RAAS ANTAGONISTS TREATMENT AND ANGIOTENSINOGEN M235T AND T174M GENE POLYMORPHISMS IN HEART FAILURE PATIENTS

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Abstract: Two molecular variants of the angiotensinogen (AGT) gene, M235T and T174M, have been linked to an increased activity of the renin angiotensin aldosterone system (RAAS). Brain natriuretic peptide (BNP) is a severity marker for HF (HF). Objectives: We analyzed the association between the presence of these genotypes and the BNP fragment (8-29) level, and also studied the prescription rates of RAAS antagonists according to these mutations. Methods: There were analyzed 42 HF patients NYHA III- IV class, aged 65.76±76 years. Results: The mean value of serum BNP fragment was 2991.24±2034.61 fmol/ml. RAAS antagonists were prescribed as follows: ACE inhibitors in 71.42% of the patients; ARBS in 16.66% of the patients and aldosterone antagonists in 88.09% of the patients. Conclusion: We found no direct correlation between the AGT gene polymorphisms and BNP fragment level in our HF patients. RAAS antagonists are indicated in all HF patients, regardless the mutation present.

Cuvinte cheie: *polimorfism genetic, insuficienta cardiaca*

Rezumat: *Două variante moleculare ale genei angiotensinogenului (AGT) M235Tşi T174M au fost asociate cu o activitate crescută a sistemului renină-angiotensină aldosteron (RAAS). BNP reprezintă un important marker al severităţii insuficienţei cardiace (IC). Obiective: Am analizat asocierea dintre prezenţa acestor genotipuri şi nivelul fragmentului BNP (8-29), precum şi rata de prescripţie a antagoniştilor SRAA, în funcţie de prezenţa acestor mutaţii. Material şi metodă: S-au luat în studiu 42 de pacienţi cu IC, clasa NYHA III-IV, cu vârsta medie 65.76±6.29. Rezultate: Valoarea medie a BNP a fost 2991.24±2034.61 fmol/ml. Antagoniştii SRAA au fost prescrişi după cum urmează: IECA la 71.42%, sartani la 16.66% şi antagonişti aldosteronici la 88.09% dintre pacienţi. Concluzie: Nu am găsit o corelaţie directă între polimorfismul genetic al AGT şi nivelul BNP în cadrul lotului studiat. Tratamentul cu inhibitori ai SRAA ar trebui să reprezinte o terapie de elecţie chiar dacă este identificată o singură formă a polimorfismului genetic.*

BACKGROUND

The gene encoding angiotensinogen (AGT) has been implicated in the pathogenesis of heart failure (HF) both through genetic linkage studies and by allelic association. Human AGT is **encoded** by **AGT gene** (1q42-q43). AGT gene is expressed in proximal **tubule** cells of the kidney. **AGT** consist of **453 amino acid** residues. It contains two potential sites for ***N***-linked glycosylation (ASN-X / THR-X Asn-X-Ser/Thr-X, amino acid positions 295 and 319). The glycosilation takes place in the liver. The glycosilated form of angiotensinogen is cleaved by renin to inactive decapeptide Angiotensin I (AT I), which is cleaved by ACE (kynase II) to active octapeptide vasopressor Angiotensin II (AT II). There are two molecular variants of the angiotensinogen gene, M235T and T174M, one encoding threonine (T) instead of methionine (M) at position 235 (M235T) and the other encoding methionine instead of threonine at position 174 (T174M) (2,4) .

A mutation in exon 2 of the gene AGT M235T has been associated with elevated levels of AGT, with 235TT homozygotes having between 10% and 20% more plasma AGT than 235MM individuals (1,2,3,4,5).

Studies demonstrated that AGT variants M235T and T174M were independently predictive of moratlity in HF patients, and also, their combined presence in HF patients doubles the mortality rates, and is associated with an increased risk for HF in these individuals, in comparison with those having only one mutation. In a recent study, on ICC patients, patients with AGT M235T genotypes were considered, according to the AGT polymorphism, in 2 separate groups: combinations of M235T and T174M genotypes (235TT and/or a 174M allele) were combined into a single "high-risk population" group and compared with the remaining "low-risk population" genotype combinations (AGT 235MM or MT combined with 174TT) (6).

The study concluded that these high-risk combinations predicted mortality independent of established risk factors, including age, gender, ethnicity, previous history of HF or myocardial infarction (MI), LVEF, renal dysfunction, ß-blocker and spironolactone treatment, and plasma N-BNP or AT II levels. The two mutations combined, also conferred a significantly greater risk than either genotype alone (6). It was estimated that nearly 25% of deaths in HF subjects could be attributed to the AGT M235T and T174M polymorphisms, demonstrating that the AGT genotypes have a significant impact of on mortality in HF (5,6). Also, in patients with an AGT 235TT genotype who had recognized hypertension before the

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onset of HF, the age of diagnosis of hypertension was approximately 10 years earlier than the rest of the population (5,6). These patients were significantly younger when admitted to hospital with HF, suggesing that the AGT 235TT genotype might accelerate the development of hypertension (5,6). The investigators suggest that these individuals should be treated early for hypertension to delay or prevent the onset of HF (4,6,7).

In some studies, plasma levels of AGT have been shown to be 10% to 20% higher in subjects with the M235T variant, although no change was found in plasma AGT in association with the T174M variant (8,9,10). Tissue AGT levels have not been investigated in association with M235T or T174M genotypes, individually or in combination. There is a possibility that AGT M235T and T174M variants may increase the level of activation of the RAAS within the heart and kidney, and by this promoting cardiac remodeling and renal impairment and, consequently, influencing the outcome in HF (8).

Studies are trying to identify the cardiac signaling pathways altered in association with these AGT gene variants, to explain the mechanism underlying the association between the "high-risk" AGT genotype combinations and increased mortality in HF, and also to determine their clinical use as prognostic markers (8).

It has been proved that these molecular variants have an increased prevalence in the European population (33%), and they may have clinical relevance in the setting of HF (6).

OBJECTIVES

The purpose of our study was to determine whether there is an association between the presence of the M235T and T174M genotypes independently and the BNP fragment (8-29) level, and also, the combined influence of those molecular variants on the level of BNP fragment (8-29), considered an important diagnostic and prognostic marker in HF patients. We also evaluated the prescription rates of renin–angiotensin (RAAS) antagonists according to the presence of AGT mutations.

MATERIAL AND METHODS

There were analyzed 42 HF patients, included in NYHA III- IV functional class and 22 subjects without cardiovascular disease (controls). The diagnosis of HF was established in all patients according to the ESC Guidelines for Diagnosis and Treatment of Acute and Chronic HF, 2008.

Differences between quantitative variables were examined using Student test (independent-sample T test), and for qualitative variables we used χ^2 test. A p value less than 0.05 was considered significant from statistical point of view.

RESULTS

The main characteristics of patients included in the study were as shown in Table1. Genotype combination frequencies for the AGT M235T and T174M polymorphism were as shown in Table 2.

In controls, genotype frequencies for the M235T polymorphism were as follows: homozygote TT-2 subjects (9.09%), heterozygote TM-4 subjects (18.18%) and for the T174M polymorphysm: heterozygote TM 2 subjects (9.09%), and homozygote MM-0 subjects. There were no pathogen mutation combinations found.

The average value of serum BNP fragment levels was 2991.244 ±2034.611 fmol/ml for the entire group.

When considering the genotype combination of the AGT M235T and T174M polymorphisms, the serum level of BNP fragment, was surprisingly higher for the for the negative MM-negative TT combination (4427.25±2669.95fmol/ml), in comparison with the heterozygote MT- heterozygote TM combination (1600.33±893.77fmol/ml), the heterozygote MT-negative TT combination (3417.143±2170 fmol/ml), the homozygote TT- heterozygote TM combination (2088.33± 1252.94 fmol/ml) and the homozygote TT- negative TT combination (3177.5 ± 2490fmol/ml) - $p<0.05$.

Table no. 1.The main characteristics of the patients included in the study

Number of patients	42
Gender (males%)	61.90
Glycemia (mg/dl)	98.16±27.39
Cholesterol (mg/dl)	162.36±38.34
LDL (mg/dl)	104.88±28.16
HDL (mg/dl)	35.68±9.57
TG (mg/dl)	109.12±56.41
HTA %	57.69
DM %	19.23
FE %	42.28±8.69
LVESD (mm)	49.92±13.36
LVEDD (mm)	64±10.88
NYHA III class (%)	57.69
NYHA IV class (%)	42.31
BNP fragment (8-29) (fmol/ml)	2815.488±2031.26
Beta blockers %	69.04
ACE inhibitors %	71.42
ARBS %	16.66
Aldosterone antagonists %	88.09
Nitrates %	73.80
Diuretics %	95.23
Antiplatlets %	61.90
Anticoagulants %	61.90
Calcium blockers %	9.5

Table no. 2. Genotype combination frequencies for the AGT M235T and T174M polymorphism

M235T+T174M	**MT-M235T* TM-T174M***	**MT-M235T* TT-T174M****	**TT-M235T*** TM- T174M***	**TT-M235T*** TT-T174M****	**MM-M235T** TT-T174M****
Frequencies	10p (20%)	13p (26%)	7p (14%)	10p (20%)	10p (20%)

We also analyzed each genotype separately, and we obtained the same results. Serum BNP levels are significantly higher in patients negative for AGT M235T mutation- negative MM (4427.25±2669.95fmol/ml), in comparison with the homozygote TT patients (2677.1429±2062.625fmol/ml), and the heterozygote MT patients (2285.62±1448.459fmol/ml)- $p<0.05$. In regard to the T174M genotype, we found a significantly higher value of the BNP fragment in negative TT patients

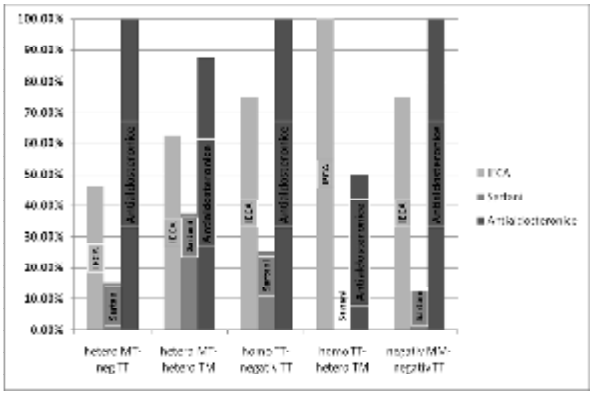
(3348.3793±2190.70fmol/ml), then in heterozygote TM patients (1764.2±1007.1868fmol/ml)- $p<0.05$.

The treatment prescribed to HF patients, including RAAS antagonists (ACEI, ARB's and aldosterone antagonists), but also beta blockers, loop diuretics and nitrates is represented in Table1.

The prescription rates of renin–angiotensin (RAAS) antagonists according to the presence of AGT genetic polymorphism M235 was as follows: heterozygotes- ACEIs 38.48%, ARBs- 17.24%, aldosterone antagonists- 68.96%; homozygotes: ACEIs- 85.71%, ARBs- 14.2%, aldosterone antagonists- 85.79%. In heterozygotes patients for the AGT 174 mutation the prescription rate for RAAS antagonists was: ACEIs-73.33%, ARBs- 20%, aldosterone antagonists- 80%.

The prescription rate of RAAS antagonists according to the presence of the genetic mutations combination is presented in Chart 1. Aldosterone antagonists were more frequently prescribed, exept for the homozygote group TT M235-heterozygote T174, followed by ACEIs and ARBs.

Figure no 1. The prescription rates of renin–angiotensin (RAAS) antagonists according to the presence of AGT mutations



DISCUSSION

The predisposition for cardiovascular diseases is given by the cumulative effects of genetic polymorphisms, modulated by external risk factors (3,4). These genetic mutations can also have an influence on therapeutic response (medication and diet changes) in patients already diagnosed with cardiovascular diseases. Genetic polymorphisms in the renin-angiotensin-aldosterone system (angiotensinogen, ACE, AT1 and AT2 receptors of angiotensin II) are of great importance, because of its role in modulating cardiovascular hemodynamic. There were described genetic polymorphisms for angiotensinogen, ACE, AT1, AT2 and AGII receptors, renin, and recently angiotensin converting enzyme type 2 (EC2) (1,2,3,4).

Plasmatic changes in angiotensinogen levels, and aslo its structural variety, have an influence on AGT synthesis (5,11,12,13). The positive correlation between plasmatic AGT and high blood pressure have been proved by studies that observed the interrelation between DNA in, or near the angiotensinogen locus and BP. These facts were also demonstrated by the NHLBI Family Heart Study, in 1997 (14). The impact of the angiotensinogen gene (AGT) M235T polymorphism on plasma AGT levels and essential arterial hypertension has been reported (14).

A recent study at Rehabilitation Hospital Cluj-Napoca analyzed the M235T polymorphism in AGT gene in Romanian patients with essential hypertension as well as controls. In the study groups, the M235T variant was found more frequently in hypertensive patients (81,57%), than in control subjects (66,66%). There were identified 52,63% M235T heterozygotes and 28,94% T235T homozygotes in the hypertensive group (15, 16). In our study, M235 mutation was present in 76% of the patients (32% heterozygotes and 44% homozygotes). The results of this study, and the ones mentioned before suggest a strong association of the M235T polymorphism in the gene encoding AGT with essential hypertension (15, 16, 17,18,19,20,21,22).

The allele frequency of the AGT M235T polymorphism for the general population was reported by the latest studies to be 34% MM, 47% MT and 19% for TT. In our study we obtained the following results: 67.44 % MM, 42.42 % MT and 30.30% for TT. It has also been described a two fold increase in mortality in HF patients having this mutation (6).

A second variant within the AGT gene, T174M, has also been associated with hypertension, with the M allele associated with an increased risk HTA (8,23).

In the study conducted by Pilbrow et al the genotype frequencies for the T174M polymorphism were 79% TT, 20% TM and 1% MM. The 174M allele was associated with increased mortality independent of established prognostic indicators such as smoking, hypertension, DM type 2, high levels of AGII, creatinine or cholesterol (6).

In our study, the heterozygote form was present in 34.88% of the patients. We had no homozygote patients for the MM allele.

Previous studies concluded also that these high-risk combinations (M235T şi T174M) predicted mortality in HF patients, independent of other risk factors (6).

We found no direct correlation between the angiotensinogen M235T and T174M gene polymorphisms and BNP fragment serum level in our HF patients, the two molecular variants of the angiotensinogen gene, individually, and also in combination being paradoxically associated with decreased serum levels of BNP fragment.

The Pilbrow study, found no correlation between the plasmatic N-BNP level and any of the combination of the two AGT gene variants. Also there was no direct association between the BNP level and the M235T and T174M variants independently (6).

With regard to the RAAS antagonists, the authors consider that these should be administered to all "high risk" patients (the 235TT allele si ≥ 1 174M alleles). All the patients included in the study received ACEIs and spironolactone (70.8% ACEIs and 9% spironolactone in "high risk" group, and 70.4% ACEIs and 10.7% spironolactone in "low risk" group) (6,23, 24, 25). On the other hand, none of the patients received ARB's. In our study, most of the patients received aldosterone antagonists, except the homozygote TT M235T-heterozygote T174M group, followed by ACEIs and ARB's.

In our study, all patients received RAAS antagonists: 71.42% ACEIs, 16.66% ARB's and 88.09% aldosterone antagonists.

However, our study is limited in terms of the small sample of subjects. Further and more extensive studies are required to clarify whether there is a association between T174M and M235T gene polymorphisms, BNP levels and the treatment with ACEI, ARB's and antialdosteronic diuretics.

CONCLUSIONS

We can say that the association of the two genetic mutations was detected in less then 25% of the HF patients. The prescription of RAAS antagonists drugs is mandatory in all HF patients, regardless the presence of the above mentioned pathological genotypes, M235T and T174M and in no relationship with natriuretic peptides level

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