The impact of inflammation on Resistin, IL-6 and CRP in Acute Myocardial Infarction Patients.

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Summary:

Background: Inflammation contributes across the spectrum of cardiovascular disease, including the earliest steps in atherogenesis. Myocardial Infarction (MI) is most commonly due to occlusion (blockage) of a coronary artery following the rupture of a vulnerable atherosclerotic plaque. It has been suggested that the adipose tissue may play an important role in mediating this chronic inflammatory process, human resistin, is a 12.5-kDa protein, it found in the inflammatory zone. Interleukin-6 (IL-6) is a pro-inflammatory cytokine, it secreted by T cells and macrophages to stimulate immune response. C-reactive protein (CRP) is a protein found in the blood, the levels of CRP rise in response to inflammation.

Objective: to determine the role of Resistin in Acute myocardial infarction patients and its effects on IL-6 and CRP.

Patients and Methods: The study included 50 patients with Acute Myocardial Infarction and 40 healthy subject as control, levels of resistin, Interleukin-6 (IL-6) and CRP were measured.

Results: The levels of resistin, IL-6 and CRP were significantly elevated with ($p<0.001$). There was positive correlation between resistin with IL-6 and CRP in acute myocardial infarction patients.

Conclusions: There was significantly increasing in levels of resistin, in acute myocardial infarction patients and this increasing may be related to inflammation. Resistin positively correlated with pro-inflammatory factor (IL-6 and CRP) so it have inflammation properties may consider a cardiovascular risk factor.

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Introduction:

Inflammation contributes across the spectrum of cardiovascular disease, including the earliest steps in atherogenesis. This recognition has had a profound impact on understanding the atherothrombosis as more than a disease of lipid accumulation, but rather as a disorder characterized by low-grade vascular inflammation. This concept can be used to predict future cardiovascular risk.[1] Myocardial Infarction (MI), commonly known as a heart attack, 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. [2] Adipose tissue is increasingly recognized as a key regulator of energy balance, playing an active role in lipid storage and buffering, and synthesizing and secreting a wide range of endocrine products that may be directly involved in the pathogenesis of the complications associated with obesity. Yet the mechanisms that relate fat mass to vascular disease are poorly understood. Obese individuals demonstrate vascular endothelial dysfunction , which is central to the pathogenesis of atherosclerosis and predicts cardiovascular risk.[3] Interleukin-6 (IL-6) is secreted by T cells and macrophages to stimulate immune response to trauma, especially burns or other tissue damage Leading to inflammation. is also a "myokine," a cytokine produced from muscle, and is elevated in response to muscle contraction. [4] IL-6 is pro-inflammatory cytokine, IL-6's role as an anti-inflammatory cytokine is mediated through its inhibitory effects on TNF-alpha, and activation of IL-1 and IL-10. [4] C-reactive protein (CRP) is a protein found in the blood, the levels of which rise in response to inflammation (an acute-phase protein). Its physiological role is to bind to phosphocholine expressed on the surface of dead or dying cells (and some types of bacteria) in order to activate the complement system. CRP is synthesized by the liver.[5] Resistin is an adipocytokine that may link insulin resistance, inflammation and atherosclerosis. Resistin up-regulates the expression of adhesion molecules in endothelial cells and promotes smooth muscle cell proliferation. Serum resistin levels were reported to be high in patients with coronary artery disease, especially unstable angina. [6]

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Subjects:

This study was performed during the period from December 2009 to April 2010. This study includes fifty patients with Acut Myocardial Infarction (AMI) were admitted to Cardiac Care Unit (CCU) at Medical City Teaching Hospital and Ibn –ALbetar Hospital. Patients with age rang (20-78) years. Blood samples were taken from the patients after thorough examination. Subjects with a history a AMI or diabetes mellitus or any chronic diseases were excluded from the study. Forty healthy

individuals were taken as a control group. They were comparable with patients group by their age, sex and BMI.

Blood collection and laboratory analysis: From each patient and control, five ml venous blood was collected from patients and control. Samples were collected between (8-9 A.M.) after 10 hours fast. Blood was left to clot and then centrifuged at 4000 rpm for 5 minutes to separate the serum and dispensed into tightly closed Eppendorf tubes and stored at -20 C° until assayed. Each serum sample was analyzed for Resistin, Interleukin-6 (IL-6) and CRP by using ELISA kit from United States Biological Company.

Statistical analysis:

Statistical analysis was performed by statisticians with the SPSS 15.01 Statistical Package for Social Sciences and also Excel 2003. Data analysis was done using chi- square test for tables with frequencies, while 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 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:

Subjects with incident AMI were older and were more likely to be male with a history of hypertension or smoking. The results showed that patients with AMI have significantly higher levels of (resistin, IL-6 and CRP) with ($p<0.001$) as shown in table (1)**.** The results showed that there was strong positive correlation between resistin with IL-6 in female patients group (r=0.723), in female control group (r=0.511), in male patients group (r= 0.781), in total patient group (r=0.715) and in total control group (r=0.508). The results showed that there was strong positive correlation between resistin with CRP in female patients group(r=0.543), in male patients group (r=0.509) and in total patients (r=0.610) as shown in table-2.

Table 1: the comparison between groups for (resistin, IL-6 and CRP)

parameters	Female patients Mean±SR NO.=16	Female Control Mean±SR NO.=16	P-value	Male Patient Mean±SR NO.=34	Male Control Mean±SR NO=24	P-value	Total Patients Mean±SR N0.=50	Total Control Mean±SR N0.40	P-value
IL-6 Pg/ml	87.64 ±8.70	33.946 ±7.158	<0.001	84.97 ±2.34	32.567 ±4.229	<0.001	85.89 ±3.35	33.13 ±8.06	<0.001
CRP mg/L	23.72 ±5.08	15.19 ±2.79	<0.001	20.60 ±6.76	10.825 ±5.588	<0.001	22.34 ±6.26	12.75 ±1.29	<0.001
Resistin ng/ml	8.18 ±2.19	4.82 ±1.240	<0.001	9.53 ±2.30	4.515 ±1.109	<0.001	9.05 2.10±	4.64 ±1.93	<0.001

Table (2) the correlation between resistin with (IL-6 and CRP) 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
CRP	0.543	0.498	0.509	0.419	0. 610	0.420
IL-6	0.723	0.511	0.781	0.660	0.715	0. 508

Discussion:

The study results revealed a significantly higher level of resistin among AMI patient in male and female. There was no significant difference between male and female patients group as shown in table (1) This results found agree with a study done by Islamabad and Gujranwala in 2010[7] they found strong association between the elevated resistin levels and CAD. High levels of resistin may negatively influence the atherosclerotic process through several mechanisms. Resistin directly activates the endothelium through up regulation of adhesion molecules, an effect that is antagonized by adiponectin. Resistin also induces production of the proinflammatory cytokines endothelin-1, monocyte chemoattractant protein (MCP)-1, and pentraxin by endothelial cells. It also promotes migratory activity of vascular smooth muscle cells. In macrophages resistin facilitates lipid accumulation, thereby promoting formation of foam cells. Furthermore, resistin induces production of the proinflammatory cytokines tumor necrosis factor-α and interleukin (IL)-12 by macrophages through activation of nuclear factor κB. [8] As in table (1) our results revealed that there was significant increasing in resistin in AMI patients, and so it agree with this theory. Sabir Hussain, et al., in 2010[10] found anther effect of resistin on cardiomyocytes, they found that resistin could increases the cell surface area of cardiomyocytes and could also involved in the activation of hypertrophic signal transduction processes (eg, MAPKs). Also they reported that hyper-resistinemia may contribute to the development of pathologic cardiac hypertrophy; resistin- transduced ventricular cardiomyocytes lead to the impairment of myocytes relaxation. A negative effect of resistin on the cardiac function is also supported by the association of the cytokine with high risk in patients with congestive heart failure. [10] In this study it has been noticed that the

patients with higher level of resistin have the longest time to stay in CCU. So according to the relation of resistin with NO that mentioned by Noboru Toda et al., 2010,[9] The results in table (2) showed that there was significantly positive correlation between resistin with IL-6 and that agree with study done by (HU Wen-lan, et al., 2009)[11], who found positive relationships of resistin levels with plasma inflammatory markers, such as IL-6 in cardiovascular disease patients. Inflammatory responses stimulated resistin secretion, and resistin could also promote production of pro-inflammatory mediators such as interleukin-6 (IL-6) partially by activation of nuclear factor-κB signaling pathway, hence aggravate the pro- inflammatory response by a positive feedback. [11] Increased pro-inflammatory cytokines can stimulate leptin and resistin production, stimulated CRP production, potentially induce chronic low-grade inflammation and contribute to the insulin resistance that develops in obese patients. [12] Inflamed vessel walls may be the common denominator for both AMI and stroke. Atherosclerosis may be considered as an inflammatory disease a longstanding inflammatory process results in an accumulation of macrophages and lymphocytes in the atherosclerotic lesions. A decrease in strength of the vessel wall by various factors involved in the inflammatory process, such as activation of inflammatory cells, cytokines and proteolytic enzymes, might be of crucial importance in the pathogenesis of both stroke and AMI. [13] As shown in table (1) it was found that IL-6 significantly higher in patients group than control groups. IL-6 impaired fibrinolysis due to high levels of plasminogen activator inhibitor-1, microalbuminuria, and small dense low density lipoprotein particles. And more recent additions to the list of metabolic syndrome members have been markers both of endothelial cell activation, such as von Willebrand factor, and of acute phase activation, namely fibrinogen, C-reactive protein and IL-6. The influence of proinflammatory cytokines on vascular endothelium is well recognised, and include stimulating expression of adhesion molecules and inhibiting endothelial-dependent nitric oxidemediated vasodilatation. IL-6 has been shown to enhance fatty lesion development in mice. [14] A study by (M. Mohsen Ibrahim et al 2010) [14] suggested that IL-6 is a pro-atherogenic cytokine. As he observed that increasing in IL-6 are associated with enhanced plaque formation. Increased in IL-6 was also associated with reduced collagen content in the plaques, blunted synthesis, and release of IL-10 and diminished recruitment of inflammatory cells into the atherosclerotic plaque. [14] There suggestion was agreed with results of table (2), from which a positive correlation was found between IL-6 and resistin. Elevated levels of IL-6 are also a primary stimulant of soluble intercellular adhesion molecule-1 (sICAM-1), which mediates the attachment and migration of leukocytes across the endothelial surface.[15] Juan F. Navarro and Carmen Mora., in 2005 [15] mentioned that IL-6 may contribute to the development of atherosclerosis through various metabolic, endothelial, and coagulant mechanisms. In this respect, it is of interest that both TNF-α and IL-6 inhibit lipoprotein lipase production in adipocyte cell lines, thus mediating lipolysis and dyslipidemia. [15] Patients with persistently elevated IL-6 levels demonstrate a worse in-hospital outcome. Raised levels of IL-6 are often found correlated to CRP levels, consistent with IL-6 being the main stimulant for the hepatic production of CRP. [16] In normal conditions, the heart does not express cytokines. However, during an ischemic event , they may be up to 50 times in the culprit ischemic region and up to 15 times in the adjacent (non ischemic) zones. In the early post (Myocardial Infarction) MI phase, a certain degree of cytokines production is physiological, because in this phase cytokines play an important cyto-protective role by reducing cell apoptosis. [17] That agrees with results in table (1) and contributed to explanation the significantly higher level of IL-6 in AMI patients. As shown in table (1) it was found that CRP significantly higher in patients group than control groups.CRP binds to a large number of autologous and extrinsic ligands, including native and modified plasma lipoproteins, phospholipids, and apoptotic cells, which are present in the atherosclerotic lesions. When bound to ligands, CRP activates the classic pathway of complement, a major player in the immune and inflammatory response, and reacts with Fcγ receptors on phagocytic cells. Both CRP and complement are known to colocalize in human atherosclerotic lesions, which suggests that CRP, by activating the complement, may be an active participant in atherosclerosis development. [18] In vitro experiments showed that CRP modulates the activity and expression of multiple factors implicated in atherogenesis. It stimulates the production of endothelin-1 (ET-1) in endothelial cells in vitro. CRP may also facilitate leukocyte adhesion and internalization into the arterial wall by stimulating the expression of vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1, E-selectin, and monocyte chemoattractant protein-1. [18] **CRP** increases smooth muscle cell (SMC) migration and neointimal formation after carotid angioplasty in rats. It may also activate nuclear factor-κB and activator protein-1 (AP-1) and up regulate angiotensin type 1 receptors (AT1-R) in human vascular SMCs, which could mediate many of the proinflammatory effects of angiotensin (Ang) II. (Ang II has been reported previously to be associated with increased expression of both VCAM-1 and collagen in animal models. [19], this is the mechanism of CRP in developing atherosclerosis that explain the relation of elevated level of CRP as in table (2) in patients with AMI.

CRP can also effect on plasminogen activator inhibitor-1 (PAI-1 is a key regulator of fibrinolysis by inhibiting tissue plasminogen activator (tPA). [19] The fact that foam cells in early lesions stain positively for the CRP-R as well as CRP is

consistent with the hypothesis that CRP participates in foam cell formation by opsonizing lipid particles. Co localization of CRP with so-called enzymatically degraded LDL (E-LDL) was demonstrated in early atherosclerotic lesions.[20] Although there is evidence that foam cell formation by E-LDL is in part due to lipoprotein uptake via a scavenger receptor–mediated pathway, cellular uptake of E-LDL may be accompanied by the uptake of bound CRP and mediated by the CRP-R. CRP is internalized by macrophages via the endosomal route and is partially degraded, followed by recycling of the CRP-R. CRP may be an important component of the plasma proteins insulating the arterial wall preceding the so-called initial atherosclerotic lesion, which is characterized by the first appearance of monocyte-derived macrophage foam cells. Monocyte infiltration into the arterial wall is a 2-step process that involves adherence to the activated endothelium first and directed migration to a chemotactic gradient second. Diffusely deposited CRP may generate a chemotactic gradient within the arterial wall, attracting monocytes that have transmigrated the endothelium. This finding can agree with our finding because our patients with elevated level of CRP have bad prognosis. Nonetheless, early accumulation of native CRP in insudated areas may partly explain some of the phenomena in atherosclerotic lesion formation that are hitherto not understood. First, in addition to other chemoattractants, eg, monocyte chemotactic protein-1, CRP may act as a chemoattractant for blood monocytes in vivo. Second, CRP is known to inhibit neutrophil chemotaxis and the binding of neutrophils to endothelial cells. [21] Further, C-reactive protein has been shown to exert direct adverse effects on the vascular endothelium by reducing nitric-oxide release and increasing endothelin-1 production, as well as by inducing expression of endothelial adhesion molecules. (22)

Conclusions:

There was significantly increasing in levels of resistin in acute myocardial infarction patients and this elevation may related to inflammation. Resistin is positively correlated with pro-inflammatory factor (IL-6 and CRP) so it has inflammation properties and so it may consider it as a cardiovascular risk factor.

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