STUDY OF THE LEVELS OF CYTOKINES IN PATIENTS WITH HEART DISEASES IN TIKRIT CITY, IRAQ

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Abstract

This study was conducted on heart diseases patients in Cardiac Care Unit (C.C.U.) in Salah- Aldin General Hospital in Tikrit City-Salah-Aldin governorate. The study began in 31 December 2018 to 1st September 2019 and included 90 subjects divided in two groups, the first group was 80 patients with different heart diseases (20 Heart Failure (HF), 20 Myocardial Infarction (MI), 20 Unstable Angina(UA), 20 Stable Angina (SA) were attended from Salah- Aldin General Hospital, while the second group was (10) apparently healthy subjects. blood samples was collected from patients and healthy subjects and then serum separated for testing. The present study was designed to obtain more clarification of some Immunological changes in Iraqi patients with heart diseases, and find out the role of some Immunological parameters (pro-inflammatory cytokines like, Interleukin - 6(IL-6), Interleukin 1 Beta (IL-1β) and Tumor Necrosis Factor-alpha (TNF-α) in serum of Iraqi patients with heart, the result of Immunological parameters include: Interleukin-6, Interleukin 1β and Tumor necrosis factor- alpha There were a high significant increasing (P<0.01) in the concentration of these parameters in all Heart disease groups as compared with control healthy subjects. The highest concentration of all above Immunological parameters were found in heart failure group followed by myocardial infarction group and followed by unstable angina group while the lowest concentration was instable angina group when compare with control healthy subjects for all Immunological parameters. The study aimed to explore the physiological connection between Immunological parameters, interleukin-6 and interleukin 1β and tumor necrosis factor – alpha with Heart diseases patients in Tikrit city. It can be conclude that the serum interleukin-6 (IL-6), interleukin-1β (IL-1β) and Tumor necrosis factor – alpha (TNF-α) There were a high significant increasing (P<0.01) in the concentration of these parameters in all Heart disease groups as compared with control healthy subjects.

Key words: Heart diseases, inflammation, TNF-α, IL-6, IL-1β.

Introduction

Heart disease is a chronic inflammatory reaction process, and the patients with stable angina, unstable angina and even myocardial infarction are accompanied by varying degrees of systemic inflammatory response (Chen et al., 2017). Inflammation plays a central role in many heart and vascular diseases, including heart failure (Braunwald, 2008), myocardial infarction (Hansson, 2005), arrhythmias (Francis et al., 2016), pericarditis (Elamm et al., 2012), and myocarditis (Imazio and Gaita, 2015), inflammation in the vessel wall is associated with heart and vascular disease and activation of several cytokines like IL-6-signaling pathway has been shown to be important in the atherosclerotic process, plaque building and plaque destabilization (Libby, 2002). An inflammatory role following tissue injury stimulating the release of cytokines like tumor necrosis factor (TNF), interleukin (IL-1B), IL-6, (Yan and Gao, 2012). The pathogenesis of MI and UA completely that the role of the immune system in the pathogenesis of atherosclerosis and the primordial role of the immune system and inflammation in atherosclerosis gained its importance only for last 3 decades (Libby, 2012). Inflammatory markers ( interleukin-6 and tumor necrosis factor-α) were associated with increased HF risk (Gopal et al., 2012).
Material and Methods

This study was achieved by collecting 90 blood samples included (80) patients (40 male and 40 female) diagnosed with Heart disease and (10) subjects as Control (5 male and 5 female). patients study carried out in Cardiac Care Unit (C.C.U.) in Salah-Aldin General Hospital in Tikrit City from 31 December 2018 to 15 September 2019 on study population age ranged from (38-75) years old.

A total number of 90 subjects were participated in the present study, distributed as follow:-

**Group 1:** (80) Patients with heart disease distributed into 4 subgroups:

a. Heart Failure patients (20), (10 male and 10 female)

b. Myocardial Infarction patients, (20) (10 male and 10 female)

c. Unstable Angina patients (20) (10 male and 10 female)

d. Stable Angina patients (20) (10 male and 10 female)

**Group 2:** (10) Ten subjects, apparently healthy as controls (5 male and 5 female).

Serum samples treatment

Approximately 5 ml of fasting human blood was collected from each subject (patients and control) and transferred into sterilized test tubes and allowed for 30 minute to clot at room temperature, the sample was centrifuged for 15 minutes at 3000 rotations per minute and the serum was immediately separated and stored at (-20 °C) till used for interleukin 6 (IL-6), interleukin 1β (IL-1β) and Tumor necrosis factor-alpha (TNF-α).

Determination of interleukin - 6 (IL-6) in serum

Interleukin - 6 concentration in serum of heart diseases patients was estimated depends on kit procedure is an Enzyme-Linked Immunosorbent Assay (ELISA) from Koma BIOTICH, Company (Korea ) (Koma BIOTICH Korea. Human TNF-α ELISA Kit, K 033194: 8).

Determination of interleukin-1β (IL-1β) in serum

Interleukin-1 β (IL-1β) concentration in serum of heart diseases patients was estimated depends on kit procedure is an Enzyme-Linked Immunosorbent Assay (ELISA) from Koma BIOTICH, Company (Korea) (Koma BIOTICH Korea. Human IL-1B ELISA Kit, K 0331131:8).

Determination of Tumor necrosis factor – alpha (TNF-α) in serum

Tumor necrosis factor – alpha (TNF-α) concentration in serum of heart diseases patients was estimated depends on kit procedure is an Enzyme-Linked Immunosorbent Assay (ELISA) from Koma BIOTICH, Company (Korea) (Koma BIOTICH Korea. Human TNF-α ELISA Kit, K 033194: 8).

Statistical analysis

All data were presented as a mean and standard deviation, (SD). P value less than 0.05 was accepted as a significant value. Unpaired Student T test was used to compare between the mean of measured variables.

Results and Discussion

1. Interleukin - 6 (IL-6)

Table 1 shows the concentrations of (IL-6 pg/ml) in a Heart disease groups. There was a high significant increasing (P< 0.01) in the concentration of (IL-6 pg/ml) in all Heart disease groups as compared with control healthy subjects. The highest concentration of IL-6 pg/ml was found in heart Failure group (615.6±7.8 pg/ml), followed by Myocardial Infarction group (575.2±6.4 pg/ml), followed by Unstable Angina group (525.4±5.5 pg/ml), while the lowest group is Stable Angina group (490.1±6.6 pg/ml).

This results was agreed with study by (Groot et al., 2014) who indicated that higher levels of IL-6 at 24 h were associated with lower left ventricle ejection fraction (LVEF) of HF. also this study was agreed with (Plenz et al., 2001) that have shown plasma IL-6 levels to be strongly associated with adverse outcomes in HF patients, presumably via directly induced myocardial dysfunction.

This study was agreed with (Senguttuvan et al., 2019) who reported that serum levels of IL-6 were increased in Indian patients with Acute Coronary syndrome ACS including Acute Myocardial Infarction (AMI) and Unstable angina (UA) as compared to healthy controls. Also this study agreed with (Ritschel et al., 2016; Hirota et al., 2004; Orn et al., 2009) they shows high levels of IL-6 with heart failure and acute myocardial patients. IL-6 is produced by the macrophages and monocytes in reaction to other inflammatory cytokines which contain
inflammatory markers (interleukin-6 and tumor necrosis factor-α), were associated with increased HF risk (Gopal et al., 2002). Inflammation involving the IL-6 pathway has also been shown to play a major role in heart failure development and long-term mortality and myocardial remodeling process occurring after an acute myocardial infarction (Huang et al., 2015). Results of (Ritschelet al. 2016) suggest that components of the IL-6- trans-signaling pathway may play an important role in both the inflammatory response pattern accompanying an acute STEMI as well as in the risk of developing new cardiovascular events.

2. Interleukin-1β (IL-1β)

Table 2 shows the concentration of IL-1β pg/ml in a Heart disease groups. There was a high significant increasing (P<0.01) in the concentrations of IL-1β pg/ml in all Heart disease groups as compared with control healthy subjects. The highest concentration of IL-1β pg/ml was found in Heart Failure group (6530.8±61.8 pg/ml), followed by Myocardial Infarction group (6225.5±51.3 pg/ml), and followed by Unstable Angina group (6185.3±45.6 pg/ml), while the lowest group is Stable Angina group(6098.1±39.6 pg/ml) as compared with control healthy subjects (3225±31.5 pg/ml).

The results of IL-1β agreed with (Szekely and Arbel, 2018), who indicated that increased level of IL-1β in serum of patients with acute and chronic HF, The cytokine hypothesis of heart failure suggests that a precipitating event triggers activation of pro-inflammatory cytokines, which leads to detrimental effects on left ventricular function and accelerates the progression of heart failure. Some mechanisms relating IL-1 to impaired systolic function have been purposed. IL-1β was shown to decrease the beta-adrenergic responsiveness of all type calcium channels in a Cyclic Adenosine Mono Phosphate (cAMP) independent mechanism (Liu et al., 1999).

Patients with heart failure (HF) demonstrate a marked increase in a variety of pro-inflammatory cytokines, including IL-1, with increasing levels according to the degree of disease severity, independent of whether the etiology is ischemic, hypertensive, idiopathic dilated cardiomyopathy, or inflammatory, recombinant human interleukin-1 competitive receptor antagonist that blocks the biologic effects of interleukin-1, thereby reducing systemic inflammatory responses (Yndestad et al., 2006). Patients with Coronary Artery Disease CAD and Acute Coronary syndrome ACS have higher levels of IL-1β and Interleukin-1 Receptor I (IL-1RI) compared with normal people. Within the CAD patients, those with myocardial infarction have the highest levels, compared with unstable angina (UA) or stable angina pectoris (SA) patients (Liu et al., 2015).

3. Tumor Necrosis Factor-β (TNF-β)

Table 3 shows the concentration of TNF-α in Heart disease groups. There was a high significant increasing (P<0.01) in the concentrations of TNF-α in all Heart disease groups as compared with control healthy subjects. The highest concentration of TNF-α was found in heart failure group (1025.5±58.1 ng/ml), followed by myocardial infarction group (875.1±44.1 ng/ml), and followed by unstable angina group (825.2±33.4 ng/ml), while the lowest group is stable angina group (805.1±29.2 ng/ml) as compared with control healthy subjects (414.1±24.2 ng/ml).

The results of TNF-α in this study was agreed with (Torre-Amione et al., 1996) and (Chung et al., 2003) they indicate that Serum levels of tumor necrosis factor-α (TNF-α) are increased in patients with heart failure, and the increase is directly correlated with the severity of disease. TNF-α is produced by the failing heart (possibly due to an increase in ventricular wall stress (Palmieri et al., 2008) and may contribute directly to the evolution and progression of heart failure (Torre-Amione et al.,1996) and (Chung et al., 2003). In addition, TNF-α can cause pathological changes in the myocardium, including ventricular remodeling, interstitial fibrosis, and cardiomyocyte apoptosis (Bradham et al., 2002) and...
(Chung et al., 2003).

Also this study was agreed with (Tsutamoto et al., 2000) who indicated that plasma levels of TNF-α and IL-6 were increased in the 23 Congestive Heart Failure CHF patients compared with normal subjects and significantly decreased after 14 weeks of treatment with an angiotensin II (Ang II) type 1 receptor antagonist (candesartan cilexetil) decreased plasma levels of the immune markers such as TNF-α, IL-6, and that it improved the biological compensatory action of endogenous cardiac natriuretic peptides in patients with mild to moderate Congestive Heart Failure CHF.

Results of this study was agreed with (Senguttuvan et al., 2019) who showed serum levels of TNF-α were increased in Indian patients with ACS including AMI and UA as compared to healthy controls. also this study agreed with (Ridker et al., 2000) study who explained effects of increased TNF-α after many months post MI were studied. They found that patients who are clinically stable but with ele-vated increased TNF-α levels had recurrent coronary events indicating a role of the persistence of inflammatory in stability in such pa-tients. TNF-α levels were found to be higher in patients with MI (STEMI) as com-pared to UA (NSTEMI). This underlines the fact that patients with UA (NSTEMI) might have slow indolent on-going inflammation as compared to patients with MI (STEMI) where anti-inflammation tries to overtake inflammatory cytokines. Previously, study (Heinisch et al., 2005) showed persistently increased TNF-α after 15 days in patients with ACS as compared to stable angina and healthy controls.

References


