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Relation of IL-1β, IL-5, IL-6 and IL-8 with stable angina pectoris

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Abstract-Background: The inflammatory mediators released by injured endothelium enhance the interaction of circulating leukocytes with endothelial cells and contribute to development and progression of atherosclerosis. In this study we designed to determine some interleukins (IL-1β, IL-5, IL-6 and IL-8) as diagnostic markers instable angina pectoris.

Methods: The study was carried out on 30 patients who were diagnosed with stable angina pectoris (18 males and 12 females).They visited Nasiriya Heart Center throughout the period from the beginning of November 2021 to the end of April 2022, and 20 age matched healthy person (13 males and 7 females). IL-1β, IL-5, IL-6, IL-8 were assayed by an enzyme-linked immunosorbent assay (ELISA). Troponin I (cTnl) was determined by using monoclonal cTnI-specific antibody. D-dimer by electro-chemiluminescence method. CK-MB by monoclonal CK-MB-specific antibody. Myoglobin by monoclonal MYO-specific antibody.

Results: Male and female patients with stable angina showed significantly elevated serum levels of troponin (P<0.001), D-dimer (P<0.001) and (P<0.01) respectively, CK-MB (P<0.01), myoglobin (P<0.01), IL-1β (P<0.01 and <0.05 respectively) and IL-6 (P<0.05), with non-significant changes in the levels of IL-5 and IL-8 in comparison with healthy male and female individuals respectively.

Conclusion: In addition to troponin, D-dimer, CK-MB and myoglobin; the proinflammatory mediators such as IL-1β, and IL-6 are helpful diagnostic markers instable angina pectoris.

Keywords—Angina pectoris, cytokines, inflammatory

I. INTRODUCTION

Cardiovascular disease is responsible of the most mortality worldwide and accounted for about 31.9% of the total deaths in the United States in 2010. Atherosclerosis involves many pathophysiological steps including endothelial activation, monocyte differentiation and formation of foam cell, immune cell recruitment, fibrotic plaque development due to foam cell death, migration, and proliferation of smooth muscle cells, plaque rupture, and thrombosis (Abdul-Mounther et al., 2010). Foam cells play a central role in the pathogenesis of atherosclerosis. Specifically, the formation and accumulation of foam cells in the subendothelial space of a damaged artery is one of the early key steps responsible for the development of atherosclerosis (Birck et al., 2013). Cytokines are a diverse group of low-molecular weight proteins, which are categorized into several classes such as the interleukins, chemokines, interferons, tumor necrosis factors, transforming growth factors, and the colony-stimulating factors. Several cytokines play a role in the pathophysiology of atherosclerosis and coronary heart disease (Biasucci et al., 1998; Mizia-Stec et al., 1999; Yang et al., 2021). The inflammatory mediators releasing by injured endothelium enhance interaction of circulating leukocytes with endothelial cells and contribute to development and progression of atherosclerosis (Huh et al., 1996; Munn et al., 1995). Interleukin 1 (IL-1) families are involved in the accumulation of inflammatory cells, platelet aggregation, which accelerated the formation of plaques, myocardial cell apoptosis, and ventricular remodeling following myocardial infarction. Interleukin 6 (IL-6) showed a prognostic value in patients with the acute coronary syndrome. It gradually become a commonly used indicator in clinical practice and is strongly associated with the severity of acute coronary syndrome (McLaren et al., 2011; Mourouzis et al., 2020).

This study designed to determine some interleukins (IL-1β, IL-5, IL-6 and IL-8) as diagnostic markers of stable angina pectoris.

II. MATERIALS AND METHODS

The study was conducted on 30 patients who diagnosed with stable angina pectoris (18 males and 12 females).They visited Nasiriya Heart Center for the period from the beginning of December 2021 to the end of April 2022, and 20 age matched healthy person as a control group (13 males and 7 females). Patients who had a history of autoimmune or inflammatory disease, cancer, immunosuppression, or prior treatment with statins were excluded from the analysis since these disorders had the potential to independently alter

the parameters of our study. Blood samples were collected from patients who had arrived at the emergency department and then separated a serum, after that stored at -20 ◦C until used for the measurement of IL-1β, IL-5, IL-6, IL-8, troponin, D-dimer, CK-MB and myoglobin. IL-1β, IL-5, IL-6, IL-8 assayed by enzyme-linked immunosorbent assay (ELISA), according to the manufacture protocol of BTL, China, (https://www.bt-laboratory.com). Troponin I (cTnl) was determined by monoclonal cTnI-specific antibody. D-dimer measured by electro-chemiluminescence method. CK-MB by monoclonal CK-MB-specific antibody. Myoglobin by monoclonal MYO-specific antibody, according to the operational manual of Nipigon Health corp. Canada, (https://nipigonhealth.com). The study was approved by the ethical committee of the postgraduate studies of Southern Technical University- Basrah, Training and Human Development Centre/ Thi-Qar Health Office and Nasiriyah Heart Centre. Furthermore, it performed after taking informed, written consent of the participants.

Statistical analysis

The statistical significant differences were determined using SPSS (version 26) and independent t-test (two-tailed) for normally distribution variables, whereas the Mann-Whitney *U* used for those variables that were not normally distributed, and significant at p<0.05.

III. Results

Table 1 showed that male patients with stable angina in comparison with healthy male individuals of the same age group (57.93±6.98 VS 51.77± 7.01 years) showed high significantly elevated serum levels of troponin (0.59±0.13 VS 0.03±0.004 ng/ml, P<0.001), D-dimer (408.40±42.23 VS 353.15±84.94 ng/ml, P<0.001).

It is also showed a significant change in the levels of CK-MB (6.60±1.33 VS 3.72±1.47, P<0.01), myoglobin (62.33±10.38 VS 53-23±11.41 ng/ml, P<0.01), IL-1β (116.80±19.21 VS 88.79± 12.36 pg/ml, P<0.01) and IL-6 (5.57±0.73 VS 4.46±0.79 ng/L, P<0.05)

On other hand there is no significant changes in the serum levels of IL-5 (9.52±1.70 VS 10.28±1.69 ng/L) and IL-8 (8.42±0.85 VS 8.25±0.76 ng/L).

TABLE 1: THE SERUM LEVELS OF IL-1B, IL-5, IL-6, IL-8, TROPONIN, D-DIMER, CK-MB AND MYOGLOBIN IN MALE PATIENTS AND HEALTHY CONTROL

Parameters	Healthy control	Patients with stable angina	P. value
No	13	18	
Age	51.77± 7.01	57.93±6.98	NS*
IL-1β (pg/ml)	88.79± 12.36	116.80±19.21	P<0.01
IL-5 (ng/L)	10.28±1.69	9.52±1.70	NS*
IL-6 (ng/L)	4.46±0.79	5.57±0.73	P<0.05
IL-8 (ng/L)	8.25±0.76	8.42±0.85	NS*
Troponin (ng/ml)	0.03±0.004	0.59±0.13	P<0.001
D-Dimer (ng/ml)	353.15±84.94	408.40±100.23	P<0.001
CK-MB (ng/ml)	3.72±1.47	6.60±1.33	P<0.01
Myoglobin (ng/ml)	53-23±11.41	62.33±10.38	P<0.01

* NS: non-significant

The results of female patients with stable angina in comparison with healthy female individuals of the same age group (50.33±8.65 VS 47.85±6.59) also revealed significant elevation of serum levels in each of troponin (0.47±0.16 VS 0.02±0.001 ng/ml, P<0.001), D-dimer (387.54±79.00 VS 366.28±86.99 ng/ml, P<0.01), CK-MB (5.42±1.37 VS 4.35±0.46 ng/ml, P<0.01), myoglobin (63.09±17.46 VS 45.14±9.66 ng/ml, P<0.01).

Also there is significant elevation in the levels of IL-1β (103.01±19.94 VS 93.72±12.09 pg/ml, P<0.05) and IL-6 (5.35±0.62 VS 4.97±0.75 ng/L, P<0.05).

While there is no significant changes in the serum levels of IL-5 (10.02±1.37 VS 10.45±1.31 ng/L) and IL-8 (8.23±0.84 VS 7.90±0.94 ng/L).

TABLE 2: THE SERUM LEVELS OF IL-1B, IL-5, IL-6, IL-8, TROPONIN, D-DIMER, CK-MB AND MYOGLOBIN IN FEMALE PATIENTS AND HEALTHY CONTROL.

Parameters	Healthy control	Patients with stable angina	P. value
No	**7**	**12**	
Age	**47.85±6.59**	**50.33±8.65**	**NS***
IL-1β (pg/ml)	**93.72±12.09**	**103.01±19.94**	**P<0.05**
IL-5 (ng/L)	**10.45±1.31**	**10.02±1.37**	**NS***
IL-6 (ng/L)	**4.97±0.75**	**5.35±0.62**	**P<0.05**
IL-8 (ng/L)	**7.90±0.94**	**8.23±0.84**	**NS***
Troponin (ng/ml)	**0.02±0.001**	**0.47±0.16**	**P<0.001**
D-Dimer (ng/ml)	**366.28±86.99**	**387.54±79.00**	**P<0.01**
CK-MB (ng/ml)	**4.35±0.46**	**5.42±1.37**	**P<0.01**
Myoglobin (ng/ml)	**45.14±9.66**	**63.09±17.46**	**P<0.01**

* NS: non-significant

IV. DISCUSSION

There are many studies have included lipid plaque formation, platelet formation, injury response, and inflammation, were proposed to explain the pathogenesis of coronary heart disease. However, the inflammation theory has been given a lot more attention recently. In this study we designed to determine some interleukins as diagnostic markers instable angina (Soehnlein & Libby, 2021).

In the current study, troponin, D-dimer, CK-MB and myoglobin were significantly elevated in male and female patients with stable angina.

It is well known that individuals with acute ischemia illness have increased transaminase activity. The American College of Cardiology (ACC) and the European Society of Cardiology (ESC) in 2000 stated that troponin I and T and CK-MB are indicators of choice for diagnosis (Babuin & Jaffe, 2005). Even in the absence of increased CK-MB values, troponin levels were found to have elevated in a high percentage of individuals who presented with symptoms of acute coronary syndromes (Ottani et al., 2000). CK-MB is one of three forms (isoenzymes) of the enzyme creatine kinase (CK). It is primarily found in heart muscle cells, but small amounts are found in skeletal muscles. It is a cardiac marker used to assist diagnoses of an acute myocardial infarction, myocardial ischemia or myocarditis (Kemp et al., 2004). Determination of CK–MB isoenzyme, assessed within

24 hours of symptom onset, has a 98% predictive value for myocardial necrosis (Alpert et al., 2001).

Myoglobin is a haem protein, located in the cytoplasm of both cardiac and skeletal muscle cells, constituting about 2% of the total muscle protein. Its relatively low molecular weight (17 kDa) and cytoplasmic location ensure its rapid release into the circulation; the plasma concentration is elevated 2–3 h after myocardial injury (Alpert et al., 2001; Kagen et al., 1975; Kemp et al., 2004)

The National Academy of Clinical Biochemistry (NACB) in the US, and the (ESC)/(ACC) Committee for the Redefinition of Myocardial Infarction, both recommend the use of plasma myoglobin as early markers of myocardial damage (Collinson et al., 2003).

Furthermore, increased d-dimer is indicative of a hypercoagulable state associated with acute coronary syndromes. D-dimer levels reflected a systemic prothrombotic state and focal vessel wall-related fibrin formation with unstable atherosclerotic plaque activity (Davies, 1990).

This study revealed that IL-1β and IL-6 were significantly elevated in male and female patients with stable angina. Recent studies revealed atherosclerosis is a chronic inflammatory process and in all stages of atherosclerosis inflammation was present (Libby, 2012; Taleb, 2016). IL-1β possesses many effects in all stages of atherosclerosis. In the initiation of atherosclerosis, it stimulated inflammatory response in endothelial cells, reflected by increased chemokines secretion and expressions of adhesion factors, and promotes the accumulation of inflammatory cells in blood vessels and their invasion into the intima (Abbate et al., 2012; Bevilacqua et al., 1985).

Many studies revealed that drugs targeting inflammatory factors improved outcome in patients with myocardial infarction, and administration of the monoclonal IL-1β–neutralizing antibody (canakinumab) can reduce the recurrence of cardiovascular events by 17% (Nakahara & Strauss, 2017; Ridker et al., 2017). Furthermore, IL-1β promotes the gene expression of many inflammatory mediators, it induces cyclooxygenase-2 (COX-2) formation leads to production of prostaglandin. In addition, the generation of IL-6 and matrix metalloproteinase (MMP) are also induced by IL-1β (Beltrami-Moreira et al., 2016; Dinarello, 2009; Loppnow & Libby, 1990).

Interleukin 6 exhibited a broad range of biological activities in multiple systems. IL-6 was possessed both pro-inflammatory and anti-inflammatory effects according to the type of injury and the tissue in which it is acting (Harker et al., 2011; Kamimura et al., 2003; Tedgui & Mallat, 2006).

As in our results, many authors showed that IL-6 is elevated in patients with coronary heart disease and can be consider as a marker of inflammation related to cardiovascular risk (Mendall et al., 1997; Ridker et al., 2000; Zakai et al., 2007). IL-6 may play pro- and anti- atherogenic effects in pathophysiology of atherosclerosis. It stimulated vascular smooth muscle proliferation and activated endothelial cell and platelet, while, its atheroprotective activity included upregulated LDL receptor gene expression resulted in lowering of LDL (Davì & Patrono, 2007; Gierens et al., 2000; Morimoto et al., 1991; Wung et al., 2005). Atherosclerotic thrombosis is intimately associated to the acute reactants such as fibrinogen, C-reactive protein (CRP), and plasminogen activator inhibitors, which are also increased by IL-6 (Libby, 2013; Suzuki et al., 2001).

V. CONCLUSIONS

This study revealed that male and female patients with stable angina recorded a significant increase of IL-1β and IL-6 , while IL-5 and IL-8 levels showed insignificant changes. Accordingly, these proinflammatory markers are helpful diagnostic markers and follow up indicators instable angina pectoris, in addition to troponin, D-dimer, CK-MB and myoglobin.

Conflict of interests

Authors have no conflict of interests.

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