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CAN ANEMIA BE A CONFOUNDER IN NT-PROBNP INTERPRETATION?

Mudhafar Sami Khazaal

Medical Physiology Department, College of Medicine, University of Karbala, Iraq
ORCID ID: 0000-0002-7212-6118
Corresponding author
E-mail address: mudhafar.sami@uokerbala.edu.iq

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ABSTRACT

Heart failure is a major clinical issue of concern worldwide. Many biomarkers are used to aid the diagnosis of heart failure patients. One of the increasingly used biomarkers is the biologically inactive NT-proBNP. Several confounding factors affect plasma NT-proBNP levels, and hence, the validity of its interpretation. Heart failure and anemia are well correlated, as evidenced by variety of studies. In, anemia can yield some degree of cardiac stress that leads to the expression of NT-proBNP levels above the cut-off values.

This study aimed to test whether anemia can be associated with an elevated peptide level in patients without heart failure. A cross-sectional study in which a total of 108 randomly selected patients, irrespective of their hemoglobin levels, were recruited. Males comprised 39.8% of the total, and the patients were divided based on their hemoglobin levels into anemic (55 patients) and non-anemic (53 patients) groups. Plasma NT-proBNP levels were assessed using ELISA, and the results were interpolated and analyzed against hemoglobin levels. The NT-proBNP level was significantly higher in patients with anemia ($p < .0001$) where it was 268.7 ± 38.9 vs. 93.55 ± 17.4 ng/dl for both the anemic and non-anemic groups, respectively. Hemoglobin level showed a negative correlation with plasma peptide level ([F (1, 107) = 28.63, p < .0001)], with an *r2* of 0.358.

Anemia is inversely correlated with the plasma NT-proBNP level. In fact, anemia can raise the peptide level beyond its recommended cutoff levels making the interpretation of the raised NT-proBNP more challenging.

INTRODUCTION

Brain natriuretic peptide (BNP) is a hormone with a pleiotropic activities that belongs to a well-known family called "the natriuretic peptides" the members of which share a common functional similarity despite their different genetic backgrounds [1]. Under various stressful events, particularly in volume overload, the myocardial cells synthesize both the BNP and its derivative the N-terminal pro-brain natriuretic peptide (NT-proBNP) and release them into circulation [2].

From the technical point of view, there are several confounding factors that affect the clinical interpretation of an elevated NT-proBNP. For example, the peptide level is elevated in patients with reduced renal function probably secondary to the plasma volume expansion and the reduced renal clearance after decreased glomerular filtration rate [3]. Obesity can raise the NT-proBNP level [4]. Atrial wall stress induced by atrial fibrillation also increases NT-proBNP level [5]. Yet, the most important cause for an elevated NT-proBNP is the heart failure (HF) [6].

Heart failure is frequently associated with anemia which is as defined as a level of hemoglobin below 13 g/dl in males or below 12 g/dl in females [7]. Anemia adds a further burden to the patient`s prognosis, and an increased risk of death. The relationship between anemia and heart failure is hard to explain since both of them are well correlated [8]. Anemia can be a predisposing factor to heart failure and can, for instance, be a result of it.

Owing to the tissue hypoxia caused by the reduced oxygen carrying capacity of hemoglobin, the body will take corrective measures as a compensatory mechanism. Some of these compensatory mechanisms involve an increased cardiac activity to reduce the blood circulatory time. Anemia is indeed one of the hyperdynamic situations that will stimulate the cardiomyocytes to release NT-proBNP [9].

PATIENTS AND METHODS

This study was of a cross-sectional type that was conducted at Al-Hussain teaching hospital/ Karbala/ Iraq, from the period of October 2020 to September 2021. A total of 108 patients were enrolled in this study after obtaining the approval of the ethical committee for medical research at Karbala directorate of health.

After full and comprehensive explanation for their part in the study, all of the enrolled patients gave their consent to participate. Patients were recruited from medical wards and outpatient clinic. The selection was randomized where every third patient to show up at the outpatient clinics was chosen.

Exclusion criteria;

The exclusion was based on the careful history taking stressing upon the kind of medication taken by the patients in case of any, careful evaluation of the patients records, thorough physical examination, and relevant investigations such as chest X-rays, echocardiogram, ECG. etc. The study excluded the following:

- Patients with frank heart failure as evidenced by the patient`s medical record or by finding a low ejection fraction (EF %) < 40% after doing an echocardiographic exam using Vivid 7, GE Medical Systems, Horton, Norway.
- Patients with ischemic heart disease (IHD).
- Renal insufficiency (serum creatinine > 2mg/dl).
- Patients with an age of more than 74 years to exclude age related variability in the peptide level.

After sterilizing the cubital fossa for venesection, 5 cc of venous blood were withdrawn from the median cubital vein and immediately translocated in potassium EDTA containing tubes. The hemoglobin (Hb) level was measured as a part of the complete blood count (CBC) using Mindray BC 20s Hematology Analyzer provided by Mindray inc., Shenzhen, China. Within 12 hours of collection, blood aliquots were stored by deep freezing at – 80 C using TUS 80-100 deep freezer provided by Labortechnik, Germany. For the analysis of NT-proBNP level, kits provided by LSBio, USA were used. The plasma NT-proBNP levels from all patients were measured by enzyme linked immunosorbent assay (ELISA). A level of less than 125 pg/ml was used as a normal cutoff for patients aged 0-74 years old [10]. According to Hb levels, patients were categorized in two groups:

1. ***Group-A: (Non-anemic patients):*** this group involved 53 patients with Hb ≥ 12.5 g/dl for men and of ≥ 11 g/dl for women. The mean age for this group was (54.22 ± 12.61 years), males comprised about 45.2% (n=24).
2. ***Group-B: (Anemic patients):*** this group comprised 55 patients with Hb of <12.5 g/dl for men and of < 11 g/dl for women. The mean age for this group was (57.67 ± 13.44 years), males comprised (35.5%) (n=19) of this group.

Statistical analysis

Statistical analyses were conducted using SPSS®/PC software package (version 25.0) provided by (IBM®, SPSS Inc., Chicago, IL, USA). Results are presented as means ± SD. A *p* value < 0.05 was considered as a reference point of statistical significance. Comparisons between both groups were made with the use of t-test. Analysis of the effect of variables on the NT-proBNP level was done using multivariate regression analysis.

RESULTS

Table (1) reveals the basic anthropometric data for the participants. The table shows no significant statistical differences between both groups with respect to age, male ratios, and body mass index (BMI).

Table (1): the baseline characteristics of the participants.

Parameter	*Non-anemic group (n=53)*	*Anemic group (n=55)*	*All participants (n=108)*	*P value*
Age (years)	54.22 ± 12.61	57.67 ± 13.44	55.21 ± 12.46	0.305
Male ratio (%), n	45.2 (n=24)	35.5 (n=19)	39.8 (n=43)	0.254
BMI (Kg/m2)	26.2 ± 3.1	28.1 ± 4.3	27.94 ± 4.3	0.0183
Hb level (g/dl)	13.22 ± 2.2	10.8 ± 1.9	10.99 ± 2.49	< 0.0001*
NT-proBNP (ng/l)	93.55 ± 17.4	268.7 ± 38.9	142.09 ± 66.96	< 0.0001*
Comorbidities				
- AF	2	1	3	
- Septic shock	1	0	1	
- Pulmonary embolism	2	1	3	
- Systemic hypertension	9	5	14	
- LVH	4	2	6	
- Pulmonary hypertension	1	0	1	
	0	1	1	
EF (%)	45.88 ± 4.07	44.67 ± 3.22	44.89 ± 4.02	0.118

* Highly significant statistical difference between at *p* value of ≤ 0.05.
All data are expressed as mean ± standard deviation unless mentioned otherwise.
BMI = Body mass index, Hb = hemoglobin, AF = atrial fibrillation, LVH=left ventricular hypertrophy, EF = ejection fraction.

The mean age of all participants was (55.21 ± 12.46) years and their Hb concentration was (10.99 ± 2.49) g/dl. Subjecting both, the age and Hb level for linear regression analysis showed that there was no significant correlation between both variables where the slope was - 0.013 (95% CI, 0.19 to 0.22), *r2* = 0.001, as shown in figure (1).

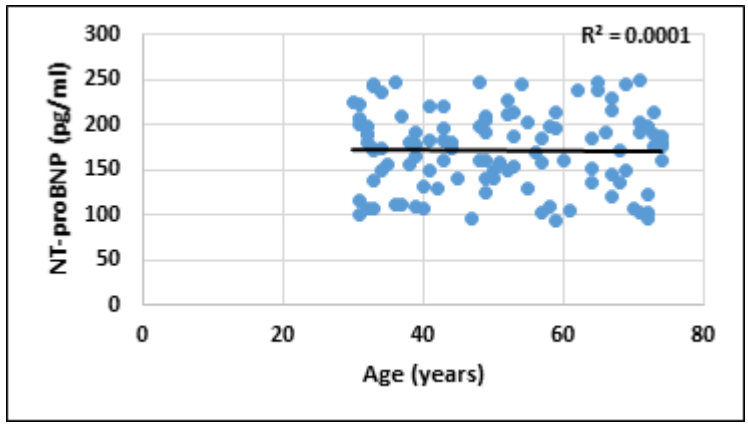

Figure 1: the regression analysis between the age of participants and their hemoglobin level. r2 =0.001. The regression line is depicted by a solid dark line.

The results also revealed that there was no correlation between the age of patients and their NT-proBNP level were the regression analysis [F (1, 99) = 0.0012, *p*=.972] and *r2*= 0.025, as shown in figure (2).

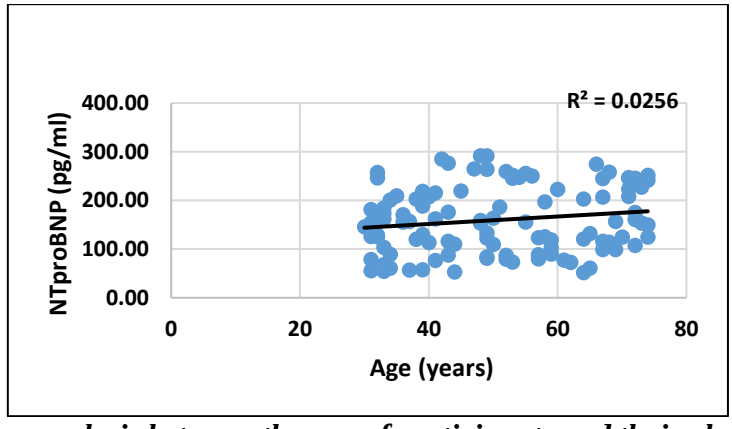

Figure 2: the regression analysis between the age of participants and their plasma NT-proBNP level. r2 = .0256. The regression line is depicted by a solid dark line.

The hemoglobin level of all patients was (10.99 ±2.5) g/dl, range (7.2-15.8). There was a significant statistical difference between both groups in respect to their NT-proBNP levels where anemic patients revealed a statistically significant higher NT-proBNP when compared to the non-anemic patients (268.7 ± 38.9 vs. 93.55 ± 17.4) ng/dl for both groups respectively. The linear regression analysis between the hemoglobin level and plasma NT-proBNP revealed an overall statistically significant regression [F (1, 107) = 28.63, $p < .0001$)], with an *r2* of 0.358, as shown in figure (3).

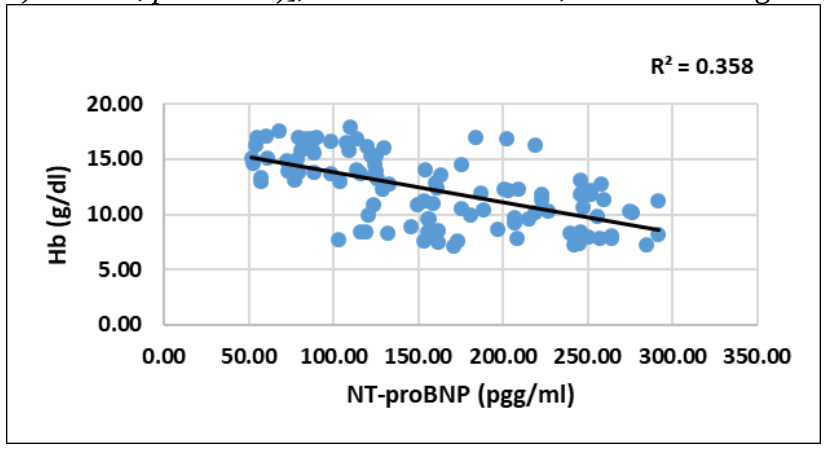

Figure 3: the negative correlation between the hemoglobin level and the plasma NT-proBNP level. r2 = 0.358. The trend line is shown as a solid dark line.

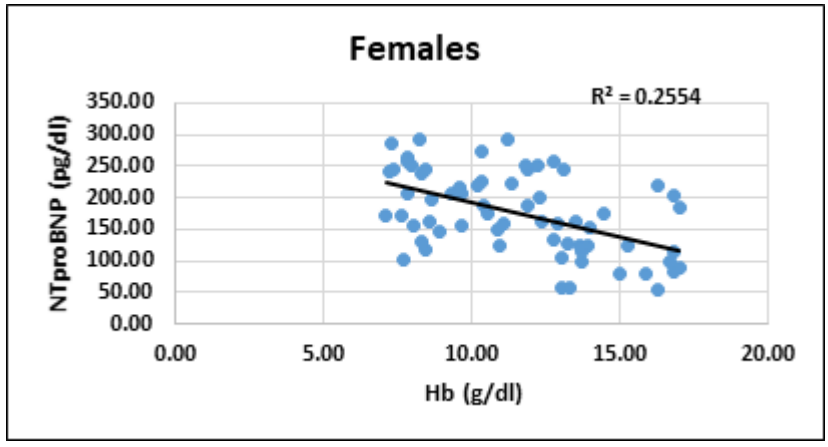

Figure 4: the result of regression analysis between hemoglobin level and plasma NT-proBNP level in anemic females. r2 =0.055.

In order to rule out the effects of other confounding factors on the raised NT-proBNP level, the study conducted a multiple linear regression analysis. The following variables were adjusted: age, gender, comorbidities. The regression analysis conducted after adjusting for the other variables revealed that only systemic hypertension, hemoglobin level, and NT-proBNP were significantly associated. After excluding the 10 patients with systemic hypertension, still, there was a significant statistical correlation between the 2 variables, namely, the NT-proBNP and hemoglobin [F (1, 97) = 0.813, $p < .0001$)] with an $r2$ =0.384.

From the physiological point of view, there is a gender variation in Hb level between males and females with females having relatively lower values compared to males. Therefore, the impact of reduced Hb level on the plasma NT-proBNP was investigated independently. Females registered significantly lower values of hemoglobin concentration (11.54 ± 1.98 vs. 13.18 ± 1.77, 95% CI= 0.471 to 2.82, p =0.006) after independent paired t-test. Running simple linear regression to evaluate the effect of hemoglobin on the peptide level in females resulted in a negative slope ($r2$ = 0.255, 95% CI = 0.121 to 0.261, p <0.001) as shown in figure (4). Meanwhile, the regression analysis returned a more negative regression in males ($r2$ = 0.428, 95% CI = 0.121 to 0.261, p <0.001) as explained in figure (5). Despite the gender variability, nevertheless, both slopes were statistically significant (p <0.001).

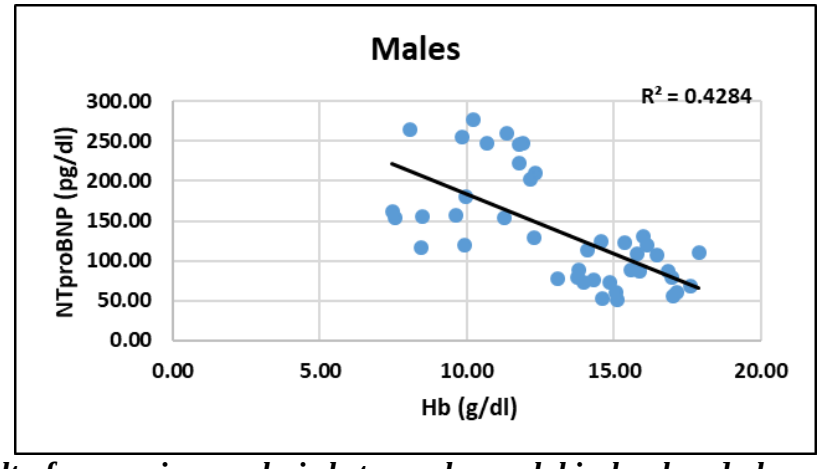

Figure 5: the result of regression analysis between hemoglobin level and plasma NT-proBNP level in anemic males. r2 = 0 .428.

The current study was able to identify different reported conditions for the peptide elevation, as shown in table (1), such as atrial fibrillation (AF), sepsis, pulmonary hypertension, pulmonary embolism, left ventricular hypertrophy (LVH), and systemic hypertension. Other common causes of raised NT-proBNP such as heart failure, renal failure, and acute coronary syndrome (ACS) were already ruled out from the study.

The cardiac contractility, as reported by the EF%, was of no significant statistical difference between both groups (45.88 ± 4.07 vs. 44.67 ± 3.22, $p = 0.118$) for anemic and non-anemic groups respectively.

DISCUSSION

After excluding most of the factors that are associated with raised NT-proBNP level, especially heart failure, the study revealed an association between anemia and elevated peptide level. From the hypothetical point of view, varying degrees of anemias, irrespective to their etiology, may impose a sort of hemodynamic adaptive mechanisms that eventually lead to cardiac stress as it is evident by the elevation of the NT-proBNP level.

There was a negative correlation between both of the aforementioned variables where the lessor the hemoglobin concentration the higher the NT-proBNP level. This inverse relationship couldn't be explained to the age of the patients, at least in the context of this study since the linear regression analysis has ruled out the impact of age on the raised NT-proBNP (Fig. 1).

These findings are consistent to those reported similar studies [11]. For instance, B Tirmenstajn-Jankovic *et. al*. reported that anemia has associated independently with the raised NT-proBNP level in their study population and regarded anemia as a confounding factor [12]. On the other hand, a study found that after examining patients with heart failure, there was an inverse relationship between both BNP and Hb level. Despite the fact that it is expected to find an elevation in the BNP level, nevertheless, their reported value for correlation coefficient (*r*) between both variables was close to that reported by this study (0.41 vs 0.39) [13].

It is imperative to recognize the effect of reduced Hb level on the NT-proBNP marker since the latter is being increasingly used as a tool for diagnosing and managing patients with heart failure.

Anemia may causes the patient to complain from non-specific symptoms like unexplained fatigue, poor exercise tolerance, dyspnea upon exertion. Thus, anemia may produces a clinical picture that has the same symptomatology of that of heart failure.

Since the natriuretic peptides are used as a tool for aiding the diagnosis of heart failure, it is essential to understand the effect of anemia on the plasma concentrations of these natriuretic peptides.

Several studies have reported variable results for anemia prevalence in heart failure population. It may reaches 30% in stable patients with heart failure and up to 50% in those with decompensated heart failure [8]. In another study, it was 41.9% [14]. Anemia seems to deteriorate the clinical outcomes in patients with heart failure. Et al. found that anemia is associated with worsening functional class, more enhanced symptoms, and increased morbidity and mortality. Also, alterations in hemoglobin levels of HF patients modified the course of their disease [15].

The etiology of anemia in heart failure patients is not clear and may be due to several interacting factors. Heart failure may induces activation of proinflammatory cytokines like tumor necrosis factor, neurohormonal changes, and altered renal function that lead to a defect in iron metabolism, resistance to erythropoietin action, and depressed bone marrow function yielding anemia of chronic disease [16]. The hemodilutional effect of water retention, secondary to heart failure, may add to the pathogenesis of anemia in heart failure. Additionally, the renal affection as a frequent comorbid condition that accompanies heart failure will produces less erythropoietin hormone [17].

Anemia per se can be an aggravating factor in heart failure. It is well known that anemia results in reduced blood viscosity, nitric oxide mediated systemic vasodilation in response to the reduced oxygen content of the blood, increased heart rate, and reduced resistance to blood flow. All these factors will increase the cardiac output causing an increased myocardial oxygen demand and myocardial hypertrophy.

Left ventricular hypertrophy (LVH) and chronic heart failure are well-known causes for the raised natriuretic peptide. The finding of elevated NT-proBNP levels in the scope of this study can't be attributed to the LVH or HF since such cases were already excluded beforehand.

The elevated NT-proBNP is caused by many other factors apart from the failing heart. These factors include obesity [18], respiratory distress, pulmonary embolism, intracranial hemorrhage, and renal insufficiency. [19] In the same manner, the raised peptide level can't be attributed to previously mentioned factors since those were scrutinized for their effect using multiple linear regression analysis

Valvular heart diseases augment the level of NT-proBNP in patients with HF upon comparison to those with no valvular pathology. The current study wasn't able to identify such a correlation between both variables since it included only one patient with a mitral regurgitation. Other patients were already ruled out while recruiting. Therefore, it is safe to conclude that valvular pathologies didn't participate significantly to the elevated peptide level [20].

The current study included several limitations. Heart failure with preserved ejection fraction (HFpEF) is associated with high natriuretic peptide titer [21]. While this study excluded patients with overt HF based on their medical records, it can't tell for sure that none of the recruited patients has an occult HF. Even when ejection fraction (EF %) was calculated for each participant, yet the possibility of HFpEF remains questionable. The study neither classified anemia based upon etiology nor it took the duration of anemia in account. It is possible that different types of anemias have different impact on the NT-proBNP level.

CONCLUSION

The study concluded that in patients with no HF, anemia is inversely correlated with the plasma NT-proBNP level. In fact, anemia can raise the peptide level beyond its recommended cutoff levels making the interpretation of the raised NT-proBNP more challenging.

Data access: Data supporting this study cannot be made available due to medico legal obligation from the hospital and the personal desire of the participants not to share their information.

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Conflict of Interest Statement:

The study declares that there is no conflict of interest.

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