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Evaluation of serum concentration Interleukins in Patients with Myocardial Infarction by ELISA Technique.

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ABSTRACT

To evaluate the serum concentrations of proinflammatory cytokines interleukin (IL-1, IL-6, IL-8, IL-18), tumour necrosis factor α (TNF-α), C-reactive protein (CRP), and anti-inflammatory cytokine interleukin 10 (IL-10) regulatory in patients with AMI, and to assess the correlation between anti-inflammatory and pro-inflammatory cytokines in AMI.

Thirty patients (13 women and 17 men) with (AMI) enrolled in this study and 30 as control group (10 women and 20 men). There aged varied between 40-60 years old. In this study were measured serum levels of proinflammatory cytokines interleukin (IL-6, IL-1, IL-18, and IL-8), TNF-α, and anti-inflammatory cytokine interleukin 10 (IL-10) in both patient and in control groups by using ELISA technique.

Serum levels of interleukine-6, IL-1α, IL-8, and IL-18, CRP, and TNF-α were significantly higher whereas serum IL-10 L level in AMI patients in compares with control group.

This study shows a significantly increase in IL-1, IL-18,IL-6,andIL-18 and decrease in IL-10 in the circulation of AMI.

Keywords: Acute Myocardial Infarction, Interleukins, and cytokines.

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تقييم مستوى التركيز المصلي للانترلوكينات باستخدام تقنية ELISA في المرضى الذين يعانون من احتشاء عضلة القلب

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الملخص

في هذه الدراسة قمنا بتقييم التركيز لسيتوكينات الموالية للالتهابات انترلوكين (IL-1، IL-6، IL-8، IL-18)، عامل نخر الورم (TNF-α) α)، بروتين سي التفاعلي (CRP)، و المضادة للالتهابات انترلوكين 10 في الاشخاص المصابين باحتشاء عضلة القلب الحاد وباستخدام تقنية الاليزا، وتقييم العلاقة بين السيتوكينات المضادة للالتهابات والموالية للالتهابات في احتشاء عضلة القلب الحاد في ثلاثون مريضا (13 امرأة و 17 رجلا) باحتشاء عضلة القلب الحاد (المجموعة الأولى) المسجلين في هذه الدراسة و 30 (10 امرأة و 20 رجلا) كمجموعة تحكم (المجموعة الثانية) الذين تتراوح أعمارهم بين 60-40 سنة.

اظهرت النتائج بان مستويات انترلوكين 6، كان IL-1α، IL-8، و IL-18، CRP، وTNF-α أعلى بكثير في احتشاء عضلة القلب الحاد مقارنة بمجموعة التحكم. بينما كان هناك انخفاض ملحوظ في المستو المصل IL-10 L مستوى في المجموعة الأولى مقارنة بمجموعة التحكم.

هذه الدراسة تظهر زيادة كبيرة في IL-1، IL-18، IL-6، IL-18 وانخفاض IL -10 مصل المرضى الذين يعانون من احتشاء عضلة القلب الحاد .

الكلمات الدالة: احتشاء عضلة القلب, الانترلوكينات, السيتوكينات.

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1. Introduction

Acute myocardial infarction is myocardial necrosis occurs when blood flow stops to a fragment of the heart leading to the damage of the cardiac muscle. The last decade its part in the focus of attention immunological from cardiologists [1]. AMI is associated with an inflammatory process. The signs of inflammation are inspected as well possible tools for predicting cardiovascular risk. Reperfusion of infarcted myocardium and ischaemia due to inflammatory response to injury is linked with the stimulation of IL-6 and TNF-α, which is complicated in recruiting and motivation of inflammatory cell, encourages the liver to yield CRP and which have adverse inotropic impact interceded by nitric oxide synthase in myocardium[2].

The aim of research was to assess the levels of anti-inflammatory and pro-inflammatory cytokines in AMI.

2. Subjects and Methods

Thirty patients (13 women and 17 men) with AMI who were admitted to Tikrit Teaching Hospital/ Tikrit/ Iraq were enrolled to this study, and 30 (10 women and 20 men) healthy individuals were considered as control group, their ages were varied between 40-60 years. Five milliter of the venous blood was withdrawn from both patient and control groups, and transferred in to non-heparinized sterile tubes. The levels of IL-6, IL-1α, IL-18, IL-10 and IL-18 were measured by enzyme-linked immunosorbent assay (ELISA) kits from United States Biological-Company.

Statistical analysis: Statistical analysis was performed using SPSS-21 Unpaired t test was done to evaluate significant variance between means. $P < 0.05$ was considered statistically significant.

Results: The study group included 30 (13 women and 17 men) patients of acute myocardial infarction with a mean age of 57.46 ± 10.95 year, and control group comprised of 30 (10 women and 20 men) healthy individuals with a mean age of 51± 10.47 year.

The results of Table (1) indicate the mean of serum interleukine-6, IL-1α , IL-8, and IL-18 ,CRP and TNF-α levels was significantly higher in acute myocardial infarction (32.3±1.42 pg/ml), (35.5±5.65 pg/ml), (101.25±14.16 ng/ml), (314.36±23.4 pg/ml), (1.102±.22 mg/dl) and (33.19±4.95 pg/ml) respectively than in control group (5.89±0.20 pg/ml), (12.09±2.26pg/ml), (11.1740±1.99ng/ml), (102.22±6.83 pg/ml), (0.75±0.15 mg/dl) and

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(9.1±1.03 pg/ml) respectively. Also the results showed a significant decrease in serum IL-10 level in AMI patients (26.± 6.01 pg/ml) in comparison with control group (34.23±5.99 pg/ml).

Table (1): Immunological parameters of study groups.

Parameters	Patients (Group1) Mean ± SD	Control(Group 2) Mean ± SD
Interleukine-6 (pg/ml)	32.3±1.42*	5.89±0.20
Interleukine-1α (pg/ml)	35.5±5.65**	12.09 ±2.26
Interleukine-8 (ng/ml)	101.25±14.16**	11.17±1.99
Interleukine-10 (pg/ml)	26± 6.01**	34.23±5.9
Interleukine-18 (pg/ml)	314.36±23.4**	102.22±6.83
CRP (mg/dL)	1.102±.2215**	0.75±0.15
TNF-α (pg/ml)	33.19±4.95**	9.1±1.03

*P< 0.01, **P< 0.0001

Correlation within Parameters

The results revealed that there was positive correlation between IL-1 with IL-8(0.316), IL-6(0.497), IL-10(0.757), and CRP (.085) in AMI group. Also there was positive correlation between IL-8 with IL-6 (.488), IL10 (.848) and CRP (885). IL-6 with IL-10, and CRP (0.834), (0.228) respectively, also IL-10 with CRP (0.775). TNF with IL-1 (0.449), IL-8 (0.647), IL-6 (0.656), IL-10 (0.045), and CRP (0.666).

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Table (2): Correlation within Parameters

parameters	Patients (r)	Control (r)
IL-1 with IL-8	0.316	0.198
IL-1 with IL-6	0.497	-0.129
IL-1 with IL-10	0.757	0.059
IL-1 with CRP	0.085	-0.319
IL-8 with IL-6	0.488	0.132
IL-8 with IL-10	0.848	0.037
IL-8 with CRP	0.885	-0.028
IL-6 with IL-10	0.834	-0.040
IL-6 with CRP	0.228	0.227
IL-10 with CRP	0.775	-0.054
TNF with IL-1	0.449	-0.144
TNF with IL-8	0.647	0.087
TNF with IL-6	0.656	0.085
TNF with IL-10	0.045	-0.368
TNF with CRP	0.666	0.077

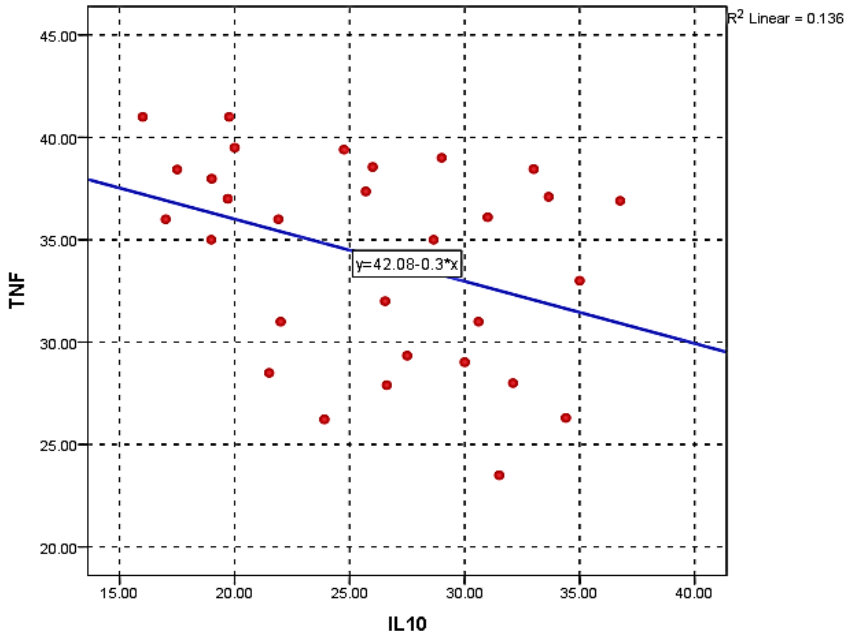

Fig. (1): Correlation between TNF and IL-10 in patients with AMI

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3. Discussion

Acute myocardial infarction (AMI) is defined as a part of acute coronary syndrome characterized by a typical clinical syndrome consisting of chest pain, dyspnea with rise and fall in troponin or creatine kinase–MB [3]. In current data, IL-6 level of AMI patient groups showed a significant increase while IL-10 decrease as compared with the control group. Few other authors verified the same in their studies [4-5]. There are few studies which found a low level of IL-10 in AMI [4, 6-7].

IL-10 is persuaded in the reperfused myocardium and may moderate the reaction to injury by down-regulating levels of TNF-α and IL-6. Stimulation of IL-10 impedes mRNA for IL-6 in reperfused myocardium, however the expression of IL-6 is prolonged in myocardial ischemia [8]. Malarstig *et al*. [9] who established that raised IL-10 was an indicator of poor outcome and promote systemic inflammation in patients with acute coronary syndrome. In recent years, a new notion of imbalance between pro-inflammatory and anti-inflammatory factors has emerged, favoring pro-inflammatory factors, leading to atherosclerotic plaque rupture [6-7].

In the current work, serum level of IL-8 was found to be significantly elevated in patients with AMI. The findings come in a deal with study conducted by the Kanda *et al.* [10] Who elucidated that the serum levels of assessment of IL-8 has a clinical relationship of CAD and the sign was useful pointer for the recognition of unstable angina.

Serum level of Interleukin-18 was significantly elevated in patients grouping comparison with control group. Interleukin-18, primarily Locate as interferon gamma encouraging factor in macrophages [11]. It plays crucial role in the inflammatory sequence and in the immunities processes due to its capability to stimulate the production of IFN-gamma in T lymphocytes, which are thought to plays a critical role in the disruption atherosclerotic plaque [12], so the positive impact of the discourage IL-18 on plaque development and composition, and continued benefit to the heart in terms of cardiac remodeling and preserved function was described by Whitman *et al.* and Hartford *et al* [13-14].

Interleukin-1 (IL-1) produced within tissues and has multifunctional cytokine contributes to local inflammatory reactions, primarily complicated in the regulation of inflammatory processes, it mediates stimulation of fibroblast growth, and differentiation of B and T cells [15-16]. Serum level of IL-1 was found to be significantly elevated in patients with

AMI.These results were agreement with a study done by Miyao *et al*. [17], Guillen *et al*. [18] Interleukin-1 might be donated to the wound healing in cardiac repair mechanism after the stroke [19].

Results of this study showed significantly increase in TNF-α level in myocardial infarction patients. These results harmony with results obtained by Mizia-Stec et *al*. [20]. Tumor necrosis factor-α has a role in the pathogenesis of atherosclerotic plaques. It increases the chemokine and scavenger receptor expression. We observed significant increase in CRP level in AMI patients, AMI motivate a systemic inflammatory response with enhanced synthesis of CRP, which prompts release of growth factor and cytokine, those mediators affect necrosis expansion and stimulate CRP expression [21-22].

4. Conclusion:

This study demonstrations a pointedly increase in IL-1, IL-18, IL-6, and IL-18 and decrease in IL-10 in the circulation of AMI patients these biomarkers may be valuable in diagnosis AMI.

References

[1] Anjuman G, Muhammad A, Abdullah A," ***Changes in Inflammatory Markers Concentration in Diabetic and Non-diabetic Patients with Myocardial Infarction***", Adv. Biores, 3, 73, (2013) .

[2] Ahmad MI, Sharma N., "***Biomarkers in Acute Myocardial Infarction***", J Clin Exp Cardiolog, 3, 222, (2012).

[3] Entedhar R. Sarhat, Thuraia R., Dina N. , "***Study of serum levels of Melatonin, Paraoxonase, Oxidative stress in Iraqi patients with Acute Myocardial Infarction***", European Academic Research, 4(1), 112, (2016).

[4] Biasucci L. M, Vitelli A. , Liuzzo G. Elevated levels of interleukin-6 in unstable angina. Circulation, 94, 874, (1999).

[5] Dizdarević-Hudić L, Kusljugić Z, Baraković F, Brkić S, Sabitović D, Jahić E, Isabegović M, Smajić E, Hudić I, Divković K, "***Correlation between interleukin 6 and interleukin 10 in acute myocardial infarction***", Bosn J Basic Med Sci., 9 (4), 301, (2009).

[6] Shibata M., Endo S., "***Inada K. Elevated plasma levels of interleukin-1 receptor antagonist and interleukin-10 in patients with acute myocardial infarction***". J. Interferon Cytokine Res, 17, 145, (1997).

[7] Alam S.E., Nasser S.S., Fernainy K.E. Cytokine imbalance in acute coronary syndrome. Curr. Opin. Pharmacol., 4, 166, (2004).

[8] Frangogiannis N. G., Mendoza L. H.," ***Lindsey M. L. IL-10 is induced in the reperfused myocardium and may modulate the reaction to injury***", J. Immunol, 165, 2798, (2000).

[9] Malarstig A., Eriksson P., Hamsten A., "***et al. Raised interleukin-10 is an indicator of poor outcome and enhanced systemic inflammation in patients with acute coronary syndrome***", Heart, 94, 724, (2008).

[10] Kanda T, Hirao Y, Oshima S, Yuasa K,Taniguchi K, Nagai R et al," ***Interleukin-8 as a sensitive marker of unstable coronary artery disease***", Am J Cardiol, 77, 304, (1996).

[11] Pasupathi Palanisamy, Y. Yagneswara Rao, Jawahar Farook, et al. "***Oxidative Stress and cardiac Biomarkers in patients with acute myocardial infarction***", European Journal of Scientific Research, 27, 275, (2009).

[12] Gupta S, "***Does aggressive statin therapy offer improved cholesterol-independent benefits compared to conventional statin treatment***", Int J Cardiol, 96(2), 131, (2004).

[13] Whitman S, Ravisankar P, "***Daugherty A. Interleukin-18 enhances atherosclerosis in apolipoprotein E mice through release of interferon-gamma. Circ Res***", 90(2), 34, (2002).

[14] Hartford M, Wiklund O, Hultén LM, Persson A, Karlsson T, Herlitz J, Hulthe J, "***Caidahl K. Interleukin-18 as a predictor of future events in patients with acute coronary syndromes***". Arterioscler Thromb Vasc Biol, 30 (10), 2039, (2010).

[15] Arai K, Lee F, Miyajima A, et al. "***Cytokines: Coordinators of immune and inflammatory responses***", Annu Rev Biochem, 59, 783, (1990).

[16] Lord PCW, Wilmoth LMG, Mizel SB, Mccall CE. "***Expression of interleukin-1α and β genes by human blood polymorphonuclear leukocytes***", J Clin invest, 87, 1312, (1991).

[17] Miyao Y, Yasue H, Ogawa H, Misumi I, Masuda T, Sakamoto T, "***Morita E: Elevated plasma interleukin-6 levels in patients with acute myocardial infarction***", Am Heart J. 126, 1299, (1993).

[18] Guillen I, Blanes M, Gomez-Lechon MJ, "***Castell JV: Cytokine signaling during myocardial infarction: sequential appearance of IL-1 beta and IL-6***", Am J Physiol, 6(2), 229, (1995).

[19] Heymanss, Luttuna A, Nuyens D, Theilmeier G, reemer E, Moons L, Dyspersin GD, Cleutjens JP, Shipley M, Angellilo A, Levim, Nube O, Baker A, Keshet E, Lup F, Herbert JM, Smits JF, Shapiro SD, Base M, Borgers M, Collen D, Daemen MJ, "***Carmeliet P:Inhibition of plasminogen activators or matrix metalloproteinases prevents cardiac rupture but impairs therapeutic angiogenesis and causes cardiac failure***", Nat Med, 5, 1135, (1999).

[20] Mizia-Stec K, Gasior Z, Zahorska-Markiewicz B, et al. "***Serum tumor necrosis factor-alpha, interleukin-2 and interleukin-10 activation in stable angina and acute coronary syndromes***", Coron Artery Dis, 14, 431, (2003).

[21] Frangogiannis NG, Smith CW, "***Entman ML.The inflammatory response in myocardial infarction"***, Cardiovasc Res, 53, 31, (2002).

[22] Nian M, Lee P, Khaper N, "***Liu P. Inflammatory cytokines and post myocardial infarction remodeling***", Circ Res, 94, 1543, (2004).

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