Study of the Correlation between Brain natriuretic peptide and Each of Malondialdehyde, Ceruloplasmin and Transferrin in patients with Heart failure(HF) in Thi-Qar Governorate

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ABSTRACT:

Objective: Heart failure is a chronic condition where the heart is unable to provide the different organs with adequate circulation of blood due to abnormality in the heart and changes in the hemodynamic, kidney, nerve and hormone system. The present study is designed to determine the levels of Brain natriuretic peptide **(BNP)**, Malondialdehyde **(MDA)**, Ceruloplasmin **(Cp)** and transferring **(Tf)** in patients with systolic Heart failure **(HF)** with Hypertension and healthy individuals.

Subjects: Serum Brain natriuretic peptide, malondialdehyde, ceruloplasmin and transferrin were measured in 68 patients heart failure and 68 supposedhealthy subjects .

Results: The levels of serum Brain natriuretic peptide, malondialdehyde and ceruloplasmin were revealed significant increase in patients with heart failure as compared to control group whereas the levels transferrin showed a significant decrease in heart failure patients in comparison to control subjects(P ≤0.05).This study was also revealed the correlation between the concentrations of the measured parameters and BNP.

Conclusion: This study revealed significant increase BNP in patients with (HF) and Serum BNP may have a significant diagnostic role in heart failure.Lipid peroxidation and oxidative stress is more prominent in patients with (HF) comparison to healthy individuals , and there is a disorder in antioxidant system in patient with heart failure .according to the levels of ceruloplasmin and transferring

Keywords: Heart failure, Brain natriuretic peptide, Malondialdehyde, ceruloplasmin**, and transferrin .

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INTRODUCTION:

Heart failure is a complex syndrome and the most common reason for hospitalisation and rehospitalisation among older adults (Lloyd-Jones et al.,2010). Heart failure is a chronic condition where the heart is unable to provide the different organs with adequate circulation of blood due to abnormality in the heart and changes in the hemodynamic, kidney, nerve and hormone system (McMurray et al.,2012). Heart failure is associated with poor prognosis and treatment is complex and contains multi-drug regimens and lifestyle changes (McMurray et al.,2012). Adherence to treatment is essential for clinical outcomes such as survival and health-related quality of life (van der Wal et al.,2005). Factors such as cognitive dysfunction and comorbidities can affect adherence to treatment (Moser et al.,2012).There are many causes of HF which vary in different parts of the world. The main causes for developing HF are ischemic heart disease, hypertension, diabetes and coronary artery disease. There are several pathophysiological pathways that are the main causes for developing HF. However, there seems to be a point where a structural remodeling of the myocardium starts, which leads to a pump dysfunction or HF (McMurray et al., 2012). Patients with heart failure may have a number of symptoms, the most common being breathlessness, fatigue, exercise intolerance, and fluid retention (Badgett et al., 1996).For the diagnosis of HF a variety of diagnostic tests is available including assessment of clinical signs and symptoms of HF, laboratory blood tests, radiological examinations, electrocardiography and echocardiography (Maisel et al., 2002). In

1988 a new cardiac natriuretic peptide, B-type Natriuretic Peptide (BNP) was discovered, (Tsutamoto et al., 1997) which in the following years was shown to have prognostic properties (Cowie et al., 1997), and later also appeared to have diagnostic properties in the emergency department (Richards et al., 1998), and out-patient settings (Clerico et al., 2002). Brain natriuretic peptide (BNP)There are two types of cardiac natriuretic peptides secreted by the heart: atrial (ANP) and brain (BNP). BNP is secreted constitutively from myocytes, with secretion increasing in response to pressure and volume overload in the ventricles, such as that noted to occur in heart failure patients. (Tang, 2007).BNP is a biologically active peptide of 32 amino acids and has vasodilator and natriuretic properties. BNP is cleaved from the 108-amino acid pro-brain natriuretic peptide released from the cardiac ventricles in response to stretching of the chamber. The second remnant after cleavage, N-terminal pro-brain natriuretic peptide (NTproBNP), is a 76-amino acid peptide with no known biological function which circulates at higher concentrations than BNP and may represent cardiac status over longer periods (Hobbs et al., 2002).In recent years, plasma levels of BNP have attracted increasing interest as markers of ventricular dysfunction (Chrysohoou et al., 2010). Indeed, measurement of plasma BNP concentrations has been shown to be useful in the diagnosis, risk stratification, and management of adult patients with congestive heart failure (Parcharidis, 2011). Moreover, BNP may be useful in various other conditions in adults, such as hypertrophic cardiomyopathy,(Panou et al., 2006) left ventricular (LV) remodeling after

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myocardial infarction, arrhythmogenic right ventricular (RV) dysplasia,(Koch *et al.*, 2006) while finally it may be a strong predictor of mortality in adults with congenital heart disease (CHD) (Giannakoulas *et al.*, 2010).BNP and NTproBNP are useful to support clinical judgment for the diagnosis or exclusion of HF, in the setting of chronic ambulatory HF (Kober *et al.*, 2008), or acute decompensated HF (Abraham *et al.*, 2011).

Malonaldehyde (MDA), thiobarbituric acid reactive substance (TBARS), lipid hydroperoxides (LH), and 4-hydroxyalkenals (4-HNE) are the example of lipid peroxidation by-products which have been used as biomarker of lipid peroxidation level, many investigations have shown that MDA, TBARS, LH and 4-HNE are directly linked to increase the rates of lipid peroxidation (La Monte *et al.*, 2000).

$$H_2O_2 + Fe^{2+} \rightarrow \cdot OH + OH^- + Fe^{3+}$$

Oxidative stress is characterized by an increased concentration of oxygen-derived products that provoke critical, even irreversible, cell injury, oxygen reduction leads to the synthesis of reactive intermediate compounds such as the superoxide anion, hydroxyl radical, hydrogen peroxide and peroxidative derivatives of polyunsaturated fatty acids (PUFA) such as conjugated dines, lipid hydroperoxides and malonyldialdehyde (MDA) (Caimi *et al.*, 2003). Oxidation of circulating low density lipoprotein (LDL) has been linked to the initiation and pathogenesis of atherosclerosis and ultimately to the pathogenesis of cardiovascular disease (Nuttall *et al.*, 1999).Ceruloplasmin (Cp):It is an important protein that circulates in plasma

as a major copper ion transporter, (90-95%) of human plasma copper is associated with the ceruloplasmin as a no dialyzable fraction and the remaining 5-10% of plasma copper is fairly loosely attached to albumin and histidine, but only a trace of copper is present as free Cu^{++} (Burtis and Ashwood, 1996). Normally, ceruloplasmin is synthesized in the liver, and secreted into plasma (Takahashi *et al.*, 1984) Ceruloplasmin, the multifunctional copper containing enzyme, possesses a significant oxidase activity directed toward ferrous ions (Curzon *et al.*, 1961).The oxidase activity increases during inflammation, infection, and injury which suggests that serum Cp possibly acts as an antioxidant and as an acute phase protein (Fleming *et al.*, 1991). It is implied that during exposure to oxidative stress, substantial inactivation of Cp may occur and free copper ion may be released (Choi *et al.*, 2000). Therefore, the damaged Cp may cause the augmentation of free radical-mediated damage to other macromolecules upon exposure to oxidative stress, thus, Cp is a very important component of the cellular defense mechanism against toxicity (Kang, 2006). Transferrin (Tf) :Serum iron, total iron-binding capacity (TIBC), and calculated transferrin saturation (TS) tests are used to screen for and monitor conditions of iron deficiency and iron overload, although the usefulness of these tests for diagnosing iron deficiency can be debated (Guyatt *et al.*, 1992).Transferrin is a major plasma protein of biological interest because of its evolutionary history and because of its multi regulatory control (Bowman *et al.*, 1988). Transferrin synthesis and storage are regulated by iron levels, estrogens, and nutritional status (Morgan, 1983). Transferrin transports ferric iron into cells by receptor-mediated endocytosis, a unique

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process by which transferrin and its receptor are reutilized repeatedly in iron delivery, in humans, serum transferrin concentration decreases in iron overload and increases in chronic iron deficiency (Vostreis *et al.*, 1988).

Design of study:

This study conducted at AL-Hussein Teaching Hospital in Thi-Qar, especially, in the coronary care unit (CCU) , Biochemistry Laboratory, the Unit viruses in the main blood bank in Thi-Qar, and specialist clinics at the period between 1/11/2012 to 1/10/2013. It included (136) subjects, control (68) and patients (68) .The study has been conducted on total number of supposed healthy individuals and patients:

HF group : 68 patients with systolic Heart Failure with Hypertension [34 males and 34 females] with age range (40—76).

control group : control group, consist of 68 supposed healthy subjects [34 males and 34 females] with no history of systematic illness at age range (40 –75).   Blood samples were collected after the echocardiogram had been recorded . About (8mL) of blood samples of Heart failure patients and controls were taken and allowed to clot at room temperature in empty disposable tubes centrifuge to separate it in the centrifuge at 3000 rotor per minute (rpm)for 10min,the serum samples were separated and stored at (-20ºC) for later measurement of biochemical parameters, unless used immediately.

Methods
Serum Brain natriuretic peptide(BNP) was estimated by enzyme linked immunoassay method by ELISA Reader, USA using kit supplied by RayBiotech, Inc. USA.

Lipid peroxidation Marker (Serum MDA)
Lipid peroxidation is determined using the thiobarbituric acid method. In this method, MDA level of the serum was measured by the following procedure according to a modified method of (Fong *et al.*,1973).It concentrations were calculated usingthe extinction coefficient of MDA (εMDA) equal to (0.156 x18 nmol /ml) .

Serum Antioxidants
Serum Cp concentration was measured by the method of (Menden *et al.*,1977) which using the extinction coefficient of Cp (ε Cp) equal to (0.68) to calculate it concentration Also, the **Serum Tf** concentration was measured by colorimetric method (Burtis *et al.*, 1999). in which an excess of iron is added to the serum to saturate the Tf. The unbound iron is precipitated with basic magnesium carbonate. After centrifugation the iron in the supernatant is determined.The concentration of iron remaining is assayed and the result expressed as total iron binding capacity (TIBC) . The serum Tf concentration was calculated from the following equation:() , (Gambino ,*et al* . , 1997)

SerumTf (gm/L) = TIBC (µmol/L)/ 25.1 .

Statistical Analysis
 Statistical analysis was done using the software **SPSS** version 15.0,the results were expressed as mean ± standard deviations (mean ± SD). Tow way ANOVA-test was used to compare parameters in different s 0.05) we significant. Person correlation coefficient (r

) was used to test the correlation relationship among the different parameters in each patients group.

RESULTS:

In this study we measured the level of BNP, MDA, Cp , and Tf among patients with heart failure and healthy individuals. also the correlation between the

concentration of BNP and (MDA, Cp, and Tf) were evaluated in this study. The levels of serum Brain natriuretic peptide, malondialdehyde, and ceruloplasmin Showed significant increase among patients heart failure as compared to control group whereas the levels of transferrin showed a significant decrease in heart failure patients in comparison to control subjects.

Table (3 – 1):- Serum Brain natriuretic peptide concentrations of (control) and (HF) groups

Group	n	BNP concentration (pg/ml) mean± SD
Control	68	101.00 ± 34.01^{b}
HF	68	478.84 ± 72.39^{a}
LSD		20.54

Each value represents mean ± SD values with non-identical superscript
(a , b or c … etc.) were considered significantly differences (p ≤ 0.05).
HF: Patients with Heart Failure group.
LSD : Low significantly differences .

Table (3 – 5):- Serum Malondialdehyde concentrations of (control) and (HF) groups

Group	n	MDA concentration (nmol/mL) mean± SD
Control	68	14.54 ± 1.69^{b}
HF	68	23.93 ± 3.85^{a}

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Table (**3 –6**)**:-** Serum Ceruloplasmin concentrations of (control) and (HF) groups

Group	n	Cp concentration (g/L) mean± SD
Control	68	2.84 ± 0.52^{b}
HF	68	3.94 ± 0.59^{a}
LSD		0.2

- Legend as in table (3- 1)

Table (**3 – 7**)**:-** Serum Transferrin concentrations of (control) and (HF) groups

Group	n	Tf concentration (g/L) mean± SD
Control	68	3.45 ± 0.55^{a}
HF	68	2.53 ± 0.29^{b}
LSD		0.194

- Legend as in table (3- 1)

Correlation relationship between BNP and all parameters in patient and control groups .
Figure(1) shows the positive correlation relationship between BNP and MDA in patient and control group patient with coefficient correlation (r = 0.20) , control with coefficient correlation (r = 0.57).
Figure(2) shows the positive correlation relationship between BNP and Cp in patient and control group patient with coefficient correlation (r = 0.09) , control with coefficient correlation (r = 0.10).
Figure(3) shows the negative correlation relationship between BNP and Tf in patient and control group patient with coefficient correlation (r =-0.123) , control with coefficient correlation (r =-0.648).
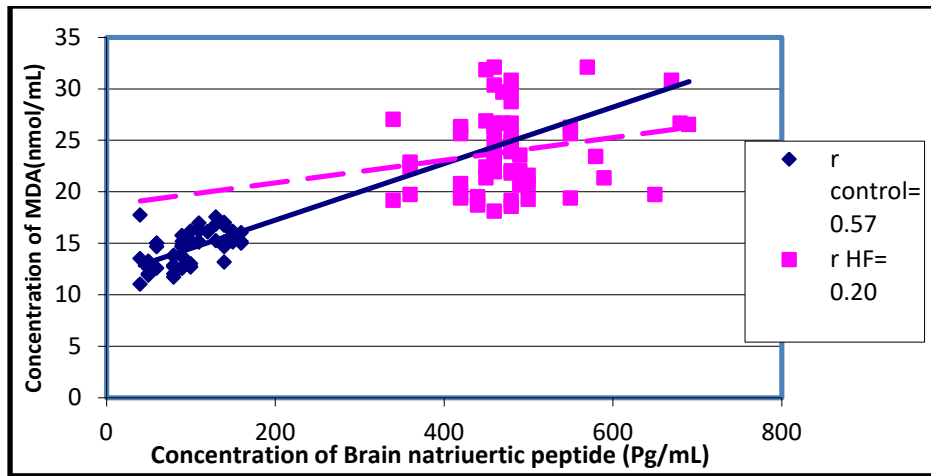
Figure (1): Correlation relationship between BNP and MDA in patient and control groups.
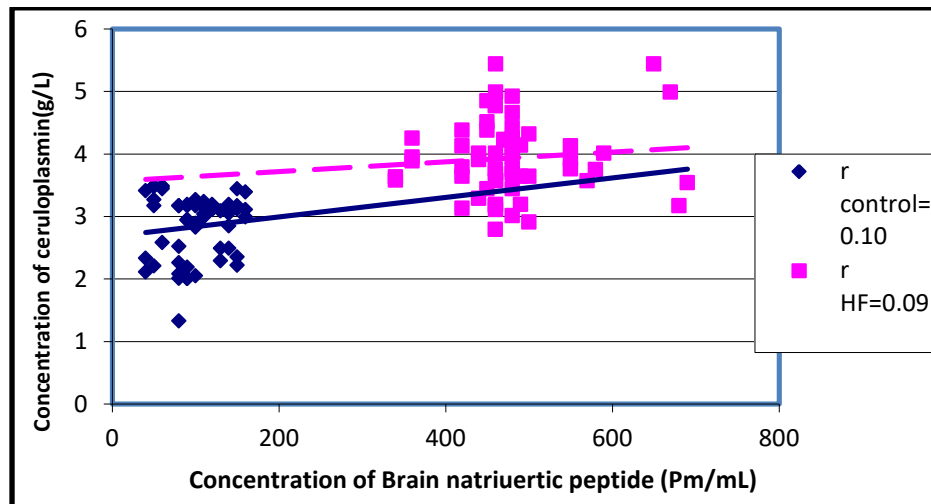

Figure (2): Correlation relationship between BNP and Cp in patient and control groups.

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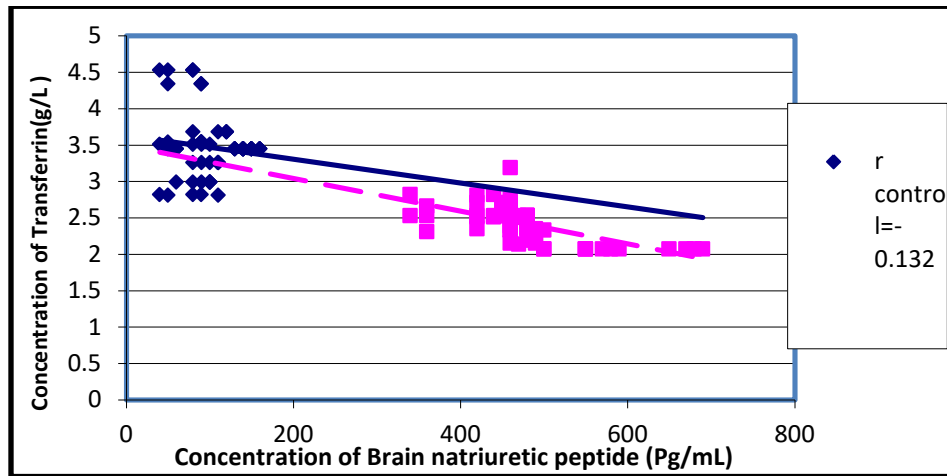Heart failure(HF) in Thi-Qar Governorate

Figure (3-15): Correlation relationship between BNP and Tf in patient and control groups.

DISSCUSSION:

Natriuretic peptide hormones, a family of vasso active peptides have emerged as important candidate for development of diagnostic tools and therapeutic agents in cardiovascular diseases for diagnosis of heart failure and left ventricular dysfunction, where few focus on the measurement of circulation BNP and its N-terminal fragment of pro-hormone (Cheng *et al.*, 2001). Brain type natriuretic peptide (BNP) is a cardiac hormone with diuretic, natriuretic, and vasodilator properties that is secreted mainly by the ventricles in response to volume expansion and pressure load (Cowie and Mendez ,2002).BNP,which is produced by the cleavage of a precursor protein into BNP and the biologically inactive peptide N-terminal precursor protein, causes natriuresis,diuresis, vasodilatation, and smooth muscle relaxation (Levin *et al.*,1998). Unlike the atrial natriuretic peptide, whichis stored in the cardiac atria

and ventricles, the cardiac ventricles are the major source of plasma BNP,suggesting that BNP may be a more sensitive and amore specific indicator of ventricular disorders thanother natriuretic peptides. Moreover, BNP release seems to be in direct proportion to ventricular volume expansion and pressure overload (Maeda *et al.*, 1998).Plasma concentrations of BNP increase in variouspathologic states, particularly those involved in increased cardiac chamber wall stretch and expanded fluid volume (eg, in cases of heart failure, renal failure, or primary hyperaldosteronism), or reduced peptide clearance (eg, in case of renal failure). BNP seems to have clinical utility in terms of excluding the diagnosis of heart failure in patients with symptoms of breathlessness or fluid retention and mayprovide prognostic **Thi-Qar Medical Journal (TQMJ):Vol(8)** withheart

(Koglin, *et al.*, 2001; . Cheng,2001) .

Also,there is some evidence that it may be useful formonitoring heart failure therapies (Maisel, 2003).Generally, in HF the levels of MDA was significantly higher than those who had no history of HF. (Puspha *et al.*, 2005). suggested that reperfusion of the infarct myocardium leads to oxidative stress and there was a highly significant enhancement in the level of MDA. Our results are in accordance with previous report .(Rajesh *et al.*, 2011). Oxidative degradation of lipids is known as lipid peroxidation, and one of the most abundant carbonyl products of lipid peroxidation is MDA, which is an index of oxidative damage (Cavalca *et al* ., 2001). Increased lipid peroxidation may occur as a result of increased free radical generation and suppressed scavenging mechanism (Jayakumari *et al.*, 1992). A possible cause of this increase in CP in HF patients is the impairment of the oxidant-antioxidant balance in favour of the oxidants. Serum CP levels were reported to be an independent risk factor for cardiovascular diseases (Fox *et al.*, 2000). ceruloplasmin is an acute phase protein and is synthesized by the liver in response to tissue damage and inflammation (Sirajwala *et al.*, 2007). Antioxidant terminates the chain of the reaction by removing free radicals and inhibits other oxidation reaction by oxidizing themselves. The balance between free radicals formation and antioxidant activity is called oxidative stress. When the oxidative stress is unbalanced in favor for free radicals, such as acute and chronic exercises, damage occurs to many cellular membranes such as the heart and skeletal muscles (Jenkinson *et al.*, 1999). In humans, increased plasma transferrin levels are found in iron deficiency anemia whereas decreased plasma transferrin occurs in conditions resulting in increased iron stores (Morgan *et al.*, 1983). The low plasma transferrin concentration found in humans with increased iron stores may be due to a negative feedback of storage iron levels on transferrin synthesis (Aisen, 1984). The total iron binding capacity was found to be significantly low in these groups reflecting large volume of iron. The high levels of stored iron found in the subjects of coronary heart disease as compared with controls, suggest that there is a cumulative risk of high stored iron in the development of coronary heart disease

Conclusions:

From the data in this study we can conclude the following points:-Serum BNP can consider a diagnostic marker of in heart failure.Lipid peroxidation positively associates with BNP levels .Disorder the antioxidant system in patient with heart failure .according to the levels of (ceruloplasmin and transferrin) .

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