± 1.6***	34.40 ± 1.0***
LDL-C (mg/dl)	133.33 ± 5.27***	159.73 ± 0.01***	176.93 ± 0.92***
VLDL-C (mg/dl)	19.6 ± 0.02 NS	21.97 ± 0.30 NS	22.04 ± 0.84 NS
Non HDLC (mg/dl)	152.30 ± 5.92***	182.0 ± 0.13***	198.07 ± 0.56***
IL-6 (ng/ml)	80.50±4.50***	92.80±2.95***	266.11±7.16***
hs-CRP (mg/dl)	10.2 ± 1.3***	15.0 ± 2.5***	19.8 ± 2.5***

**P< 0.05, **P< 0.001, ***P< 0.0001, NS: not significant.*

Table (3): Correlations between lipoprotein (a) and othervariablesunder study

Lp (a)	Correlation coefficient (r)	Lp (a)	Correlation coefficient (r)
Age (years)	0.85**	**TAG (mg/dl)**	0.20 NS
BMI (Kg/m^2)	0.93**	**HDL-C (mg/dl)**	- 0.92**
SBP (mmHg)	0.48 NS	**LDL-C (mg/dl)**	0.90**
DBP (mmHg)	0.39 NS	**Non HDL-C (mg/dl)**	0.91**
Duration (years)	0.30 NS	**IL-6 (ng/ml)**	0.98**
TC (mg/dl)	0.86**	**hs-CRP (mg/dl)**	0.92**

*** P <0.001,NS: not significant.*

Discussion:

In this study, dyslipidemia showed that hypertensive patientshadhigher levels of serum TC, and LDL and significantly lower level of HDL-C which is well documented in the study of Onwubuya et al. [22]. Further analysis of these lipid and lipoprotein indices in different grades of hypertension showed a statistically significant difference among the levels of non HDL-C in grade 1, 2, 3 as compared to the control, but there was no significant difference in TAG and VLDL between these grades.

Serum Lp (a) had been found in harmony with Sonal et al.[23]. This dysregulation of lipid metabolism may contribute to the pathogenesis of atherosclerosis and CVD and to the progression of heart disease. Lipoprotein (a) has properties in common with LDL-C but contains a unique protein, apo (a), which is structurally different from other apolipoproteins[24].Circulating levels of Lp (a) are extremely resistant to common lipid lowering therapies, and there are currently no robust treatments available for reduction of Lp (a) apart from plasma apheresis, which is expensive and labor intensive [25]. The clinical interest in Lp(a) is largely resulting from its role asa cardiovascular risk factor. Lipoprotein (a) levels have been associated with CVD in numerous studies[26,27]. Compared with small, dense LDL particles, Lp (a) is characterized bya different protein content (17-29% verse 26-31%),a longer half-life in *vivo* and an accentuated athero-thrombotic potential, probably due to its

preferential accumulation within atherosclerotic plaque [28].In the current study,a significant correlation was observed between Lp (a) levels and the other CVD risk factors, such as BMI which is in agreement with the study of Sharma et al[29].

Recent diagnosis of lipid disorders and cardiovascular risk should be based on the indicators which present full impact of all plasma lipid components involved in atherogenesis. Non HDL-C is used as an estimation of the total number of atherogenic particles in plasma, which represents [VLDL+ intermediate density lipoprotein(IDL) +LDL-C] and relates well to apo B levels.In fact, Rana et al. suggested it to be better indicator than LDL-C in predicting cardiovascular events and has been shown to predict coronary heart diseases similar to apo B levels[30]. Thus, not only lowering LDL-C, but also nonHDL-C is an important target of prevention and treatment of CVD.

Previous epidemiologic studies have connected higher plasma concentrations of inflammatory markers including CRPandIL-6 to increased SBP and DBP or hypertensive status [31,32].

This study established that hypertensive patients had higher levels of inflammatory markers. High sensitive CRP, the most widely studied inflammatory marker, may stimulate endothelial activation by decreasing the expression and activity of nitric oxide synthase[33], facilitating release of endothelin-1 and reducing endothelial cell survival and differentiation[34].

Some studies highlight the possibility that arterial stiffening may precede progress of hypertension suggesting that inflammation may play a role in arterial stiffness [35,36]. All of this data suggest that vascular inflammation plays a role in pathophysiology of hypertension and may potentiate the proatherogenic effects of hypertension.

Conclusions:

The data of the present study confirm the role of Lp (a) and some inflammatory markers as predictor of the severity of coronary atherosclerosis, suggesting that Lp (a) levels should be determined in patients with arterial hypertension, especially in those with atherogenic dyslipidemia and high levels of hs-CRP and IL-6. ElevatedLp (a) levels ≥30 mg/dl beside high levels of hs-CRP and IL-6reportedly predict subsequent CV events incidence with stable coronary disease, especially in patients with suboptimal LDL-C control ≥70 mg/dl

References:

1- Ehret GB, Munroe PB, Rice KM, et al.Genetic variants in novel pathways influences blood pressure and cardiovascular disease risk. Nature. 2011;478(7367):103-109.

2-Al-Saweer A. Hypertension in clinical practice. Educatin-family physician corner.Bahrain Medical Bulletin.2011;33(1):38-43.

3-Ginter E and Simko V. Enigmatic lipoprotein (a) and cardiovascular diseases. BratislLekyListy. 2010; 111(10):570-573.

4- Siekmeier R, Scharnagl H, Kostner GM, Grammer T, Stojakovic T, and Marz W. Variation of Lp (a) plasma concentrations in health and disease. Open ClinChem J. 2010; 3:72-79.

5-Nordestgaard BG, Chapman MJ, Ray K, Boren J. et al. Lipoprotein (a) as a cardiovascular risk factor: current status. Eur Heart J. 2010; 31(23):2844-53. 6-AshfaqF, GoelPK, SethiR, KhanMI, AliW, and IdrisMZ. Lipoprotein (a) levels in relation to severity of coronary artery disease in north Indian patients.2013; 14(1): 12-16

7-Watson T, Goon PK, and Lip GY. Endothelial progenitor cells, endothelial dysfunction, inflammation, and oxidative stress in hypertension. Antioxid Redox, Signal, 2008; 10: 1079-1080.

8- Ferguson-Smith AC, Chen YF, Newman MS, May LT, Sehgal PB, and Ruddle FH.Regional localization of the interferon-beta 2/B-cell stimulatory factor 2/hepatocyte stimulating factor gene to human chromosome 7p15-p21. Genomics. 1988; 2(3): 203-208.

9- Leal VD and Mafra D. Adipokinesin obesity. ClinChimActa.2013; 419:87-94.

10- De Heredia FP, Gomez-Martínez S, and Marcos A. Chronic and degenerative diseases.Obesity, inflammation and the immune system.5thInternational Immuno-nutrition Workshop. Proc Nutr Soc. 2012; 71:332-338.

11- Everett BM, Kurth T, Buring JE, and Ridker PM. The relative strength of C-reactive protein and lipid levels as determinants of ischemic stroke compared with coronary heart disease in women. J Am Coll Cardiol. 2006; 48(11): 2235-2242.

12-Sanchez RA, Ayala M, Baglivo H, Velazquez C, Burlando G, Kolmann O, et al. on behalf of the Latin American expert group. Latin American guidelines on hypertension. J Hypertens. 2009; 27(5):905-922.

13- World Health Organization. International Society of Hypertension: guideline formanagement of hypertension. Guideline subcommittee.Journal of Hypertension.1999;17:151-183.

14- Phillips ML, Lembertas AV, Schumake VN, Lawn RM, Shire SJ, and Zioncheck TF. Physical properties of recombinant apolipoprotein (a) and

its association with LDL to form an Lp (a)-like complex. Biochem.1993;32(14):3722-8.
15-Richmond W. Analytical reviews in clinical biochemistry: the quantitative analysis of cholesterol. Ann. Clin. Biochem.1992;29(26):577-597.
16-Fossati P and Prencipe L. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clin. Chem. 1982;28(10):2077-2080.
17-Burstein M, Scholnick HR, and Scand MR. Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions. Journal Clinical Lab.Invest. 1982;11(6):583-595.
18-Friedewald, William T, Robert I, Levy, and Donald S.Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge.Clinical chemistry.1972;18(6):499-502.
19-HermansMP, Sacks FM, Ahn SA, and Rousseau MF. Non-HDL-cholesterol as valid surrogate to apolipoprotein B100 measurement in diabetes: Discriminant ratio and unbiased equivalence. Cardiovascular Diabetology.2011;10(20):1-7.
20-Shimada MA, Andoh K, Hata K, Tasaki K, Araki Y, Fujiyama Y., and Samba T. IL-6 secretion by human pancreatic periacinarmyo fibroblasts in response to inflammatory mediators. J. Immunol. 2002; 168 (2):861-868.
21-Pearson TA , Menash GA , Alexander RW, Anderson JL, Cannon RO, Criqui M, et al. Marker of inflammation and cardiovascular disease. Circulation. 2003; 107(3): 499-511.
22-Onwubuya EI, Anisiuba BC, Osuji CU, and Ahaneku JE. Changes in lipids and lipoprotein indices in relation to the severity of hypertension in newly diagnosed hypertensive Nigerians.ISRN Cardiology.2012;2012, ID 972341, 7 pages
23-Sonal S, Ranjana M, and Gupta RC.Serum lipids, lipoproteins and Lp (a) in hypertenstion. Journal of Advance Researches in Biological Sciences.2011;3(2):128-130.
24-Galvano F, Malaguarnera M, Vacante M, Motta M, Russo C, Malaguarnera G, et al. The physiopathology of lipoprotein (a). Front Biosci (Schol Ed).2010;2:866-75.
25-RichesKand Porter KE. Lipoprotein(a): cellular effects and molecular mechanisms, Cholesterol. 2012;2012(923289):1-10.
26- Dube JB, Boffa MB, Hegele RA, and Koschinsky ML. Lipoprotein(a): more interesting than ever after 50 years,Current Opinion in Lipidology. 2012;23(2):133-140.
27-Serban C, Dragan S, Mozos I, Noveanu L, Susan L, Christodorescu R, Pacurari A, Caraba A, and Romoşan I. Lipoprotein (a): an emerging cardiovascular risk factor in hypertensive patients. International Journal of Collaborative Research on Internal Medicine & Public Health.2011;3(10):733-742.
28-Lippi G and Guidi G. Lipoprotein (a): an emerging cardiovascular risk factor. Critical Reviews in Clinical Laboratory Sciences. 2013; 40(1):1-42.
29Sharma S, Merchant J, and Fleming SE. Lp (a)-cholesterol is associated with HDL cholesterol in overweight and obese African American children and is notan independent risk factor for CVD. Cardiovascular Diabetology.2012;11(10):1-7
30-Rana JS, Boekholdt SM, Kastelein JJ, and Shah PK.The role of non HDL cholesterol in risk stratification for coronary artery disease. Curr Atheroscler. Rep. 2010;14(2):130-134.
31-Bermudez EA, Rifai N, Buring J, Manson JE, and Ridker PM. Interrelationships among circulating interleukin-6, C-reactive protein, and traditional cardiovascular risk factors in women. Arterioscler ThrombVasc Biol. 2002; 22(10):1668-1673.
32-Kamel HM, Amin AE, and El-Adawy AR. Endothelial and some cytokine inflammatory markers as risk factors of hypertension in postmenopausalWomen.Life Science Journal. 2014;11(3):220-223.
33-Verma S, Wang CH, Li SH, Dumont AS, Fedak PW, Badiwala MV, et al.A self-Fulfilling prophecy: C- reactive protein attenuates nitric oxide production and inhibits angiogenesis. Circulation. 2002;106(8):913-919.
34-Engeli S, Feldpausch M, Gorzelnia KK, Hartwig F, Heintze U, Janke J, et al. Association between adiponectin and mediators of inflammation in obese women. Diabetes. 2003; 52(4): 942-947.
35- Kampus P, Muda P, Kals J, et al. The relationship between inflammation and arterial stiffness in patients with essential hypertension. Int J Cardiol. 2006; 112(1):46-51.
36- Kim JS, Kang TS, Kim JB, et al. Significant association of C-reactive protein with arterial stiffness in treated non-diabetic hypertensive patients. Atherosclerosis. 2007; 192(2):401-6.