Homocysteinemia in Heart Failure

Mohammed Adnan Khaleefah(BSc)[1], **Ismail Ibrahim Latif**(PhD)[2], **Ali Mousa Jaffar (**PhD)[3]

[1] College of Medicine, University of Diyala, Baqubah, Iraq
[2]College of Medicine, University of Diyala, Baqubah, Iraq
[3]College of Medicine, University of Diyala, Baqubah, Iraq

Correspondence Address:
Dr. Mohammed Adnan Khaleefa
College of Medicine, University of Diyala, Baqubah, Iraq
email: drmohammed922@gmail.com mobile 009647702966903

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Abstract

Background: Heart failure represents a major health problem leading to considerable mortality morbidity. High total homocysteine concentrations have been related to increased risk of atherosclerosis and its sequel, including coronary heart disease, cardiovascular mortality, and stroke. Brain Natriuretic peptide is a member of a group of peptide hormones similar in structure, used as a specific marker for diagnosing heart failure. This marker increases gradually with the progression of the grade of heart failure.

Objective: The current study aims to measure and investigate the difference in the level of homocysteine between patients with heart failure and without, to find the relationship between the level of homocysteine and grade of heart failure, and to find out the correlation between homocysteine levels with Brain Natriuretic peptide levels.

Patients and Methods: This case-control study involved 200 participants, 100 patients with a history of chronic heart failure, and 100 control participants.A self-constructed questionnaire form prepared to collect selected variables, the participant's weight, height, temperature, electrocardiography measured by trained staff, Brain Natriuretic peptide used as a specific marker for diagnosing heart failure. Blood samples were taken early in the morning from the patient and control groups, after at least 12-hours of fasting and a 20-minute rest. Analysis of all samples of blood was performed in the Biochemistry Laboratory of Baqubah teaching hospital to measure the levels of homocysteine by Human Homocysteine ELISA kit.

Results: The sample was selected homogenously in terms of age and gender. Overweight, obesity, current and ex-smoker, history of hypertension, and history of diabetes mellitus were significantly higher in patients with heart failure than control. The fasting mean homocysteine level was significantly higher in patients (20.049nmol/ml) than in control (2.734nmol/ml). The

fasting mean homocysteine level was significantly elevated in the patients with a positive history of hypertension and diabetes mellitus (p-value ≤0.05) than patients with a negative history. The level of homocysteine was correlated significantly and positively with the level of Brain Natriuretic peptide(r=0.39, p=0.001). The fasting mean homocysteine level was significantly increased with an increase in the severity (grades) of the heart failure (p-value = 0.001).

Conclusion: There was a relationship between the homocysteine level with the development, and severity (grades) of the heart failure as well as significantly and positively correlated with the Brain Natriuretic peptide level.

Keywords: Heart Failure; Homocysteinemia; Brain Natriuretic peptide

Introduction

Heart failure (HF) is a clinical syndrome characterized by elevated mortality, recurrent hospitalization, reduced life quality, and a complex therapeutic regimen[1].Heart failure is a major public health problem affecting over 23 million people worldwide[2]. In spite of new therapeutic advances, the mortality and morbidity after the occurrence of heart failure stay substantial[3].As a result, avoidance of heart failure by detection and managing the risk factors and preclinical stages of the disease is a priority[4]. After the establishment of a diagnosis clinically, the etiology of heart failure influences the treatment selection and result. The related risk factors for HF development can influence morbidity and mortality, and contribute to the differences in clinical outcomes between sexual category[5,6]. Homocysteine has been discovered in 1932. Chemically it is similar to cysteine, so that, the name is homocysteine. There has been a significant correlation between hyper-homocysteinemia and cardiovascular diseases[7].It is believed that homocysteinemia leads to damage to the endothelial cell, decrease in vascular flexibility, and changes in the hemostasis process[7]. Increase homocysteine levels may lead to a strengthen the effects of risk factors like smoking, hypertension, lipid metabolism, and promote inflammation development[7].The prevalence of homocysteinemia may vary significantly depending on diet, genetic background, and age[8]. In the response to stretching of atria and ventricles or in volume overload, cardiomyocytes start to secrete atrial and brain natriuretic peptide. Brain natriuretic peptide (BNP) released from ventricular myocytes in response to dilatation or volume overload[9].At first, Pro-BNP is released after that, it splits biologically to active (BNP) and inefficient (N-terminal segment NT-proBNP)[10,11].Brain natriuretic peptide is a specific marker for heart failure diagnosis[9].

Patients and Methods

Study design: It is a case-control study with analytical elements. The data collection was conducted between the first of April and the first of July of 2019 among participants attending the medical word in Baqubah teaching hospital, Diyala city , Iraq. A convenient sample of 100 patients diagnosed

with heart failure, and admitted to the medical word in Baqubah teaching hospital were included. Other 100 volunteers matched in terms of age and gender and who attended and admitted the medical word for other causes were selected as the control group, and the reason for this sample size is the time frame. A self-constricted questionnaire form prepared by the researchers to collect information from the participants by direct interview with them. The questionnaire included information regarding selected variables like age (18-39years,40-49years, 50-59 years, 60-69years and ≥70 years), gender (male and female), medical history of hypertension and diabetes mellitus, smoking history (current smoke, ex-smoker and never smoke), family history of heart disease and history of cardiac medications. Medical history of mixed dyslipidemia was depend on lipid profile (total cholesterol>250 mg/dl, high density lipoprotein (HDL) <40 mg/dl, low density lipoprotein (LDL) >150 mg/dl and triglyceride (TG)>160 mg/dl)[12]. Pulse rate and respiratory rate were measured by the researchers manually and the blood pressure of each participant was measure by a mercury sphygmomanometer. The grade of heart failure for each patient was determined according to New York Heart Association[13].The participant's weight, height, temperature, and electrocardiography (ECG) measured by trained staff. Body mass index (BMI) was calculated by the following equation:

BMI= kg/m^2, where (kg) is a person's weight expressed in kilograms and (m^2) is their height in squared meters where underweight with BMI< 18.5 kg/m^2 , normal with BMI between 18.5 to <25 kg/m^2 , overweight with BMI between 25.0 to <30 kg/m^2 and obese with BMI ≥ 30.0 kg/m^2[14].

Serum samples Collection

Venous samples were taken early in the morning for the patient and control groups, after at least 12-hours of fasting and a 20-minute rest. The samples of blood were drawn from the median cubital vein or from another vein if this was not accessible. After cleaning the venipuncture site with iodine using concentric circles, the iodine remained in contact with the skin until dried to ensure disinfection. After that 5 ml of blood was taken and put in the gel tube, gel tube has been put in the cool box (contained ice bag) till transport to the emergency unit laboratory and separated by centrifuging for 10 minutes at 6000 rpm. After separation of whole blood the serum sample was drawn by pipette and put in theplain tube and stored in a deep freezer (-20°C).

Homocysteine level is detected by using an Enzyme-Linked Immunosorbent Assay (ELISA) kit(SunRed) used for the accurate quantitative detection of human homocysteine in plasma, cell culture supernatants, serum, cell lysates, tissue homogenates. The NT-pro BNP was detected by using a human (BNP) ELISA kit (SunRed). It is a quantitative assay for the use of urine, plasma, cell culture supernatant, serum, and tissue samples.

Statistical analysis

Statistical analysis of data was carried out by the Statistical Packages for Social Sciences (SPSS), Version 23. The P-value ≤ of 0.05 was considered to be significant statistically.

Results

The mean level of homocysteine in studied participants: The fasting mean homocysteine level was significantly higher in patients with heart failure (20.049nmol/ml) than control participants (2.734nmol/ml) p=0.0001, as in Table (1) and Figure (1). And by logistic regression test, every increase of 1.362 nmol/ml homocysteine level, there will be an increase in 3.9-time risk to get heart failure.

Table (1): Mean homocysteine levels in studied participants

Participants	Mean ±SD	Median	P-value
Patients	20.049±14.15 nmol/ml	15.611 nmol/ml	0.00001*
Control	2.734±0.346 nmol/ml	2.735 nmol/ml	

* Student – test

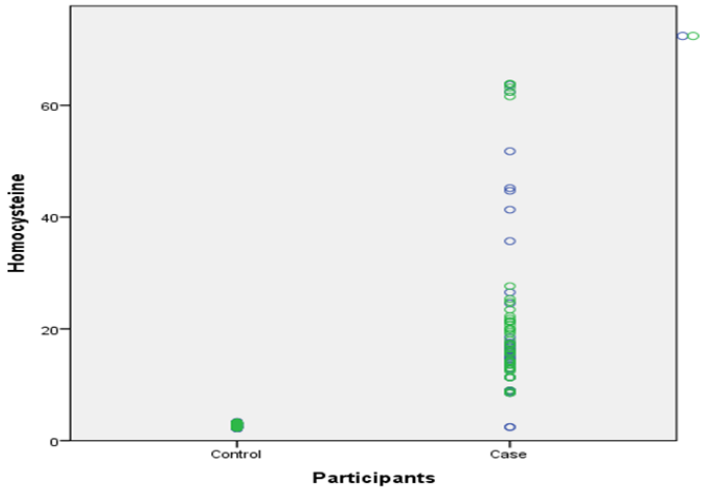

Figure (1): Fasting levels of homocysteine in the studied participants

The difference in mean level of homocysteine with medical history of patients: The mean homocysteine level was significantly higher in patients with a history of hypertension and diabetes mellitus (p-value ≤0.05). The mean homocysteine level was significantly increased with increasing in grades of heart failure (p value = 0.001), Table (2).

Table (2): Differences between the mean homocysteine levels according to the medical history of patients

Medical history		Mean level of homocystein	P value
History of hypertension	Negative	7.036 nmol/ml	0.0001*
	Positive	17.658 nmol/ml	
History of diabetes mellitus	Negative	8.941 nmol/ml	0.0001*
	Positive	17.390 nmol/ml	
Grades of heart failure	I	14.505 nmol/ml	0.001**
	II	18.088 nmol/ml	
	III	21.719 nmol/ml	
	VI	51.056 nmol/ml	

* Student t test ,** ANOVA test

Association of the levels of Pro BNP with heart failure: The Pro-BNP mean concentration ± SD was significantly higher among heart failure patients than that among control subjects, and showing a significant association (P = 0.0001), as seen in the Table (3).

Table (3): Association of levels of Pro BNP with heart failure

BNP [ng/L]		Heart failure patients	Control participants
Mean ± SD		408.713±357.384	17.575±
Mean± SE		35.738	4.836
The range		50-1600	5-160
The Percentile	05th	50.000	5.000
	25th	109.064	5.616
	50th(Median)	336.058	6.918
	75th	476.193	9.589
	95th	1144.275	118.779
	99th	1548.092	160.000
P-value		0.0001	

Association of levels of Pro BNP with the grades of heart failure: The Pro BNP mean concentration ± SD showed a statistically significant association with HF grades and increase with increasing in grade getting its highest value with grade IV HF (1182.697±284.931) with a P value of 0.0001. as seen in Table (4).

Table (4): Association of levels of Pro-BNP with the grades of heart failure

HF grade	No of patients	Pro-BNP mean concentration ± SD
Grade I	40	191.835±220.505
Grade II	39	437.321±267.474
Grade III	15	603.078±332.677
Grade IV	6	1182.697±284.931
P value	0.0001	

Association of Pro BNP concentration with homocysteine concentration:The level of the BNP was correlated significantly and positively with level of homocysteine (r=0.39 p=0.001), as seen in the Figure (2).

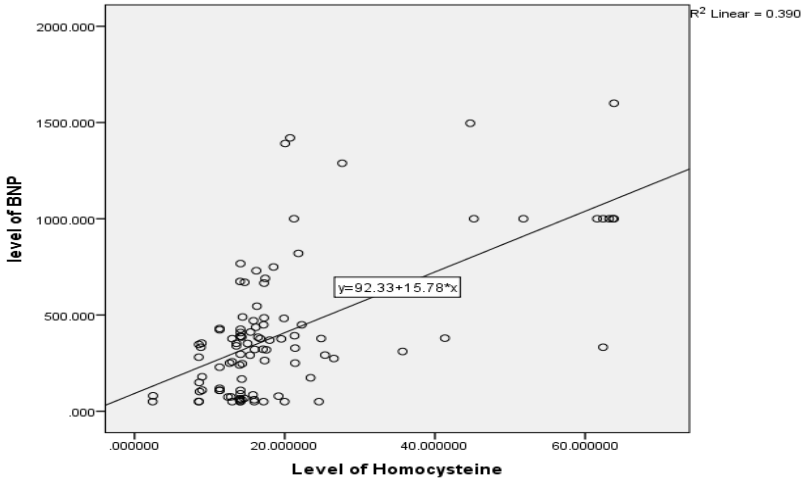

Figure (2): Correlation between the level of BNP and the level homocysteine

Discussion

Recently, plasma homocysteine has been suggested to be increased in heart failure patients potentially representing another newly recognized risk marker or risk factor. The study results are in agreement with the hypothesis that high plasma homocysteine concentrations are a significant risk factor for heart failure. Elevated homocysteine levels may promote heart failure through several mechanisms. First, elevated homocysteine concentration is a risk factor for atherosclerosis of coronary vessels. Furthermore, homocysteine can cause ischemia in myocardial tissue by encouraging the endothelial dysfunction of coronary vessels. Second, elevated homocysteine concentration in patients with acute coronary syndrome is associated with larger myocardial injury as evidenced by elevated troponin concentration. Third, the homocysteine role as a source of oxidative stress, a factor recognized to endorse myocardial dysfunction.Fourth, hyper-homocysteinemic rats have amplified cardiac fibrosis and increased activate matrix metalloproteinases, which sequentially encourage remodeling of left ventricle, a recognized originator of congestive heart failure[15].These results are supported by many other studies like The Framingham Heart Study, which was a community-based prospective cohort study of 2491 adults that accomplished, an elevated plasma level of homocysteine alone predicts risk of the congestive heart failure development in adults devoid of prior myocardial infarction[15] and another study that shown increase in plasma levels of homocysteine more than the median were associated with a 3.2-fold (95% confidence intervals 1.8 to 5.6; $p < 0.0001$) raise in cardiovascular risk16. Another clinical information point that homocysteine was associated with an elevated incidence, in addition to severity, of heart failure[17].In addition to the relationship between homocysteine and heart failure, The study findings had shown a relationship between homocysteine and the severity of heart failure (The level of homocysteine was significantly increased with an increase in the grade of heart failure (p-value = 0.001). These findings are enhanced by four other studies viewing significant associations of homocysteine with structure and function of the left ventricle[18,21]. The findings of the current study revealed that the mean fasting level of homocysteine was significantly higher in patients with hypertension, and this was comparable to another study that showed similar results and interrelated by homocysteine underwent metabolism in the body to create H_2S, which is a powerful antioxidant and vasorelaxant gas. At a high concentration, homocysteine inactivates proteins by homocysteinylation counting its endogenous metabolizing enzyme, cystathionine γ-lyase. Accordingly, decreased making of H_2S during hyper-homocysteinemia represent vascular diseases and hypertension[22]. In this study, the mean fasting level of homocysteine was significantly higher in patients with diabetes mellitus and this was similar to another study that showed similar results, which found that hyper-homocysteinemia is linked to 5-year mortality free of other major risk factors and come to be a stronger (1.9-fold) risk factor

for death in patients with type 2 diabetes mellitus than in non-diabetic ones, and interpreted by elevated homocysteine levels may apply an atherothrombotic effect by increasing oxidative stress, that may stimulate the endothelial dysfunction. Homocysteine can also influence the extracellular matrix properties and increase proliferation of smooth muscle cell. Oxidative stress is thought to be amplified in type 2 diabetes millets[23].The BNP concentration was positively and significantly correlated with the homocysteine concentration (r=0.39, p=0.001) . Other studies also found similar results, showing that increase of homocysteine levels associated with increase NT-pro-BNP levels through a link with impaired mitochondrial fatty oxidation, in two contrasted populations[24].The concentration of BNP was increased in heart failure compared to the control group, which signifies there is a statistically significant association between the concentration of BNP and heart failure (P = 0.0001). A preceding study established that BNP was significantly elevated in complicated heart failure[25]. Other prior studies in Iraq showed that BNP levels rose according to the ejection fraction, which means there is an association between ejection fraction and the level of BNP with P-value < 0.001, wherein the level of BNP increased with the reduction in ejection fraction[26]. BNP mean concentration ± SD showed a statistically significant association with heart failure grades and increased with increasing in grades getting its peak value in patients with grade IV heart failure (1182.697±284.931) with a P-value of 0.0001. There is another preceding study demonstrated that the BNP concentration elevated with the advancement of heart failure grade[27].Further studies also found same results as other studies also found similar results, showing that increase of homocysteine levels associated with increase NT-pro-BNP levels through a link with impaired mitochondrial fatty oxidation, in two contrasted populations[28,29].Other previous study show compatible results with this study, which reported that higher level of BNP in patients with class 4 and the mean level was (8270.2±6116.9) with a P=value of < 0.05[26].

Conclusions

Increase body mass index, current and past smoking, history of hypertension, and diabetes mellitus are significantly higher in patients with heart failure than the control group.

The mean fasting level of homocysteine is significantly higher in patients with heart failure than the control group, and with every increase in 1.362nmol/ml in level of homocysteine, there will be an increase in 3.9-time risk to get heart failure.

The mean level of homocysteine is significantly increased with increase in the grade of heart failure. There is a positive correlation between the level of the BNP and the level of homocysteine.

Patients with a positive history of hypertension and diabetic mellitus had significantly higher mean fasting levels of homocysteine. The mean level of BNP is significantly increased with increase in the grade of heart failure.

Recommendations

Given the novelty of these findings, the researcher likes to underscore the need for additional studies to corroborate these results.

Clinical trials will be required to examine the possibility that lowering elevated homocysteine levels through vitamin therapy with folic acid, alone or in combination with pyridoxine hydrochloride and cyanocobalamin may reduce the risk of heart failure. Future studies are suggested to explore the association of homocystiene with other cardiac and non-cardiac diseases.

It is suggested that similar studies with larger sample sizes and cover vast areas and use other variables with more laboratory diagnostic techniques to reach more accurate results. More data on intake and serum levels of folate, vitamin B12, and vitamin B6, are needed to explore the extent to which the relationship between these vitamins and serum homocysteine levels differs for the case and control participants.

Conflicts of interest: None

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