Research Article

Plasmatic Osteopontine Levels in Heart Failure Disease

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ABSTRACT

The osteopontin (OPN) is a glycoprotein composed of 300 amino acid residues, and it weighs about 400 kDa. It was identified for the first time in 1986 in osteoblasts, it is an extracellular structural protein and a member of an extracellular matrix proteins family. Our research aimed to study the plasma levels of osteopontin in patients with heart failure, and then evaluate if these levels are important in order to predict the degree of the disease progression, we aimed also from our study to compare the levels of OPN as an inflammatory cytokine with the levels of other most important inflammatory cytokines (IL-6 and TNF-α) in this case of heart failure. Blood samples were drawn from 112 heart failure patients (whatever its causes), they have been classified according to the NYHA functional classification of heart failure. Other blood samples were also drawn from 26 apparently healthy subjects as a control group. OPN, IL-6 and TNF-α were measured using sandwich ELISA assays. OPN plasma levels in heart failure patients were significantly higher than those in control group, also these increased plasma levels of OPN were compatible with the degree of the disease, which is useful in predicting the seriousness of the situation. Our study also showed significantly increased levels of IL-6 and TNF-α in patient with heart failure compared with the control group, but these increased levels were not accurate and reliable to predict the risk, especially in advanced stages of the disease. Our study showed that the OPN protein is an inflammatory cytokine which plays an important role in the inflammatory condition in the heart failure disease, it can be used to evaluate the risk of the situation and to determine the degree of the disease progression because of its distancing on the other inflammatory cytokines, especially in the advanced stages of the disease

Keywords: Heart failure, Inflammatory cytokines, Interleukin-6, Osteopontin, Tumor necrosis factor.

INTRODUCTION

Heart Failure is a clinical syndrome resulting from the inability of heart to pump adequate amounts of blood to meet the needs of the body and lungs of oxygen and other nutrients, this occurs most commonly when the cardiac output is low (the heart pumps normally about 5 liters per minute which is equivalent to 70-80 ml per beat). This term is often referred to by another which is Congestive Heart Failure because the body becomes congested with fluids.[1]

According to statistics conducted in 2006 by the American Heart Association, there are 81,100,000 people in the United States who have a pattern or more of cardiovascular diseases which include hypertension, coronary heart disease, heart failure and cardiac arrest, 5,800,000 people of whom suffer from heart failure.[1-2]

Doctors classify the stage of heart failure according to functional NYHA classification (New York Heart Association) in order to determine the optimal treatment. This classification connects the symptoms with daily physical activities and lifestyle of patient. It has four classes which range between class I and class IV in accordance with the increasing progression of the disease.[3-4]

Osteopontin (OPN), is a glycoprotein composed of 300 amino acid residues, and it weighs about 400 kDa. It was identified for the first time in 1986 in osteoblasts. It is an extracellular structural protein and a member of an

extracellular matrix proteins family. It exists in the extracellular fluids, in inflammation sites, and in the extracellular matrix of mineraltissues.[5] Osteopontingenes in humans are located on the long arm of the fourth chromosome in region 13 (4q 13). Osteopontin is synthesized by many different tissues including fibroblasts - osteoblasts - osteocytes - bone marrow cells - macrophages - neutrophils - T & B lymphocytes - smooth muscles - skeletal muscle myoblasts - endothelial cells.[6-7]

In 2010, the role of osteopontin in the inflammatory process of heart failure was identified (as an inflammatory cytokine). It was also proven that the cardiac muscle cells are an important source of osteopontin in left ventricular hypertrophy. It turned out that osteopontin increased in the ventricular cardiac muscle in rats suffering from heart failure, which suggests that osteopontin increases in patients with heart failure, and that this increase refers to the degree of disease development.[8]

Thus, the increase of osteopontin expression in heart failure has two levels:

1. Hyper-expression of osteopontin in extracellular matrix due to remodeling, fibrosis, and hypertrophy.[5]
2. Hyper-expression of osteopontin in the cardiac cells as a result of excessive dynamic load on the chambers of heart.

During the process of heart failure, the cardiac muscle is subjected to develop remodeling. Local pro-inflammatory cytokines like TNF-α and IL-6 exacerbate this process through the induction of chronic inflammation in heart.[8]

MATERIALS AND METHODS

Study groups

Heart failure group, which included 112 patients (84 males and 28 females), the average age was 57 ± 14 years. They were divided into groups according to NYHA functional classification in the following form: NYHAII included 40 patients, NYHAIII: included 43 patients, NYHAIV: included 29 patients, while we couldn't get samples for NYHAI because the patients of this group don't show any symptoms and do not usually go to hospitals. Samples of heart failure group were taken from patients diagnosed with heart failure relying on clinical symptoms, medical history, and an echocardiogram. Patients with left heart failure in particular were selected regardless of it cause, we excluded in our study many patients who suffer from medical conditions which significantly affect the studied parameters, namely: autoimmune diseases - lupus erythematosus - all kinds of tumors - renal failure - diabetes type I and II - rheumatism and arthritis - bone diseases - skin immune diseases - acute and chronic infections.

Control group

Included 26 people, male and female, and their average age was 41 ± 7.5 years. For this group, samples were selected of healthy people during blood donation, taking into consideration compatibility of age with the heart failure group.

Sampling

Blood samples were drawn during 9 months (May 5, 2010 to January 20, 2011) from patients at cardiac departments and centers of many hospitals in Damascus - Syria (Mowasat Hospital - Center for Cardiac Surgery - Assad University Hospital - Basel Center for heart diseases and cardiac surgery), 5 ml of the patient's full blood was drawn on EDTA as an anticoagulant, then samples were centrifuged for 10 minutes at 1000 xg within 30 minutes of sampling. The floating plasma was taken and divided into eppendorf tubes, then preserved at a temperature of -80°C until assays were done.

Specimen Assay

Specimen Assay took place in the blood bank laboratory of Damascus University by using ELISA apparatus type ABBOTT- tecan- sun rise.

OPN assay was done by Sandwich ELISA using two kits manufactured by American R & D systems. It was based on the use of two types of Osteopontin-specific monoclonal antibodies.

IL-6 assay was done by Sandwich ELISA using two kits manufactured by Dutch Hycultbiotech Company. TNF-α

assay was also done by Sandwich ELISA using two kits manufactured by German Demidetec Company.

Statistical analysis

Excel 2007 and SPSS 18 were used for statistical study. Parameters values were calculated using the standard curve of studied standard parameters chains which drawn using Excel 2007. Values was shown by using mean± standard error, then Pearson correlation coefficient was calculated to know the severity of correlation and its direction between variables. T test was also used to see if the difference between the variables is statistically significant (if the P values were less than 0.05) or is it just a difference resulting from luck or coincidence (if the P values were greater than 0.05).

RESULTS

Osteopontin levels of the control group ranged between 16.9 - 133.5 ng / ml, with a mean of 61.6 ng / ml and a standard error of 5.5 ng / ml. An assay for the osteopontin values was done in 112 patients with heart failure and found that it ranges from 16.5 to 473.4 ng / ml, with a mean of 137 ng / ml and a standard error of 7.9 ng / ml, and they were categorized according to the NYHA functional classification as shown in Table 1.

Table 1: Average of osteopontin values in patients group after classifying them according to NYHA functional classification

| NYHA classification	Number of patients	Mean of OPN levels (ng/ml)	Standard error of OPN levels (ng/ml)
I	0	---------	---------
II	40	115.8	8.4
III	43	120.9	9.8
VI	29	180	18.4

Measuring the levels of interleukin - 6 showed that its average value in heart failure patients was 4.6 pg / ml and a standard error of 0.43 pg / ml, and it was measured in control group with an average value of 2.9 ± 0.14 pg / ml.

Measuring the levels of TNF-α showed that its average levels in control group was 2.9 ± 0.06 pg / ml, while in heart failure group, its average levels reached 4.2 ± 0.1 pg/ ml.
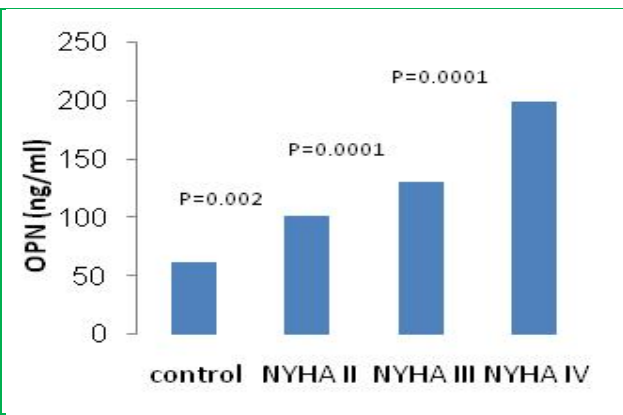
When applying T-test for comparing the osteopontin levels between study groups, a statistically significant increase was found between osteopontin levels in heart failure group and its levels in control group (P< 0.0001) (Figure 1).

When applying T-student test in order to compare osteopontin levels between control group and heart failure group after being classified according to NYHA functional classification, a statistically significant difference was found between control group and NYHAII, NYHAIII, and NYHAIV groups of heart failure patients
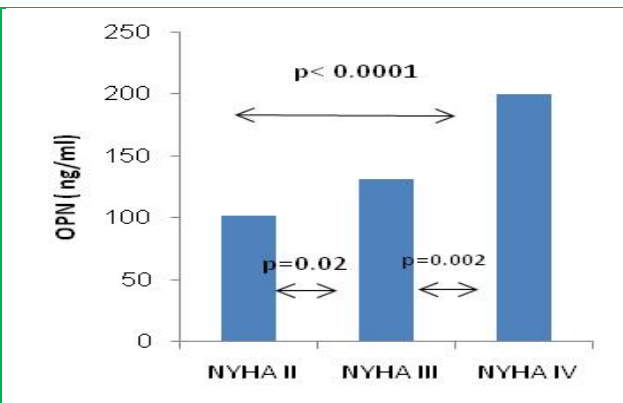
where P values for these three groups were respectively 0.002 - 0.0001 - 0.0001 (Figure 2).
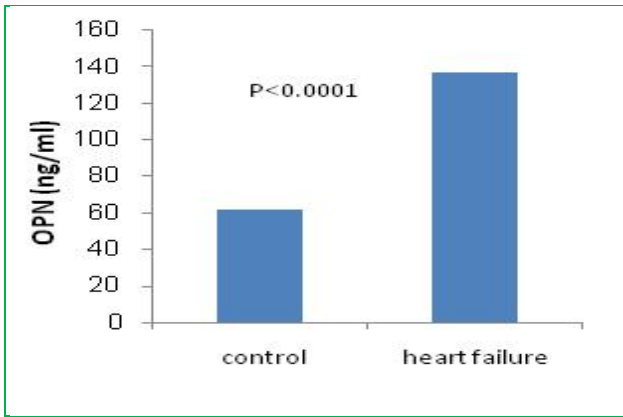
Figure 1: Average of osteopontin levels in control group and heart failure group.
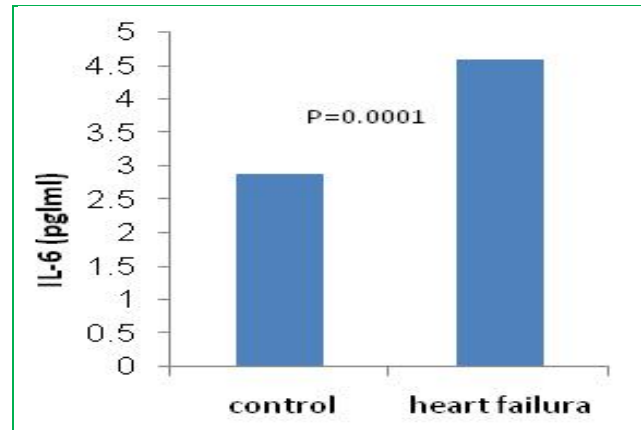

Figure 2: Average of osteopontin levels in heart failure group after being classified according to NYHA functional classification, and control group.
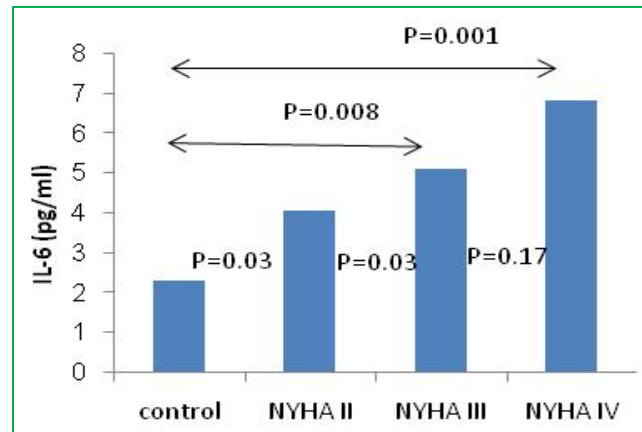

A statistically significant difference was also found between osteopontin levels in NYHAII patient group and its levels in NYHAIII patient group (P=0.02). Another statistically significant difference was found between its levels in NYHAIII and NYHAIV groups (P=0.002). And a statistically significant difference was also found between NYHAII and NYHAIV patient groups (P<0.0001) (Figure 3).

When applying T-student test for comparing IL-6 levels of study groups, a statistically significant difference was found between its levels in control group and heart failure group (P = 0.0001) (Figure 4).

A T-student test was also applied in order to compare IL-6 levels between control group and heart failure group after they had been classified according to NYHA functional classification, statistically significant differences were found between control group and NYHAII, NYHAIII and NYHAIV groups as shown in the figure. Another statistically significant difference was found between NYHAII and NYHAIII groups (P = 0.03), while there was no statistically significant difference between NYHAIII and NYHAIV groups (P = 0.17) (Figure 5).


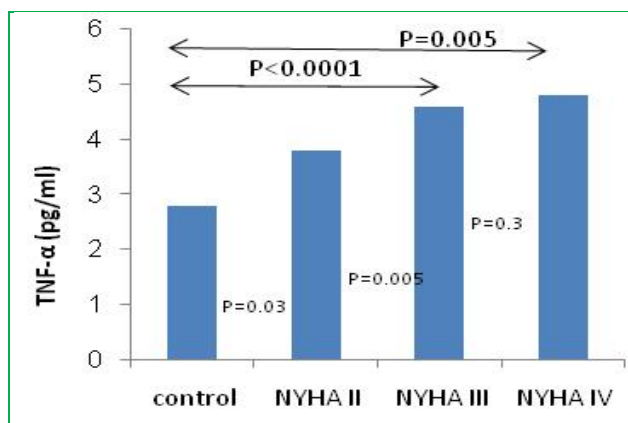**Figure 4:** Average of IL-6 levels among members of the control group and heart failure group.

Figure 5: Average of IL-6 levels in control group and NYHA groups.

When comparing TNF-α levels between study groups, a statistically significant difference was found between control group and the heart failure group (P < 0.0001) (Figure 6).

When comparing TNF-α levels between control group and the heart failure group after being classified according to NYHA functional classification, a statistically significant difference was found between control group and NYHAII, NYHAIII and NYHAIV patient groups where P values in all previous cases were respectively 0.03- 0.0001- 0.0005, as shown in Figure 7. A statistically significant difference was found between NYHAII and NYHAIII groups (P=0.005), while no statistically significant difference was found between NYHAIII and NYHA IV groups (P = 0.3) (Figure 7).

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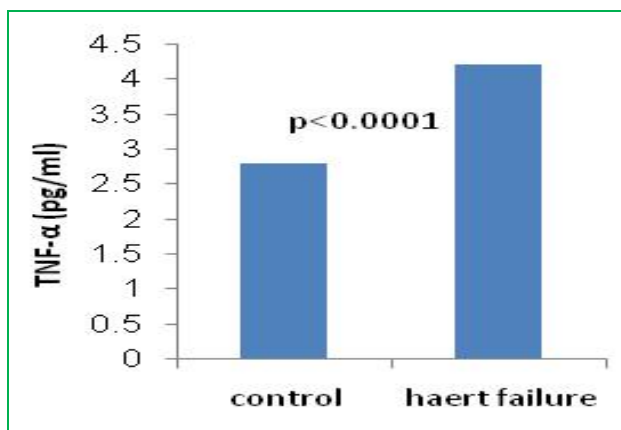
Figure 6: TNF-α levels in control group and heart failure group.

Figure 7: Average of TNF-α levels in control group and NYHA groups.

DISCUSSION

Plasmatic osteopontin levels were elevated in heart failure patients -regardless of it cause - compared with a healthy people group with a statistically significant difference (p < 0.0001), and this is consistent with researches that have examined the role of osteopontin protein as one of the inflammatory cytokines which rise in patients with heart failure.[9-10] The results of our study coincided with Mark Rosenberg and his colleagues study in 2008[11] and Stawowy and his colleagues study in 2002.[12]

The high plasmatic osteopontin levels in patients with heart failure can be explained by increasing the mechanical stress due to excessive hypertension or myocardiopathy or myocardial infarction, or increased the levels of angiotensin Π, which in turn increases in the severe of heart failure and works to induce the production of osteopontin in the cardiac muscular cells and fibroblasts which contribute in the process of cardiac muscle remodeling and fibrosis. We should not forget that the osteopontin as an inflammatory cytokine also rises in the case of heart failure in response to the inflammatory condition, thus, osteopontin secretion from the damaged areas macrophages will happen.

The results of our study showed that the plasmatic osteopontin levels in heart failure patients rise simultaneously with the progression of the disease after

the samples of this group were classified according to NYHA functional classification, as NYHAII patients show less severe symptoms than NYHAIII patients who in turn show less severe symptoms than NYHAIV patients. This compatibly rise with the degree of disease severity is statistically significant.

The results of our study coincided with Marguerite Schipper and his colleagues study in 2011[13], and Mark Rosenberg and his colleagues study in 2008[11], as well as Stawowy and his colleagues study in 2002.[12] These studies showed that the plasmatic osteopontin levels in NYHAIV patients was higher than its levels in NYHAIII patients, which in turn was higher than in NYHAII patients. This compatible increasing of plasma ticosteopontin levels with the degree of disease severity can be explained by increasing the bad influences on muscular cardiac cells coinciding with the development of the disease as a result of excessive hypertension, where the osteopontin expression rises simultaneously with the enlargement of damaged areas in the cardiac muscle. Osteopontin is produced by two important sources in this case, macrophages in the damaged areas, and fibroblasts in the case of cardiac muscle fibrosis progression. In addition, the compatible increased expression of inflammatory cytokines with the development of heart failure which in turn induces the osteopontin gene expression.[10,14-15]

The inflammatory condition affects directly the function of cardiac muscle, as we mentioned, the heart failure includes increasing of the inflammatory cytokine levels which play an important role in the pathogenic process of heart failure by influencing the contractility of the cardiac muscle, causing a hypertrophy, remodeling heart muscle, and inducing cellular apoptosis.[16]

The results of our study showed the increase of IL-6 and TNF-α levels in heart failure patients compared with healthy people. The results of our study coincided with Kalogeropoulos and his colleagues study in 2010[17], Aukrust and his colleagues study in 2005[18] and Wollert and his colleagues study in 2001.[19] All these studies showed an increased expression of the inflammatory cytokines and their soluble receptors in circulation in response to the activation of the immune elements positioned in heart, circulation, or both in heart failure patients. The results showed the important role of these cytokines in the assessment of the situation risk.

The compatible increasing of inflammatory cytokines levels with the disease severity is a point of controversy in many studies. Our study showed an increased value of TNF-α and IL-6 in NYHAII patients compared with NYHAIII patients and with a statistically significant difference, while our study did not show an increased values of TNF-α and IL-6 in NYHAIV patients compared with NYHAIII patients. These results coincided with Anita Deswal and his colleagues study results in 2001[20] on one side and disagreed with them on the other side. Anita Deswals study included a larger number of samples (one thousand two hundred of heart failure patients), and it showed an

increased levels of IL-6 in NYHAIV patients compared with NYHIII patients but this study did not show a significant increase in the levels of TNF-α in NYHAIV patients compared with NYHAIII patients, which led them to conclude that the pro-inflammatory cytokines do not have an accurate and reliable role in patients with the final phase of heart failure. Our study coincided with Anita Deswals study results for TNF-α levels, which were not significantly increased in NYHAIV patients compared with NYHAIII patients, that's probably due to the fact that IL-6 in the advanced stages of disease inhibits the secretion of TNF-α, which reduces its prognostic importance of the disease in advanced stages.

About IL-6 levels, the results of our study were different from Anita Deswals and Koller and his colleagues study in 1998.[21] Our study did not show a significant increase in the levels of IL-6 in NYHAIV patients compared with NYHAIII patients, that's perhaps due to the smaller number of our patients compared with the number of patients of those two studies, in addition to the smaller number of NYHAIV patients compared with the number of NYHAIII patients, so this difference was not clear enough.

CONCLUSION

Heart failure is a common health problem which is constantly growing. The successful management depends on early and accurate diagnosis and on predicting the serious nessin order to know which patients are more likely to be affected. Our research has shown the rise in plasmatic levels of a new marker (osteopontin) in heart failure patients, and the possibility of linking this rise with the progression of the disease, as it is an inflammatory cytokine which is as important as other inflammatory cytokines.

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