The Association Between Leptin with Lipid Profile and Troponin in Acute Myocardial Infarction Patients

Ammal Esmaeel Ibrahim*, Hadef Dhafer EL-Yassin* , Hamid Kareem Sachit AL-Janabi**

ABSTRACT :

BACKGROUND:

Fat cells, are the cells that primarily compose adipose tissue, specialized in storing energy as fat. Adipose tissue also serves as an important endocrine organ by producing hormones such as leptin, resistin, and the cytokine. Leptin is a protein hormone that plays a key role in regulating energy intake and energy expenditure, including appetite and metabolism. Acute Myocardial Infarction (AMI), is the interruption of blood supply to part of the heart, causing some heart cells to die.

OBJECTIVE:

To investigate the levels of leptin and its effect on lipid profile level in Acute Myocardial Infarction.

SUBJECTS AND METHODS:

The study included 50 patients with Acute Myocardial Infarction and forty healthy subject as control group. leptin and lipid profile levels were measured.

RESULTS:

The levels of leptin were significantly elevated in female patients group with($p=0.002$), in male patients group($p=0.018$) and in total patients group ($p=0.001$), cholesterol and LDL-C were significantly elevated with ($p<0.001$), while HDL-C was significantly lower with ($p<0.001$), there was positive correlation between leptin with , cholesterol LDL-C, triglyceride and VLDL, and there was negative correlation between leptin with HDL in acute myocardial infarction patients.

CONCLUSION:

Leptin negatively correlated with HDL and positively correlated with triglyceride and LDL this relation make this hormone act as atherosclerotic factor.

KEY WORD: leptin ,Lipid profile ,BMI and Acute Myocardial Infarction.

INTRODUCTION:

Fat cells, are the cells that primarily compose adipose tissue, specialized in storing energy as fat.[1] It is technically composed of roughly only 80% fat; fat in its solitary state exists in the liver and muscles. Its main role is to store energy in the form of fat, although it also cushions and insulates the body. Adipose tissue also serves as an important endocrine organ by producing hormones such as leptin, resistin, and the cytokine. [2]Several traditional cardiovascular risk factors track with inflammatory biomarkers, in particular central obesity and body mass index. These observations have considerable importance because adipocytes can produce inflammatory cytokines, and a common underlying disorder of innate immunity may well link obesity, accelerated atherosclerosis, and insulin resistance. [3]Leptin (Greek leptos meaning thin) is a 16 kDa protein hormone that plays a key role in regulating energy intake and energy expenditure, including appetite and metabolism. It is one of the most important adipose derived hormones. [4]Acute Myocardial Infarction (AMI), is the interruption of blood supply to part of the heart, causing some heart cells to die. This is most commonly due to blockage of a coronary artery following the rupture of a vulnerable atherosclerotic plaque, leading to ischemia. [5] Troponin is attached to protein tropomyosin and lies within the groove between actin filaments in muscle tissue. In a relaxed muscle, tropomyosin

*Department of Physiology Chemistry, College of Medicine University of Baghdad.
**Department of Internal Medicine, Collage of Medicine, University of Baghdad.

blocks the attachment site for the myosin crossbridge, thus preventing contraction. When the muscle cell is stimulated to contract by an action potential, calcium channels open in the sarcoplasmic reticulum and release calcium into the sarcoplasm. Some of this calcium attaches to troponin, causing a conformational change that moves tropomyosin out of the way so that the cross bridges can attach to actin and produce muscle contraction. Troponin is found in both skeletal muscle and cardiac muscle, but the specific versions of troponin is different among types of muscle. The main difference is that the TnC subunit of troponin in skeletal muscle has four calcium ion binding sites, whereas in cardiac muscle there are only three. [6].

SUBJECTS AND METHODS:

This study was performed during the period from December 2009 to April 2010. This study include fifty patients with Acute Myocardial Infarction (AMI) whom admitted to Cardiac Care Unit (CCU) at Medical City Teaching Hospital and Ibn –ALbetar Hospital in Baghdad. Patients, with age rang (20-78) years old. Blood samples were taken from the patients after having thoroughly examined after exclusion of subjects with a history a AMI or diabetes mellitus or any chronic diseases. Control groups contain forty age, sex and BMI matched, apparently healthy individuals, were included in this study as control group.

Blood collection and laboratory analysis. From each patient and control, five ml venous blood were aspirated from a suitable vein. Samples were collected between (8-9 A.M.) after 10 hours fast. Blood samples were divided into two parts, three ml transferred to a plain tube for (lipid profile). The remaining of blood was transferred to another sterile plain tubes for storage to measure leptin and troponin. The non heparinized blood in the plain tubes were left to clot and then centrifuged at 4000 rpm for 5 minutes to separate the serum and dispensed into tightly closed Eppendorf tubes in 1.0 ml aliquot, and stored at -20 C° until assayed. Each serum sample was analyzed for urea and creatinin to exclude kidney diseases. Lipid profile were measured by using standard methods with reagents from BioMaghreb Company – Tunisia, leptin and troponin were measured by using ELISA kits from UnitedStatesBiological-Company.

Statistical analysis Statistical analysis was performed by statisticians with the SPSS 15.01 Statistical Package for Social Sciences and Excel 2003. Data analysis was done using chi- square test for tables with frequencies, while we used independent sample t-test for tables with means and standard error. p value of ≤ 0.05 was used as the level of significance. Correlation coefficient was used to find the correlation between studied markers by using Pearson correlation. Descriptive statistics for the clinical and laboratory results were formulated as mean and standard error

RESULTS:

Lipid profile and troponin: Serum levels of lipid profile and troponin were compared between the patients groups and control group using analysis of variance t-test of significant as in table(1). The patients with AMI were found to have significantly higher serum cholesterol, LDL, LDL/HDL, and troponin ($p<0.001$) and significantly lower HDL ($p<0.001$) compared with control group.

Table 1: Comparison between studied groups for (leptin ,lipid profile and troponin)

parameters	Female patients Mean±SR NO.=16	Female Control Mean±SR NO.=16	P-value	Male Patient Mean±SR NO.=24	Male Control Mean±SR NO=34	p-value	Total Patients Mean±SR N0.=50	Total Control Mean±SR N0.40	P- value
Leptin ng/ml	73.405 ±21.86	26.48 ±6.25	0.002	46.99 ±19.36	27.933 ± 6.64	0.018	62.68 ±13.88	27.33 ±7.22	0.001
Total cholesterol mg/dl	183.76 ±49.20	126.75 ±26.295	0.003	174.03 ±43.98	99.478 ±25.225	<0.001	177.40 ±25.59	104.17 ±22.68	<0.001
Triglyceride mg/dl	142.937±14.576	138.05 ±16.62	0.484	152.96 ±11.71	133.478 ±19.153	0.051	141.26 ±12.93	137.35 ±18.87	0.744
HDL mg/dl	37.71 ±3.06	59.777 ±10.458	<0.001	29.36 ±8.82	69.737± 8.249	<0.001	39.26 ±2.50	67.26 ±11.08	<0.001
LDL mg/dl	154.36 ±6.64	100.937 ±10.959	<0.001	156.80 ±12.04	119.852 ±8.753	<0.001	149.42 ±25.50	106.26 ±2.50	<0.001
LDL/HDL	3.82	0.361	<0.001	4.95	0.313	<0.001	4.56	0.350	<0.001
VLDL mg/dl	35.387 ±6.746	27.57 ±2.32	0.508	39.58 ±6.33	26.521 ±5.737	0.051	38.23 ±8.58	27.28 ±8.64	0.744
Troponin ng/ml	34.63 ±6.51	0.0	--	38.18 ±9.95	0.0	--	30.21 ±8.36	0.0	-----

Relation between leptin with (lipid profile and troponin)

The results showed that there was strong positive correlation between leptin with cholesterol in (female patient group (r=0.517), in male patient group (r= 0.518) and total patients (r=0.662)) also with triglyceride in total patient (r=0.569), also between leptin with LDL in female patients group (r=0.571), in male patients group (r=0.516) and in total patient (r=0.519), also between leptin with LDL/HDL in female control group(r= 0.614), also with VLDL in female patient group (r=0.614) and in female control group(r=0.590) and with troponin in male patient group (r=0.518).

The positive correlation was found between leptin with cholesterol in female control group (r=0.410), in male control group (r=0.380) and in total control (r=0.409), also with triglyceride in in female patients group (r=0.479), in female control group(r=0.409),in male patient group (r=0.400), in male control (r=0.371) and in total control (r=0.390), also with LDL in female control group (0.490),in male control group (r=0.433) and in total control(r=0.466), also with LDL/HDL in female control group (r=0.395), in male patient group (r= 0.427) in male control group (r=0.398), in total patient (r=0.415) and in total control (r=0.395). while the positive correlation with VLDL found in male patient group (r=0.423), in male control group (r=0.397), in total control (r=0.471) and in total control (r=0.450), also with troponin in female patient group (r=0.409) and in total patient group(r=0.400).

The strong negative correlation was found between leptin with HDL in female patient group (r=-0.606), in female control group (r=-0.559), in male patient group(r= -0.517), in male group(r=-0.500) and total patient (r=-0.600). while the negative correlation was found between leptin with HDL in total controlgroup(r=-0.351)

Table 2: The correlation between leptin with (lipid profile and troponin) for studied groups.

parameters	Female Patients NO=16	Female Control NO=16	Male Patients NO=34	Male Control NO.=24	Total Patients NO.=50	Total Control NO.=40
Hypertension	0.433	----	0.245	--	0.557	---
Cholesterol mg/dl	0. 517	0.410	0.518	0.380	0.662	0. 409
Triglyceride mg/dl	0.479	0. 409	0.400	0.371	0.569	0. 390
HDL mg/dl	-0. 606	-0.559	-0. 517	-0.500	-0.600	-0. 351
LDL mg/dl	0.571	0. 490	0. 516	0.433	0. 519	0.466
LDL/HDL mg/dl	0. 614	0.395	0.427	0.398	0. 415	0.395
VLDL mg/dl	0. 614	0. 590	0.423	0.397	0.471	0. 450
Troponin ng/ml	0.409	----	0.518	--	0. 400	---

DISCUSSION:

There are several reasons for increased serum leptin which lead to increased the cardiovascular risk; leptin stimulates vascular smooth cell proliferation ,accelerates vascular calcification, induces oxidative stress in endothelial cells that may contribute to atherogenesis, and promotes coagulation by increasing platelet adhesiveness. [7] Furthermore, there may be hormonal pathway acting independently of either metabolic or inflammatory disturbances, with the finding that fasting serum leptin levels were independently associated with arterial distensibility. The effect of obesity on vascular function may be mediated by the hormone leptin. Obese individuals have markedly increased leptin production probably as a consequence of resistance to its function, and this agrees with our control groups, we noticed that leptin eleveated markedly in obese individual in control groups and in patients group as in table (1) and this elevation may be due to high insulin level in patients because leptin have positive correlation with insulin in addition elevated value of BMI.

Göran Hallmans., et al., in 2008[8] reported that the presence of the leptin receptor in the heart suggests that leptin could modulate cardiac function directly. Leptin may also exert other proinflammatory effects like maturation of lymphocytes into more proinflammatory phenotypes, characterized by production of proinflammatory cytokines. The most convincing evidence related to endothelial dysfunction or damage is associated with an activation of macrophages located in the vessel wall with a continued release of cytokines and proteolytic vessel wall degrading enzymes. The association with cardiolipin antibodies with stroke gives further evidence for an inflammatory component in stroke.[8]

In endothelial cells, leptin induces oxidative stress, increases production of Monocyte Chemoattractant Protein-1 (MCP-1) and endothelin-1, and potentiates proliferation. In smooth muscle cells, leptin promotes migration, proliferation and hypertrophy, this latter effect being mediated by activation of p38 mitogen-activated protein kinase. Leptin also contributes to increased activation of cytokine and its production by macrophages, neutrophils, and T lymphocytes. Finally, leptin promotes calcification of cells of the vascular wall and facilitates thrombosis by increasing platelet aggregation. Although these effects of leptin point to a proatherogenic role for this adipokine, it is important to note that obesity is associated with leptin resistance, which leads to a reduced biological response to leptin. Leptin resistance, probably mediated by alterations in leptin receptor signaling pathways and originally reported in the hypothalamus of obese subjects and experimental animals, has been attributed to extend to the peripheral effects of leptin, including those on platelets and the vascular wall. [9]

Leptin and Hypertension

In table(2) we found strong positive correlation between leptin and H.T but this correlation is less in male patients group, and this is due to the higher concentration of leptin in female compared to male, so leptin is more effective in female than male. This agrees with Rahmani Nia et al., in 2009,[10] who mentioned that cardiovascular risk factors such as hypertension and atherosclerosis, which increased with increasing obesity, were associated with increased circulating plasma leptin levels. Leptin is also produced in addition to the adipose tissue by heart, vascular smooth muscle, placental tissue, digestive epithelia and gastric mucosa. Higher leptin levels in essential hypertension may suggest a possible role for leptin in the development of atherosclerotic heart disease.[10]

(Rahmani Nia et al., 2009) [10] study showed that chronic leptin administration or overexpression of leptin produces hypertension, this supports the concept of hemodynamic actions of leptin which is due predominantly to sympathetic activation. Mechanisms responsible for increased SNA (Sympathetic Nervous Activation) remains unknown.

The receptor-mediated sympatho-excitatory effect of leptin is supported by the absence of SNA response to leptin in obese Zucker rats. (Rahmouni *et al*., 2009) [10] demonstrated an absence of renal SNA response to leptin in db/db mice that indicates that the effects of leptin on sympathetic outflow are mediated by the long-form Ob-Rb of the leptin receptor. The leptin receptor has divergent signaling capacities and modulates the activity of different intracellular enzymes. Although STAT signaling was thought to be the main pathway that mediates the leptin action in the hypothalamus, PI3-K has been found to play a pivotal role in the effect of leptin on food intake.

Kamal Rahmuni and William.,2009[11] has demonstrated that PI3-K plays a major role in the transduction of leptin-induced changes in renal sympathetic outflow. Leptin likely controls sympathetic nerve activity in a tissue-specific manner, for several reasons.

First, activation of arterial baroreceptors and hypothermia modulate differentially leptin-induced sympathoactivation to the kidney, Second, in diet-induced obese mice, lumbar SNA responses to leptin are attenuated, as compared to lean mice, whereas leptin-induced increases in renal SNA occur with the same time course and magnitude in both diet-induced obese and lean mice. Several hypothalamic neuropeptides, monoamines, and other transmitter substances have been identified as candidate mediators of leptin action in the hypothalamus. [11] Leptin-induced activation of SNA to organs such as the kidney was the first indication of the potential role of this hormone in regulation of blood pressure. The sympathetic nervous system is an important component in the control of renal function. Long-term renal sympathetic stimulation by leptin would be expected to raise arterial pressure by causing vasoconstriction and by increasing renal tubular sodium reabsorption. Sanjeev B *et al*. 2010 [12] have shown that the sympathoactivation to leptin is followed by a slow but progressive increase in mean arterial pressure. Finally, blockade of the adrenergic system inhibits the pressor response to leptin. Further evidence for the pressor effects of leptin derives from studies of transgenic mice over expressing leptin in the liver. These mice had 10-fold increases in plasma leptin and decreased body weight. Despite the decreased body weight, the transgenic mice over expressing leptin had significantly higher arterial pressure than non transgenic littermates. The transgenic mice also had increased urinary excretion of norepinephrine, a marker of sympathetic nervous system activity. The increase in arterial pressure was normalized after alpha-adrenergic or ganglionic blockade, again demonstrating the importance of the sympathetic nervous system in the presser effects of leptin.[11], this mechanism explain the effect of leptin on increasing the hypertension, in this study we found positive correlation between leptin and hypertension as in table (2)

Leptin with lipid:

In this study table (2) we found negative correlation between leptin with HDL while there were a positive correlation between leptin with (cholesterol, LDL-C, triglycerides and VLDL). leptin directly stimulates phosphorylation and activation of AMP-activated protein kinase (AMPK) in skeletal muscle, increasing phosphorylation of acetyl-CoA carboxylase (ACC) and fatty acid oxidation (at least in the early phases of AMPK activation, with later-phase activation depending on leptin functioning through the hypothalamic–sympathetic nervous

system axis). Although the autocrine or paracrine role of leptin in fatty acid metabolism has not yet been fully elucidated at the molecular level, it is known that leptin in adipocytes inhibits the synthesis of ACC, an enzyme essential (and rate-limiting) in the conversion of carbohydrates to longchain fatty acids and hence in the storage of energy as triacylglycerol. Differentiating wild-type adipocytes starved by culture in the absence of serum have lower ACC levels and lower rates of fatty acid and triacylglycerol synthesis than ob/ob cells. In addition, long-term treatment of wild-type mice with large leptin doses increases mRNA levels of the key lipolytic enzyme hormone-sensitive lipase but reduces those of the lipogenic enzyme fatty acid synthase. [13]

Hormone-sensitive lipase levels are more immediately controlled by cellular levels of cAMP, so it seems that leptin, like glucagon and catecholamines, might stimulate lipolysis primarily by increasing cAMP concentrations. Leptin-driven control of lipid metabolism has been observed not only in adipocytes, but also in other tissues that store triacylglycerol found in lean rats. Furthermore, rats lacking functional leptin receptors have high levels of acyl-CoA synthetase and glycerol-3-PO4 acyltransferase (two enzymes required for lipogenesis), but low levels of acyl-CoA oxidase (ACO) and carnitine palmitoyl transferase I (two enzymes involved in fatty acid oxidation). In view of these enzyme levels and of the high lipid contents of fa/fa non-adipocytes, it has been hypothesized that one of the functions of leptin is to keep the triacylglycerol content of non-adipocytes low, thereby protecting them from steatosis and lipotoxicity. [13], this effect of leptin on lipid metabolism agree with our results as in table (4). This table showed that there was positive correlation between leptin with cholesterol, LDL-C, triglyceride, VLDL-C, and negative correlation between leptin with HDL-C

Besides to the indirect effect of leptin on lipid metabolism and its reduction of the lipogenic effects of insulin ,addition of insulin to cultured leptin-deficient adipocytes increases the synthesis of ACC, fatty acids and triacylglycerol to a greater extent than in adipocytes that do produce leptin, possibly due in part to leptin inhibition of insulin–adipocyte binding. Like the direct effects of leptin, this action also extends to non-adipocytes: the insulin-induced increase in triacylglycerol synthesis and decrease in fatty acid oxidation in isolated mouse skeletal muscle are reduced by simultaneous administration of leptin. Leptin has been found to up regulate PPAR-a and PPAR-g, which both up regulate fatty acid binding proteins (FABPs); PPAR-a also seems to regulate fatty acid oxidation enzymes and uncoupling protein-3 (UCP3). However, findings have differed as to whether PPARs can regulate leptin gene expression or not. [13]

Leptin with Troponin:

We are the first in our country who studird a relationship between leptin and troponin, as troponin is referred to the size of infarction and table (2) showed that there was positive correlation between leptin with troponin and this mean that leptin have bad effect on prognosis of AMI patients as we showed leptin effect on vascular wall and it's relation with lipid profile. Recently study done by Ivar Rønnestad et al., in 2010 (14) found that there was a receptor for leptin in heart, also mentioned that heart itself may cause stimulate leptin because it can produce interleukins and this product is exclusively occur in heart injury, [14].

CONCLUSION :

-Leptin negatively correlated with HDL and positively correlated with triglyceride and LDL this relation make this hormone act as atherosclerotic factor.

REFERENCES:

1. Hong R, Choi DY, Do NY, Lim SC. "Fine-needle aspiration cytology of a lipoblastoma: a case report". Diagn. Cytopathol. July 2008;36: 508–11.
2. Cannon Band Nedergaard J.:Developmental biology: Neither fat nor flesh. Nature. Aug 2008: 21;454:947-48.
3. Tracy RP, Lemaitre RN, Psaty BM, et al.: Relationship of C-reactive protein to risk of cardiovascular disease in the elderly." Circulation:2002:5:1142-45.
4. Brennan AM, Mantzoros CS "Drug Insight: the role of leptin in human physiology and pathophysiology--emerging clinical applications". Nat Clin Pract Endocrinol Metab:2006:2:318–27.
5. Kosuge, M; Kimura K, Ishikawa T et al. "Differences between men and women in terms of clinical features of ST-segment elevation acute myocardial infarction". Circulation Journal. March 2006; 70:222–26.

6. Ammann P, Pfisterer M, Fehr T, Rickli H. "Raised cardiac troponins." BMJ. 2004;328:1028-29.
7. Herminia González-Navarro, Marian Vila-Caballer, María Francisca Pastor, Angela Vinué, Morris F. White, Deborah Burks, and Vicente Andrés."Plasma insulin levels predict the development of atherosclerosis when IRS2 deficiency is combined with severe hypercholesterolemia in apolipoprotein E-null mice." Cell Metab;2009:3:247-56
8. Göran Hallmans, Åsa Ågren, Gerd Johansson, Anders Johansson, Birgitta Stegmayr, Jan-Hakan Jansson, Bernt Lindahl, *Scand*."Cardiovascular disease and diabetes in the Northern Sweden Health and Disease Study Cohort evaluation of risk factors and their interactions."J Public Health :2008;31: 18-28.
9. Theodore Mazzone." Adipose Tissue and Atherosclerosis."Arterioscler Thromb Vasc Biol. 2009;27:996-1003.
10. Rahmani Nia, Hojjati, Rahnama and . Soltani." Leptin, Heart Disease and Exercise." World Journal of Sport Sciences ; 2009;2: 13-20.
11. Kamal Rahmuni and William G. Hayns."Leptin and the Cardiovascular System." Specialized Center of Research in Hypertension Genetics, Department of Internal Medicine, and General Clinical Research Center, University of Iowa, Iowa City, Iowa ;2009:5:22-42
12. Sanjeev B. Patel, Garry P. Reams, Robert M. Spear, Ronald H. Freeman and Daniel Villarreal."Leptin: Linking obesity, the metabolic syndrome, and cardiovascular disease." Current Hypertension Reports;2010;10:13.
13. Francisca Lago, Rodolfo Go´mez, Juan J. Go´ mez-Reino, Carlos Dieguez, and Oreste Gualillo." Adipokines as novel modulators of lipid metabolism. Trends in Biochemical Sciences 2009;34: 501- 16.
14. Ivar Rønnestad, Tom Ole Nilsen, Koji Murashita, Anna Rita Angotzi, Anne-Grethe Gamst Moen, Sigurd O. Stefansson, Peter Kling, Björn Thrandur Björnsson, Tadahide Kurokawa."Leptin and leptin receptor genes in Atlantic salmon: Cloning, phylogeny, tissue distribution and expression correlated to long-term feeding status ".General and Comparative Endocrinology :2010:168:55-70.