The role of B-type natriuretic peptide in the pathophysiology of severe preeclampsia

Ph.D. Thesis

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I. Introduction

Natriuretic peptides family consists of 5 structurally related molecules: atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), C-type natriuretic peptide (CNP), dendroaspis natriuretic peptide (DNP) and urodilatin. Brain natriuretic peptide (BNP) is a member of natriuretic peptide family. These peptides play an important role in the homeostasis of body fluids.The molecules of this family have a common ring structure, which consists of 17 aminoacids. It is generated by proteolytic cleavage of a pre-propeptide into a non-active N-terminal fragment of 108 aminoacids, which is further cleaved into an active hormone of 32 aminoacids and the N-terminal proBNP. BNP is produced in the cardiac ventricules and regulated by increased wall stress, causes significant decrease in blood pressure. BNP increase glomerular infiltration and natriuresis, supressing sodium reabsorption and relax the vessel's smooth musculature causing a decrease in the cardiac preload and afterload and a decrease in blood arterial pressure. BNP also reduce renin-angiotensin-aldosteron (RAA) activities and inhibit endothelin-1 production.

The BNP gene (Natriuretic Peptid Precursor B gene, NPPB gene) is positioned on the short arm of the human chromosome 1. The BNP gene contains three exons. The 5′-flanking region of the human NPPB gene was subcloned in 1996 to evaluate gene expression. Several single nucleotide polymorhisms and complex mutations were identified, some of which were shown to be associated with cardiovascular physiopathology. Other sites located in relatively distal regions of the human BNP 5′-flanking region, including shear stress-responsive elements, thyroid hormone-responsive element and the nuclear factor of activated T-cells binding site, have also been shown to participate in inducible activation of the human BNP promoter. Kosuge et al. found association between the NPPB gene (TTTC) microsatellite polymorphism and essential hypertension in women.

Preeclampsia is a heterogeneous, multisystem disesase in human pregnancy with a worldwide incidence of 5–7%. It is a pregnancy-related disorder characterized by high blood pressure and proteinuria occuring in the second or third trimester of pregnancy.It is one of the leading causes of maternal and perinatal morbidity and mortality. Even after the delivery patients with a history of preeclampsia are at elevated risk to develop cardiovascular disease in later life. It leads to peripheral tissue damage, including kidney, heart and placenta. The fetus suffers from the complications of preterm delivery andintrauterine growth retardation (IUGR).The subclassification of preeclampsia on the basis of gestational age at disease onset divide early-onset (before 34th gestational week) and late-onset preeclampsia. These two subgroups seem to have different etiologies and they are widely believed as different forms of the disease. Early-onset preeclampsia is associated with placental histopathological differences, intrauterine growth restriction, increased total vascular resistance, hypovolaemia and adverse maternal and neonatal outcomes. Higher proteinuria is associated with early-onset preeclampsia. However late-onset preeclampsia is accompanied with increased body mass index (BMI), normal or slight elevated uterine resistance index and a low rate of intrauterine growth restriction. Despite intensive research efforts, the etiology and pathogenesis of preeclampsia are not completely understood.

The aim of my work was to identify the polymorphism in the 5'-flanking region of the NPPB gene and examine the relationship between the variants of the gene and preeclampsia and the possible connection with plasma BNP levels. This polymorphism has not been studied in healthy and preeclamptic pregnancies previously. Although enhanced levels of BNP were shown in preeclampsia compared with healthy pregnancy no previous study has examined whether BNP levels shows difference between early-onset versus late-onset preeclamptic patients. I studied the relationship between intrauterin growth restriction and plasma BNP levels. I compared also the circulating levels of BNP, and standard clinical chemistry levels in patients with early-onset and late-onset preeclampsia whith those found in healthy pregnant.

II. Objectives

1. We decided to introduce a new F-PCR and DNA fragment analysis method for the fast and reliable detection of the $(TTTC)_n$ microsatellite polymorphism in the 5'-flanking region of the natriuretic peptide precursor B gene.

2. Using this technique I planned to determ microsatellite polymorphism in the in the Natriuretic peptide precursor B gene $(TTTC)_n$ in severe preeclamptic and healthy pregnants.

3. I decided to determine the plasma B-type natriuretic peptide levels in severe preeclamptic and healthy pregnants, introducing a bedside test -using a sandwich fluorescence immunoassay method- in the obstetrical, clinical practice.

I planned to answer the following questions:
4. Are plasma B-type natriuretic peptide levels higher in severe preeclamptic patients compared to healthy pregnants?

5. Are there any relations for the microsatellite polymorphism in the in the Natriuretic peptide precursor B gene $(TTTC)_n$ with plasma B-type natriuretic peptide levels?

6. Is there any difference in the allele or genotype distribution of the Natriuretic peptide precursor B gene $(TTTC)_n$ microsatellite polymorphism in early-onset and late-onset severe preeclamptic patients?

7. Is there any difference in the plasma B-type natriuretic peptide levels in early-onset and late-onset severe preeclamptic patients?

8. Is there any difference in the allele or genotype distribution of the Natriuretic peptide precursor B gene $(TTTC)_n$ microsatellite polymorphism in severe preeclamptic patients in relation to intrauterine growth restriction?

9. Are plasma B-type natriuretic peptide levels higher in severe preeclamptic patients complicated with intrauterine growth restrtiction?

10. Are there any relations between the plasma BNP levels and the clinical features or laboratory parameters of preeclampsia?

11. Which is the best cut-off value for plasma B-type natriuretic peptide concentration discriminate early-onset or late-onset preeclamptic patients from healthy pregnant women?

III. Materials and methods

1. Study population

Healthy control pregnant women (n=235) and patients with severe preeclampsia (n=220) were recruited at the 1st Department of Obstetrics and Gynecology of Semmelweis University. The patients involved in the study delivered from the 30 th June 2006 to 31st December 2010 . The Ethical Committee of the Semmelweis University approved the study, all participants were informed and they agreed to their involvement in the study.

All women with singleton pregnancies who presented to the Ambulatory Care Center were given the opportunity to participate in the study. All medical charts were reviewed to evaluate for the presence of medical conditions complicating pregnancies, with particular attention to chronic hypertension, diabetes, renal disease, and a personal history of preeclampsia. After informed consent was obtained, blood samples were collected in the third trimester. Patients were excluded from the control group, if they developed hypertensive disorder.

Severe preeclampsia was defined as new-onset, persistent hypertension ($\geq$ 160/110 mmHg) and new-onset proteinuria ($\geq$ 5000 mg/24 h) following the 20th gestational week, in the absence of urinary tract infection.

Preeclamptic patients' blood samples were collected at the time of their initial assessment for preeclampsia, before any drugs were administered, or uterine contractions started.

Intrauterin growth retardation (IUGR) was declared with a birthweight below the 10th percentile of that anticipated for gestational age. The customized centile calculator of the Hungarian Obstetrician Association was used to derive the growth centile of each infant in the study. Gestational age was established by a first trimester dating ultrasound scan for all the women.

2. DNA isolation

Three mL of peripheral blood was drawn from each patient into a potassium ethylene diamine tetra-acetic acid (EDTA) tube and stored at 4°C. Genomic DNA was extracted from 0.2 mL of samples using the High Pure PCR Template Isolation kit (Roche, Mannheim, Germany), according to the manufacturer's instructions.

Determination of the (TTTC)$_n$ polymorphism

Fluorescent PCR and DNA fragment analyses were performed using the 5'-6-FAM-AAG GAG GCA CTG GGA GAG GGA AAT-3' forward and 5'-AAT TAG CTG GGC ATG GTG GCA GGCG-3' reverse primer sequences. The PCR mix contained 10 µL of the Qiagen Multiplex PCR mix (Qiagen, Hilden, Germany) and 0.3 µM of primers in a final 20 µL volume. The PCR was performed for 32 cycles of 30 s at 95 ºC, 30 s at 60 ºC and 30 s at 72 ºC with an initial denaturation of 10 min at 95 ºC and a final extension of 10 min at 72 ºC. Four µL of the PCR products were added to 19 µL of formamid and 0.5 µL of GeneScan 500-ROX Size Standard (PE Applied Biosystems, Foster City, California, USA). The mixture was denatured at 95 ºC for 5 min and cooled to 4 ºC for 5 min. Electrophoresis was performed on ABI 3130 Genetic Analyzer by using POP7 gel (PE Applied Biosystems). The results were analyzed with Genescan Analysis software (PE Applied Biosystems).

3. Measurement of B-type natriuretic peptide (BNP) levels

The concentration of the BNP levels were measured within six hours of blood sampling by immune fluorescence method using 250 µl EDTA plasma from 50 pre-eclamptic and 50 healthy controls (Triage BNB test, Biosite Diagnostics Incorporated, San Diego,

California, USA). The Triage BNP test is a sandwich fluorescence immunoassay, comprising the Triage Meter and a disposable, single-use, plastic assay cartridge. It employs standard immunoassay techniques using recombinant murine antibodies to specifically and quantitatively detect the target protein, BNP, in EDTA-anticoagulated plasma specimens. The assay cartridge contains two different BNP-specific monoclonal antibodies. One antibody is immobilized on a solid phase (capture zone) and a second is conjugated to fluorescent nanoparticles (detection). The cartridge is engineered with integrated control features including positive and negative controls, which ensure that the test performs properly and that the reagents are functional. Several drops of EDTA plasma are introduced into the sample port using the pipette provided. The plasma flows down the diagnostic lane via capillary action taking up the fluorescent nanoparticles into suspension. BNP in the specimen binds to the antibody on the nanoparticles to form a multivalent complex which is captured by the antibody immobilized in the capture zone. Separate solid phase zones are located along the same diagnostic lane for the control assay systems. The cartridge is inserted into the Triage Meter and quantitative measurements of BNP concentration in the range from 5 to 5000 pg/mL are displayed on the meter screen and/or printout in approximately 15-20 minutes. Calibration information is relayed to the meter via a lot-specific EPROM chip (the code chip module). Values outside of this range are set to their respective range limits for analysis (e.g. any result < 5 pg/ml is set to 5 pg/ml and any result > 5000 pg/ml
is set to 5000 pg/ml). EDTA plasma samples were first thawed by placing the samples in a water bath at 21 ± 3°C for 30 - 60 minutes. Before inoculation of the sample onto the cartridge, samples were mixed by gently inverting the sample tubes for approximately three seconds. Samples were added using a transfer pipette and the inoculated assay cartridge was then placed into the Triage Meter. The Triage Meter automatically monitors progression of the immunoassay and reports the BNP concentration on completion of the assay.

4. Biochemical markers

Blood samples were taken from an antecubital vein into plain and EDTA tubes and then centrifuged at room temperature with a relative centrifugal force of 3000g for 10 minutes. The aliquots of serum and plasma were stored at -80$^{\text{o}}$C until the analyses. We analyzed the patients laboratory findings (complete blood count, renal fuction, serum electrolytes, 24 hour urine). Standard laboratory parameters (clinical chemistry levels) were determined by an autoanalyzer, using the manufacturer's kits in the Central Laboratory of the Semmelweis University. All data were collected from the medical records of the patients. Blood count was determined with Advia 120 Hematology System (Advia Centaur BNP, Siemens Healthcare Diagnostics, TarryTown, NY 10591-5097, USA). and urea, serum electrolytes and total protein and 24 hour urine protein amount with Beckman Coulter AU5800 System (Beckman Coulter, Inc. Diagnostics Division Headquarters 250 South Kraemer Boulevard Brea, CA 92821-6232, USA). Creatinine was measured with automated analyzers using the non-enzymatic, kinetic Jaffe's method.

5. Statistical analysis

Statistical analysis was performed with the STATISTICA software package (version 8; StatSoft, Inc., Tulsa, Oklahoma, USA). The normality of continuous variables was assessed using the Shapiro-Wilk's W-test. As the continuous variables were not normally distributed, non-parametric statistical methods were used. The statistical significance of the differences between patient groups was evaluated by Mann-Whitney non-parametric U-test, Fischer exact test and Pearson Chi-square (χ^2) test. $p < 0.05$ was considered as statistically significant.

Pearson Chi-square (χ^2) test was used for comparing groups of categorical data of allele and genotype frequencies. Multivariate logistic regression was carried out for

adjustment of potential confounding variables. To compare continous variables among multiple groups, the Kruskal-Wallis analysis of variance by ranks test was performed. Multiple comparisons of mean ranks for all groups were carried out as post-hoc tests. The Spearman rank order correlation was applied to calculate correlation coefficients. For all statistical analyses, $p < 0.05$ was considered statistically significant. Finally, we constructed receiver operating characteristic curves to illustrate the best cut-off value of B-type natriuretic peptide between the preeclamptic and healthy pregnants.

Data are reported as median (interquartile range) for continuous variables and as number (percentage) for categorical variables.

IV. Results

1. Study of the Natriuretic peptide precursor B gene $(TTTC)_n$ microsatellite polymorphism in severe preeclamptic and healthy pregnants

The capillary electrophoresis system allows the assignment of the fragment size with 1 bp size precision. The alleles were assigned into 12 groups based on the size distribution of the (TTTC) tetranucleotide repeats ranging from 8-repeat to 20-repeat. A 11 repeat and 16 repeat allele had the highest frequency in both group patients. In preeclamptic patients 8-repeat allele and 20 repeat allele were absent.

The overall distribution of alleles was significantly different between the control and pre-eclamptic groups (p=0.0002). We observed a lower frequency of repeat 11 alleles in controls (47.87% compared to 60.23% in severe-preeclamptic pregnants, p=0.0002). On the other hand, frequency of repeat 10 allele in healthy pregnants were higher than in the pre-eclamtics (4.26% vs. 0.68%, p=0.0005). Similarly, frequency of repeat 12 allele was higher in the control group (8.94% vs. 4.55%, p=0.0118). The frequency of the repeat 16 allele was 24.26% in healthy controls and 25% in patients having pre-eclampsia (p>0,05).

We found 35 kind of genotypes In the severe preeclamptic patients 20 form of genotypes occured, while in the control group 32 kind. The most common genotypes were the 11:11, 11:16 in both groups.

The overall genotype distribution was significantly different between the control and pre-eclamptic groups (p=0.027). The number of 10-repeat genotype carriers was significantly lower in pre-eclamptics than in the healthy pregnants (p=0.032). After adjustment for confounding factors prepregnancy BMI, maternal age, primiparity, smoking, the calculated odds ratio was 0.19 (95% CI: 0.04-0.87). Similarly, the 12-repeat genotype carriers showed significantly lower frequency in pre-eclamptics than in the healthy pregnants (p=0.037). Adjusted odds ratio was 0.53 (95% CI: 0.29-0.96). Contrary, the 11-repeat genotype carrier frequency was significantly higher in the pre-eclamptic than in the healthy pregnant group (p<0.001). Adjusted odds ratio was 2.91 (95% CI: 1.75-4.84).

About 90% of the severe preeclamptic patients (87,7%) were 11 repeat allele carriers. I compared the genotype distribution of the 11 homozygotes and 11 heterozygotes to other genotype carriers. I found that adjusted odds ratio of the 11 repeat homozygotes /OR: 3.01 (95% CI: 1.66-5.44)/ was higher than of 11repeat heterozygotes in severe preeclamptic patients /OR: 2.84 (95% CI:1.66-4.87)/.

2. Determination of plasma BNP levels in severe preeclamptic and healthy pregnants

Levels of BNP were different in normal pregnancy and severe preeclampsia. We determined the BNP levels using fluorescence immunoassay method. The concentration of the hormone was 9.75 (5.6-16.7) pg/ml in healthy control pregnants and 32.40 (13.30-65.90) pg/ml in pre-eclamptics (p<0.0001).

3. Study of the relation between Natriuretic peptide precursor B gene $(TTTC)_n$ microsatellite polymorphism and between plasma BNP levels in severe preeclamptic and healthy pregnants

In the control group, we found higher concentrations of BNP in the genotype 11:11 group (13.9 (5-21.8) pg/ml versus 9.0 (5.6-15.9) pg/ml). Similarly, in the preeclamptics, the 11/11 genotype carriers had significantly higher BNP levels than the other genotype carriers (64.5 (28.75-93.15) pg/ml vs. 17.8 (8.1-36.9) pg/ml, p<0.001).

I compared the BNP levels of the 11 homozygotes and 11 heterozygotes to other genotype carriers with with Kruskal-Wallis test. The difference was significant (p=0.03).

4. Study of the Natriuretic peptide precursor B gene (TTTC)$_n$ microsatellite polymorphism in early-onset and late-onset severe preeclamptic patients.

Among the 220 severe preeclamptic patients 127 pregnants started the symptoms before and 93 after the 34th week. In both groups all of the 8 alleles occured, which were found in severe preeclamptic patients. The observed difference between the control and late-onset preeclamptic groups (p=0.073) and the two preeclamptic groups (p=0.21) were not significant. The distribution of the alleles was significantly different between the control and early-onset preeclamptic groups (p=0.0064). We observed a higher frequency of 11 repeat alleles in early-onset preeclamptics (63% compared to 56.45% in late-onset preeclamptic pregnants and only 47.87% in control patients).

We found similar correlations by the genotype distribution. From the 20 genotypes which were found in severe preeclamptic patients, we determined 16-16 in the two study groups. 12 genotypes were identical. There was no significantly difference between the early-onset and the late-onset preeclamptic groups (p=0,195). The observed difference between the early-onset preeclamptic and control groups was significant (p=0.0137). There was no significantly difference between the control and the late-onset preeclamptic groups (p=0.366). The most common genotypes were the 11 repeat homozygotes, 11:16 and 11:12 in both groups. The frequency of the 11:16 genotype was 32.28% in early-onset preeclamptics, 36.56%, in late-onset preeclamptics, while the 11:12 genotype was 7.87% and 7.52%. Occurrence of the 11 homozygote genotype was 36.22% in early-onset preeclamptics, 27,95% in late-onset preeclamptics.

The comparison of the 11 homozygotes frequency to other genotype carriers in both study groups shows an elevated odds ratio by the early-onset preeclamptics versus controls to late-onset preeclamptics versus controls, even after adjustment of confounding factors prepregnancy BMI, maternal age, primiparity, smoking. The calculated odds ratio was 1.694 (95% CI: 1.06-2.70) vs. 1.22 (95% CI: 0.71-2.08).

5. Comparison of plasma BNP levels in early-onset and late-onset severe preeclamptic patients

The concentration of the hormone was 40.55 (28.5-82.7) pg/ml in early-onset preeclamptic patients and 24.10 (13.30-65.90) pg/ml in late-onset preeclamptics (p<0.05). We looked for a correlation with the measured concentrations and detected alleles and genotypes. In the early-onset group we found higher concentrations of BNP in the genotype 11 homozygote group with 67.05 (33.7-102.1) pg/ml and in the other genotypes 26.2 (14.3-39.5) pg/ml (p<0.0001), it was similar in the late-onset preeclamptics in 11 repeat homozgyote genotypes 55.8 (28.75-93.15) pg/ml versus others 17.1 (7.1-35.2) pg/ml (p<0.0001).

6.Study of the (TTTC)n polymorphism in the 5' flanking region of the NPPB gene in severe preeclamptic patients in relation to intrauterine growth restriction

Among the 220 severe preeclamptic patients, 155 delivered appropiate grown infants. 65 infants suffered under intrauterine growth restriction (IUGR10 percentile). Severe preeclamptic patients with a complication of severe intrauterine growth restriction -28 pregnants- yielded a subgroup (IUGR 3 percentile). In the preeclamptic group with appropriate-for gestational age infants (AGA) all of the 8 alleles occured, which were found in severe preeclamptic patients. In the IUGR 10 percentile group the 15 repeat allele was absent. In the IUGR 3 percentile group also the 12 repeat allele was missing.

The overall distribution of the alleles was significantly different between the preeclamptic patients with appropriate-for gestational age infants (AGA) and controls (p=0.0022) and between the IUGR3 group patients (p=0.0052). We observed a higher frequency of 11 repeat alleles in AGA preeclamptics (62.26% compared to 55.39% in IUGR 10 percentile, 47.87% in control patients and only 44.64% in IUGR 3 percentile group). There was no significantly difference between the IUGR 10 percentile group preeclamptic patients and controls (p=0,0781) and between the IUGR3 group patients

In the preeclamptic group with appropriate-for gestational age infants (AGA) all of the 20 genotypes occured, which were found in severe preeclamptic patients. In the IUGR 10 percentile group we found 14, in the IUGR 3 percentile group only 10 sorts of genoypes.The overall genotype distribution of the NBBP gene in between the preeclamptic patients with appropriately grown infants (AGA) and IUGR 10 percentile (p=0.0239) and between the IUGR 3 percentile group patients (p=0.0003) was significantly different.

The most common genotypes were the 11 repeat homozygotes, 11:16 and 11:12 in both groups. The frequency of the 11:16 genotype was 33.54% in the AGA preeclamptic group, 35.38%, in IUGR 10 percentile preeclamptics, while the 11:12 genotype was 9.68% and 3.08%. Occurrence of the 11 repeat homozygotes was 33.54% in the AGA preeclamptic group, 30.77% in IUGR 10 percentile preeclamptics and 25% in IUGR 3 percentile preeclamptics. The comparison of the 11 homozygotes to 11 heterozygote genotype carriers distribution in the AGA group versus healthy pregnants shows an elevated odds ratio by the AGA pre-eclamptics to control pregnants. The calculated odds ratio was 4.47 (95% CI: 2.26-8.86) vs. 4.3 (95% CI: 2.27-8.14).

7. Comparison of plasma BNP levels in severe preeclamptic patients in relation to intrauterine growth restriction

I compared the plasma BNP levels of 155 severe preeclamptic patients with appropiate grown infants and 65 preeclamptic patients with complication of intrauterine growth restriction. The concentration of the BNP was 34.4 (10.2-70.9) pg/ml in preeclamptic patients with appropriately grown infants and 29.0 (14.1-40.1) pg/ml in preeclamptic patients with intrauterine growth restricted infants (p=0.71). The concentration of the BNP was 31.2 (17.3-43.5) pg/ml in preeclamptic patients with severe intrauterine growth restricted infants.

8. Study for comparison the relations of plasma BNP levels to the clinical features or laboratory parameters of preeclampsia

There were significant differences in most of the measured parameters among the three study groups except for white blood cell (WBC), platelet count (Plt) and serum chloride levels. Hemoglobine values were significantly higher, while serum sodium levels significantly lower in early-onset preeclamptic patients than in the healthy pregnant and in the late-onset preeclamptic groups. Hematocrit and serum potassium, urea, uric acid levels were significantly higher and the total protein levels significantly lower in early-onset and late-onset preeclamptic patients than in healthy pregnant women. Furthermore early-onset preeclamptics had significantly higher 24 hour proteinuria values as compared to late-onset preeclamptic patients.

In the preeclamptic group we found a significant inverse correlation between gestational age at disease onset and plasma BNP levels (R= -0.56, p<0.001). In the control group there was no correlation between gestational age at blood collection and plasma BNP levels.

We also investigated whether plasma BNP levels of the study participants were related to their clinical features or laboratory parameters by calculating the Spearman rank order correlation coefficients. In the group of healthy pregnant women serum BNP concentrations

correlated significantly negatively with serum total protein levels and platelet count (R= -0.33 and -0.37, respectively p<0.05 for both) and positively with systolic and diastolic blood pressure (R= 0.31 and 0.32, p<0.05 for both). In late-onset preeclamptic patients also systolic and diastolic blood pressure (R= 0.64 p<0.05 and R= 0.83, p<0.001) and 24 hour proteinuria (R= 0.48, p<0.05) correlated significantly with BNP levels. There was a significant inverse correlation between plasma BNP levels of early-onset preeclamptic patients and sodium (R= -0.6, p<0.05) and total protein concentrations (R= -0.58, p<0.05). In the early-onset preeclamptic group, a significant positive correlation was observed between plasma levels of BNP and hematocrit (R= 0.59, p<0.05), serum potassium (R= 0.66, p<0.05), urea (R= 0.59, p<0.05) and 24 hour proteinuria (R= 0.62, p<0.05).

We have investigated the added value of maternal available clinical markers (BMI, maternal age) but we did not find any relations.

9. Determination off cut-off point for plasma BNP levels in severe preeclamptic patients

Using the Receiver Operating Characteristic (ROC) curve analysis we determined the best cut-off value to be 24.5 pg/ml for plasma BNP concentration based on the closest to the 100% sensitivity and 100% specificity, discriminate early-onset preeclamptic patients from healthy pregnant women (sensitivity: 95% specificity: 97.5%; area under curve (AUC) with 95% CI: 0.98 (0.91-0.99) p<0.001). The same cut off value by late-onset preeclamptic patients, yielding a sensitivity of 70% specificity: 97.5%; area under curve (AUC) with 95% CI: 0.88 (0.77-0.95) p<0.001. A BNP cut-off < 24.5 pg/ml had a negativ predictive value of 85.1% in excluding preeclampsia.

V. Conclusions

1. I introduced a F-PCR and DNA fragment analysis method for the fast and reliable detection of the $(TTTC)_n$ microsatellite polymorphism in the 5'-flanking region of the natriuretic peptide precursor B gene. The technique is fast and simple for the discrimination of the alleles.

2. I determined the (TTTC)n polymorphism in 235 healthy normotensive pregnant women and in 220 preeclamptic patients. I found 12 different alleles and 35 genotypes in our study. I observed significant differences in the distribution of the (TTTC) alleles and genotypes. I observed a lower frequency of repeat 11 alleles in controls in severe preeclamptic pregnants. On the other hand, frequency of 10 repeat allele and 12 repeat allele in healthy pregnants were higher than in the preeclamtics. The preeclamptic patients were a homogeneous population, with only 10 types of alleles and 20 types of genotypes, contrary to control group with 12 types of alleles and 32 types of genotypes. The 11 homozygote patients have a higher frequency among the severe preeclamptics. The comparison of the genotype distribution of the 11 homozygotes and 11 heterozygotes to other genotype carriers in severe preeclamptic patients shows that adjusted odds ratio of the 11 repeat homozygotes was higher than of 11 repeat heterozygotes.

3. I determined the BNP levels using sandwich fluorescence immunoassay method. This bedside test is a useful technique in the obstetrical clinical practice for preeclamptic patients.

4. I determined the BNP levels in healthy control pregnants and in severe preeclamptics. The BNP levels were significantly higher in severe preeclamptic patients than in normotensive controls.

5. The concentration of the BNP is higher preeclamptic pregnancies, it shows association with the (TTTC) genotypes. I found higher levels of BNP in those who had the genotype 11 homozygotes in both groups, significantly higher in severe preeclamptics.

6. I compared the (TTTC)n polymorphism in the 5' flanking region of the NPPB gene in early onset and late onset preeclamptic patients. The overall distribution of alleles and genotypes shows no difference between the early onset and late onset preeclamptic groups. We only observed the genotype distribution of the early onset preeclamptic patients significantly different from healthy pregnant controls.

7. Plasma B-type natriuretic peptide levels were higher in early-onset than in late-onset preeclamptic patients. This difference was highly significant. In both early onset and late onset preeclamptic gorups we found higher concentrations of plasma BNP compared to other genotype carriers.

8. I studied the (TTTC)n polymorphism in the 5' flanking region of the NPPB gene in severe preeclamptic patients in relation to intrauterine growth restriction. The overall distribution of the genotypes was significantly different between the preeclamptic patients with appropriate-for gestational age infants and between the 10 percentile and 3 percentile intrauterine growth restriction group patients. The difference between genotype distribution of the preeclamptic patients with appropriately grown infants is also significant with healthy pregnants. 10 percentile and 3 percentile intrauterine growth restriction group patients do not show such a difference.

9. I compared the plasma BNP levels of 155 severe preeclamptic patients with appropiate grown infants and 65 preeclamptic patients with complication of intrauterine growth restriction. Intrauterine growth restriction shows no connection with BNP levels in severe preeclampsia.

10. Early-onset preeclamptics had significantly higher 24 hour proteinuria values as compared to late-onset preeclamptic patients. In late-onset preeclamptic patients systolic and diastolic blood pressure and 24 hour proteinuria correlated significantly with BNP levels. There was a significant inverse correlation between plasma BNP levels of early-onset preeclamptic patients and sodium and total protein concentrations. In the early-onset preeclamptic group, a significant positive correlation was observed between plasma levels of BNP and hematocrit, serum potassium, urea and 24 hour proteinuria.

11. Using the Receiver Operating Characteristic (ROC) curve analysis, < 24.5 pg/ml seems to be a powerful discriminative indicator excluding preeclampsia. By early onset preeclamptic patients from healthy pregnant women sensitivity: 95% specificity: 97.5%. The same cut off value by late onset preeclamptic patients yielding a sensitivity of 70% specificity: 97.5%.

List of publications

Publications related to the subject of the thesis

1. **Szabó G,** Molvarec A, Stenczer B, Rigó J.Jr, Nagy B. (2011) Natriuretic peptide precursor B gene (TTTC)(n) microsatellite polymorphism in pre-eclampsia. Clin Chim Acta, 412: 1371-5. **(IF: 2,535)**

2. **Szabó G**, Rigó J.Jr, Nagy B.(2011) A natriureticus peptidcsalád élettani jellemzői és klinikai szerepe. Orv Hetil, 152: 1025-34.

3. **Szabó G,** Molvarec A, Stenczer B, Rigó J Jr, Nagy B.(2012) A natriureticus peptid prekurzor B-gén (TTTC)n polimorfizmusa súlyos praeeclampsiával szövődött terhességben. Magyar Nőorv L, 75: 22-7.

4. **Szabó G.** (2012) Biology of the B-Type Natriuretic Peptide: Structure, Synthesis and Processing. Biochem Anal Biochem, 1:e129. doi.10.4172/2161-1009.1000e129

5. **Szabó G**, Molvarec A, Nagy B, Rigó J. (2013) Increased B-type natriuretic peptide levels in early-onset versus late-onset preeclampsia. Clin Chem Lab Med, doi: 10.1515/cclm-2013-0307 **(IF: 3,009)**

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