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BNP FRAGMENT (8-29) LEVEL AND ANGIOTENSIN CONVERTING ENZYME POLYMORPHISM IN HEART FAILURE PATIENTS IN RELATIONSHIP WITH BODY MASS INDEX

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Abstract

Though increased BMI represents a risk factor for developing heart failure, in heart failure (HF) patients it paradoxically increases survival. This effect was not studied in relationship with BNP and ACE mutations. **Objective.** To investigate the relationship between BNP fragment (8-29) plasmatic level, ACE I/D polymorphism and BMI in patients with chronic congestive heart failure. **Methods.** We studied 50 patients with HF, NYHA III and IV, mean age 64.96±13.24 years, 21 patients with BMI ≥30 kg/m^2 vs 29 patients with BMI<30 kg/m^2. BMI, ACE polymorphism, the plasmatic levels of BNP fragment (8-29) were determined. **Results.** Mean plasmatic BNP fragment (8-29) level was 1891.02±1008.06 pg/mL, mean BMI value 29.09±7.59 kg/m^2, with a negative correlation between the two parameters (r=-0.46), stronger in women (r=-0.79 vs. r=-0.32 in men). Significant greater value of BNP fragment (8-29) was registered in non-obese patients (2130.77±866.58 pg/mL vs. 1547.765±1135.744pg/mL, p<0.05). The distribution of ACE mutation was: DD allele 36%, II allele 24%, and ID allele 40%. In all three groups, the patients with BMI ≥ 30kg/m^2 presented lower values of BNP fragment (8-29) levels.

Conclusion. In HF patients, there was an inverse correlation between BNP value and BMI, obese patients having lower BNP fragment (8-29) values, no matter the ACE polymorphism.

Key words: heart failure, obesity, BNP, angiotensin converting enzyme polymorphism.

INTRODUCTION

Obesity has been increasing in epidemic proportions in both adults and children (1). In adults, overweight is defined as a body mass index (BMI) 25 to 29.9 kg/m^2 and obesity as BMI ≥ 30 kg/m^2 (2). Obesity is a well-known risk factor for the development of heart

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failure (HF), but, surprisingly, obese patients with heart failure have a better prognosis than patients whose weight is normal, giving rise to the so-called "obesity paradox". Recent reports suggest that obesity also affects BNP levels, with lower circulating levels registered in those with a higher BMI (3-6). Horwich *et al.* found that obesity was associated with an increase >6-fold in the odds of having low BNP values (7). Taylor and colleagues demonstrated that this reduction in NT-proBNP levels in obesity occurred despite heavier patients having higher filling pressures than those at lower weights (8).

On the other hand, human adipose tissue expresses all components necessary for the local production of angiotensin II, which has multiple functions in adipose tissue, ranging from regulation of local blood flow to complex influences on tissue homeostasis (9). Angiotensin converting enzyme (ACE) as well as other components of the renin-angiotensin pathway have been shown to be expressed in human adipose tissue and adipocytes (10,11) and obesity and hypertension may influence the expression of these components (12). Rigat *et al.* identified a genetic polymorphism inside intron 16, consisting of the presence or absence of a fragment formed of 287 aminoacid pairs (13). The presence of this fragment defines the allele I (insertion), while its absence defines the allele D (deletion). Depending on the mode the two alleles combine, genotype EC is characterized by three types: II, DD and ID (13,14). EC serum level depends on I/D ACE gene polymorphism, higher titers being found in the DD form, which also has the highest cellular activity (13).

Objective. Even if the relationship between body mass index and NT-proBNP has already been evaluated, to our best knowledge, the one with ACE I/D polymorphism remains unclear. Thus, the purpose of the present study was to investigate, in heart failure patients, the association between the ACE I/D polymorphism- obesity- BNP fragment (8-29) level.

MATERIAL AND METHODS

Studied groups. For the inclusion into present study, all patients with heart failure and assessment of BNP fragment (8-29) level during initial presentation were selected. We performed a prospective study that included 50 patients (investigated between January - December 2008), aged 64.96±13.24 years, 68% men, and 32 % women, with chronic heart failure (defined according to the European guidelines, normal BNP value being exclusion criteria), functional class NYHA III and IV. These patients were separated according to body mass index (BMI) in two groups: group I BMI<30 kg/m^2 (29 patients) and group 2 BMI >30 kg/m^2 (21 patients).

Methods. Blood for the peptide assay was drawn into 5-mL potassium EDTA tubes and then kept at room temperature. Within 3 h, the tubes were centrifuged, and plasma was removed before being processed. If samples could not be evaluated on the same day, they were kept at -70°C. Before analysis,

every tube was inverted several times to ensure homogeneity. Samples were then analyzed in duplicate. The test is a high-specificity competitive immunoassay (Biomedica) using an immunoaffinity-purified sheep antibody specific for NT-proBNP (amino acids 8–29) immobilized to the surface of microtiter plate wells, with horseradish peroxidase as tracer. We perform the ELISA on an automated system. ProBNP is primarily synthesized in the ventricle and released into the circulation upon left ventricular stretch or wall tension. Circulating plasma forms are the amino-terminal portions of BNP fragment and NT-proBNP and BNP. BNP fragment (8-29) and NT-proBNP has longer half life than BNP.

The distribution of ACE gene insertion and deletion (I/D) was determined in all patients. Genomic DNA was extracted by a non-enzymatic method. DNA fragments were amplified by polymerase chain reaction (PCR). Genotyping for the insertion (I)/deletion (D) of the 287 bp in the ACE gene described by Evans in 2003 with minor modifications was performed in an Eppendorf thermocycler. The primers used had the following sequences: the forward primer 5'-CATCCTTTCTCCCATTTCTC -3' and the reverse primer 5'- TGGGATTACAGGCGTGATACAG-3'. The PCR conditions were: 1X PCR buffer (100mM tris- HCl, pH 8.8, 500 mM KCl 0.8% (v/v) Nonidet P40), 20ng genomic DNA, 2.0mM $MgCl_2$, 200µM dNTPs, 0.2 µM each primer, 2U Taq DNA polymerase. The PCR program was: denaturation at 95°C for 10 min, followed by 35 cycles of amplification at 94°C for 30 sec, 69°C for 30 sec, 72°C for 1 min 30 sec and a final extension step at 72°C for 2 min. The product had 290bp and the deletion formed a product of 100bp.

The local institutional Ethics Committee approved the study and all participants gave their written informed consent.

Statistical analyses

The data were analyzed using SPSS 16.0 (Demo Version). Descriptive analysis was used to evaluate the demographic and clinical characteristics of the patients. We calculated the mean and standard deviation for normally distributed quantitative variables. Differences between quantitative variables were examined using the Student test and for qualitative variables we used the chi ^{2} test. We calculated correlation coefficients in order to evaluate the relationship between variables. A p value of less than 0.05 was considered statistically significant.

RESULTS

Baseline characteristics of the patients are shown in Table 1. Plasma levels of BNP fragment (8-29) differed significantly between the matched BMI groups. Mean plasmatic BNP fragment (8-29) level was 1891.02 ± 1008.06 pg/mL and BMI 29.09 ± 7.59 kg/m² with a strong negative correlation between the two parameters (r = -0.46), relation registered in both normal weight (r= -0.67) and obese (r= -0.51) patients. The correlation was stronger in women (r= -0.79) than in men (r= -0.32). This results in a significant higher plasmatic level of BNP fragment (8-29)

Table 1. Baseline characteristics of the patients

Characteristics	
Age (yrs)	64.96 ±13.24
Male - No (%)	34 (68)
Diabetes - No (%)	10 (20)
Hypertension - No (%)	29 (58)
Smoking history - No (%)	8 (16)
Total cholesterol (mg/dL)	162.36 ± 38.34
LDL- cholesterol (mg/dL)	104.88 ± 28.16
HDL- cholesterol (mg/dL)	35.68 ± 9.57
Triglyceride (mg/dL)	109.12 ± 56.41
LVEF (%)	42.26 ± 8.69
LVEDD (mm)	64 ± 10.88
LVESD (mm)	49.92 ± 13.36

Values are presented as percentage (qualitative variables) and as mean ± standard deviation for numerical, normally distributed variables. Legend: LVEF - left ventricular ejection fraction, LVEDD - left ventricular end diastolic diameter, LVESD left ventricular end systolic diameter.

in non-obese (2130.77 ± 866.58 pg/mL) in comparison with obese (1547.765 ± 1135.744 pg/ml) patients ($p<0.05$).

The distribution of ACE mutation was as follows: DD allele - 36% (18p), II allele - 24% (12p), and ID allele - 40% (20p). No significant differences regarding the BNP fragment (8-29) levels were found between obese and non obese patients in DD and ID groups, the only exception being registered in II group ($p<0.0001$) (Table 2). But in all three groups (as per global), the patients with BMI greater than 30 kg/m^2 presented lower values of BNP fragment (8-29) levels (Table 2).

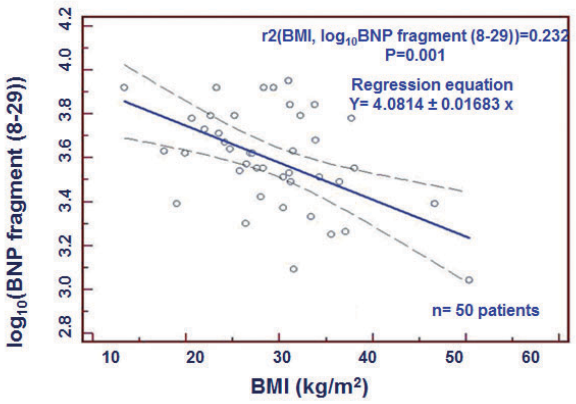

Figure 1. Correlation analysis of log10 (BNP fragment (8-29) LEVEL) vs. body mass index (BMI) as a continuous measure. Circles represent individual values of N-terminal pro-brain natriuretic peptide. The black line represents the linear regression curve fit and the dotted line its 95% confidence interval.

Table 2. BNP fragment (8-29) values according to I/D polymorphism and body mass index.

ACE I/D polymorphism	BNP (8-29) fragment level (pg/mL)			
	All patients (50 patients)	BMI<30 kg/m^2 (29 patients)	BMI≥30 kg/m^2 (21 patients)	p
DD allele (18 patients)	1743.98 1133.99	1951.25 119.04	1578.16 1247.13	0.114
II allele (12 patients)	2047.43 841.03	2408.13 811.23	1326.04 33.88	<0.0001
ID allele (20 patients)	2124.17 1064.99	2135.417 777.11	2108.64 1471.45	0.93

The results are presented as mean ± standard deviation

DISCUSSION

ACE polymorphisms have become a target for many cardiovascular studies due to the importance of ACE in the formation and degradation of vasoactive substances (14-18). ACE as well as other components of the renin-angiotensin pathway have been shown to be expressed in human adipose tissue and adipocytes (19, 20) and obesity and hypertension may influence the expression of these components (21, 22). ACE is only present in the stromal-vascular fraction of rat adipose tissue (22), whereas it is expressed in both preadipocytes and adipocytes in humans (9). The renin-angiotensin system may in turn influence the conversion of preadipocytes to adipocytes (23). Furthermore, studies have shown that there may be a connection between obesity and the DD genotype (24, 25). There was described a connection between obesity and ACE DD genotype, increased ATII level promoting obesity through conversion of preadipocytes to adipocytes (23).

In fact, increased ATII level in HF patients represents a factor of worsening prognosis and then DD polymorphism (which increases ATII level) could be associated with increased BNP. Recent evidence suggests that there may be increased clearance of circulating BNP in obesity. Natriuretic peptide clearance receptors are abundant on human adipocytes (26). Additionally, the vascularity of adipose tissue may allow for increased degradation of BNP by neutral endopeptidase (27). More recently, O-glycosylation of NT-proBNP has been shown (28). Conversely, it is also possible that overweight and obesity are associated with less robust synthesis and/or release of BNP from the myocardium. Decreased N terminal pro-BNP and pro-atrial natriuretic peptide have been observed in obesity (29,30), which may be suggestive of decreased natriuretic peptide production. Alternatively, decreased circulating BNP levels may not be a consequence of increased body weight or excess adiposity, but rather they may be a causative factor in the genotype or phenotype that leads to development of obesity. Both ANP and BNP are now recognized to be involved in fat metabolism as stimulators of lipolysis in adipose tissue (31).

In our study the BNP values were significantly high in the obese heart failure patients comparatively with non-obese ones. Horwich *et al.* reported that, although BNP levels are relatively lower in overweight and obese HF patients, BNP predicts worse symptoms, impaired hemodynamics,

and higher mortality at all levels of BMI (7). In the study of Frankenstein *et al.* the authors concluded that even if matched for NYHA, age, sex, and renal function, BMI exerts a significant and independent inverse influence on NT-proBNP in patients with HF.

NT-proBNP retained equal statistical power in all three BMI groups (32). Similarly, Hermann-Arnhof *et al.* found in the obese group, NT-proBNP concentrations statistically comparable to those in NYHA I heart failure patients (33). This will result through obesity in a protective effect upon HF patients (34).

In our study the incidence of genetic mutations in the patients with HF was: DD allele- 36% (18 patients), II allele - 24% (12 patients), and ID allele- 40% (20 patients). The incidence reported by McNamara *et al.* is somewhat different: I-18.6%; ID-50.7%, and DD-30.7%; the differences may be accounted for by the relatively small number of patients in our study (35). McNamara emphasizes that the DD mutation is present in one third of the population with high ACE levels (35).

Regarding genetic mutation ACE I/D, the presence of pathogen genotype DD (which is connected with the most elevated levels of ACE), does not influence BNP-fragment levels (8-29), irrespective of BMI values. There are no studies in literature about this fact, although there is a connection described between genotype DD and obesity, as we already stated above (7,36). Wacker *et al.* found between I/D polymorphism and obesity a significant association but only in men. It is possible that hormonal differences between sexes may alter the influence that the ACE I/D polymorphism has on weight gain (36).

CONCLUSION

In heart failure patients, there is an inverse correlation between BNP fragment plasmatic value and BMI, obese patients having the lowest BNP values no matter of the ACE polymorphism, but statistically significant only in that the relationship is not influenced by ACE polymorphism II group .

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