

Urinary excretion of brush-border enzymes of the proximal renal tubules in pregnant women with hypertensive disorders

Ocena wydalania enzymów rąbka szczoteczkowego kanalików proksymalnych nerek w moczu kobiet ciężarnych z nadciśnieniem tętniczym w ciąży

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Abstract

Objectives: The aim of our study was to evaluate urinary excretion of three brush border enzymes: gamma-glutamyl transferase, alanine aminopeptidase, and leucyl aminopeptidase in pregnant women with various types of hypertensive disorders.

Material and methods: The study included 120 pregnant women, further subdivided into four groups: 41 women at ≥ 20 weeks gestation with gestational hypertension, 28 women >20 weeks of pregnancy with preeclampsia, 21 women with chronic hypertension identified >20 weeks of pregnancy, and 30 healthy, pregnant controls.

Results: No significant differences in urinary levels of all three of the brush border enzymes were found between the groups. Also, there was no correlation between enzyme concentration in the urine and blood pressure values in any of the analyzed groups of pregnant women.

Conclusions: The obtained results suggest no damage to the brush border of the proximal kidney tubules in the early stages of disorders associated with increased blood pressure during pregnancy.

Key words: **brush border enzymes / hypertension / pre-eclampsia / pregnancy /**

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Streszczenie

Cel: Celem pracy była ocena wydalania z moczem trzech enzymów rąbka szczoteczkowego kanalików proksymalnych nerek: gamma-glutamyl-transferazy, aminopeptydazy alaninowej i aminopeptydazy leucynowej u kobiet ciężarnych w różnych postaciach nadciśnienia tętniczego w ciąży.

Materiał i metody: Badaniami objęto 120 kobiet ciężarnych, które podzielono na cztery grupy: 41 ciężarnych po 20. tygodniu ciąży z nadciśnieniem ciążowym, 28 ciężarnych po 20. tygodniu ciąży z pre-eclampsją, 21 ciężarnych z nadciśnieniem przewlekłym rozpoznany przed 20. tygodniem ciąży i 30 zdrowych ciężarnych, w niepowikłanej ciąży po ukończeniu 20. tygodnia.

Wyniki: Nie wykazano istotnych różnic pomiędzy badanymi grupami kobiet w zakresie wydalania z moczem żadnego z trzech ocenianych enzymów. Nie stwierdzono także znamiennych korelacji pomiędzy stężeniami badanych enzymów w moczu a wartościami ciśnienia krwi ciężarnych kobiet.

Wniosek: Uzyskane wyniki sugerują, że we wczesnych etapach zaburzeń związanych ze wzrostem ciśnienia tętniczego krwi w przebiegu ciąży nie dochodzi do uszkodzenia struktur rąbka szczoteczkowego kanalików proksymalnych nerek.

Słowa kluczowe: **enzymy rąbka szczoteczkowego / nadciśnienie tętnicze w ciąży / pre-eclampsia / ciąża /**

Introduction

Hypertensive disorders constitute the most common medical problem encountered in obstetric practice, complicating up to 10-15% of pregnancies and being the leading causes of maternal and perinatal morbidity and mortality [1,2,3]. Pregnancy is a relatively short period in a woman's life. However, even then, passing burden on kidneys or damage to their structures, resulting from high blood pressure and initiating a subsequent development of a chronic hypertension, may occur. Due to the need for early detection of functional and metabolic disorders in kidneys, researchers continue to look for relevant indicators of kidney damage. Assessment of enzyme activity in biological fluids is a common method used in laboratory diagnostics. Measurement of urinary enzyme activity is considered to be an useful non-invasive test to detect deterioration of renal function in the early stages [4]. It has been proven to be more sensitive for detection of subclinical tubular injury than routine tests of renal function [5,6]. Proximal tubules of the kidney have a dominant function in enzyme excretion in urine, which can be used as a marker of renal damage caused by different diseases, medicaments, or toxins [7].

Gamma-glutamyl transferase (GGT), alanine aminopeptidase (AAP) and leucyl aminopeptidase (LAP) are urinary enzymes which represent tubular function at the brush border region [7]. Increased excretion of these enzymes into the urine indicates damage to the brush border membrane, together with the loss of their microvillar structure [8,9].

The aim of our study was to evaluate urinary excretion of GGT, AAP and LAP in pregnant women with various types of hypertensive disorders.

Material and methods

The study population consisted of 120 women with singleton pregnancy, including 90 patients of the Department of Obstetrics and Gynecology, Pomeranian Medical University, hospitalized between December 2008 and January 2012 due to high blood

pressure. The remaining 30 healthy pregnant controls, matched for gestational age, were in the care of ambulatory services over the same period of time.

The study population was divided into four groups:

GH (gestational hypertension): 41 women at ≥ 20 weeks of gestation, with a new onset gestational hypertension [10].

PE (pre-eclampsia): 28 women after 20 weeks of pregnancy with pre-eclampsia defined as hypertension and proteinuria (>300 mg/day), without (25–89.3%) or with a mild (3–10.7%) liver dysfunction [10].

CH (chronic hypertension): 21 women with high blood pressure predating the pregnancy, or with increased blood pressure <20 weeks of pregnancy, admitted to the hospital due to destabilization of pressure (11 of them with proteinuria – a subgroup with superimposed pre-eclampsia) [10].

HC (healthy controls): the control group, consisting of 30 healthy, pregnant women.

Subjects were qualified to the GH or PE groups on the basis of blood pressure values of $\geq 140/90$ mm Hg, measured twice at 4-6-hour intervals on admission to the hospital. Moreover, such symptoms had to be observed for the first time >20 weeks of gestation. In the PE group, proteinuria was diagnosed subsequently. High blood pressure requiring hospitalization <20 weeks of pregnancy constituted an inclusive criterion into the CH group. None of the patients had other complications, such as pregnancy diabetes, chronic renal or cardiovascular disease, or metabolic disorders. The study was approved by the Local Ethics Committee (KB-0080/165/09) and all subjects gave their written informed consent.

First morning mid-stream urine samples were collected into sterile bottles, immediately centrifuged in 15 mL glass test tubes at 5000 rpm for 10 min., and then kept frozen in 1.5 mL polypropylene tubes at -70°C until assayed. In the GH and PE groups, the urine samples were collected at the moment of hypertension recognition, in the CH group at the moment of destabilization of pressure, and in the HC group at the time of routine ambulatory

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visits. The enzymatic activity was detected with the colorimetric method using kit assays based on the hydrolysis of AAP and LAP substrates (Sigma–Aldrich Corporation, Poznań, Poland) and of the GGT substrate (Pointe Scientific, Warsaw, Poland) by the enzymes. The reaction product was detected colorimetrically at 405 nm and quantitatively measured using Marcel Media Bio spectrophotometer (Merzet, Poznań, Poland) for AAP and LAP and UV/VIS Lambda 40 spectrophotometer (Perkin Elmer, USA) for GGT.

Urine examination was performed using LabUReader Plus analyzer (Allmed, Warsaw, Poland). In case of proteinuria (>25 mg/dL in a single urine sample), daily urine collection was performed with the loss of protein checked turbimetrically, using Architect c4000 clinical chemistry analyzer (Abbot Diagnostics, Wiesbaden, Germany). Maternal serum uric acid was measured using Architect c4000 clinical chemistry analyzer (Abbot Diagnostics). Urinary creatinine levels were estimated with tests (Pointe Scientific, Warsaw, Poland) based on Jaffe’s kinetic method. The urinary enzyme values were expressed as the ratio to urinary creatinine concentration. This relationship shows less variability than urinary enzyme excretion related to volume or time.

The results were statistically analyzed using STATA 11 software and presented as a box and whiskers plots. The Shapiro–Wilk test was used to check the distribution of the analyzed parameters. The ANOVA test was used to identify statistical significance for multiple comparisons. Analyses of differences between individual groups were assessed by Mann–Whitney U test. The strength of correlation between the parameters was measured with the use of the Spearman’s rank correlation coefficient. The p-value of <0.05 was considered statistically significant.

Results

The clinical characteristics of the study groups are shown in Table I. Maternal age was significantly higher in the CH group in

comparison to the GH and controls. Gestational age at sampling was comparable between the groups, except for the GH group in which it was significantly higher than in the CH group. Mean systolic and mean diastolic pressures were comparable between the study groups, and significantly higher than in controls. Daily urinary protein loss was higher in the PE group than in the 11 patients with proteinuria from the CH group.

The ANOVA test showed no significant differences between the groups that concerned urinary levels of all three brush border enzymes (Figures 1–3). No correlation was found between enzyme concentrations in urine and blood pressure values in any of the analyzed groups. Also, no significant correlation was found between urinary excretion of GGT, AAP or LAP and the level of proteinuria in the PE group and in the subgroup of patients with proteinuria from the CH group (n=11). No difference was observed in enzyme excretion in the CH group between the subgroups of patients with superimposed preeclampsia and without proteinuria.

A significant difference in blood uric acid levels between the groups was noted (Figure 4). The Mann–Whitney U test revealed the value of the uric acid in the PE group to be significantly higher than in GH (p<0.01), CH (p<0.05), and controls (p<0.001). Uric acid values were also significantly higher in the subgroup of chronic hypertensive patients with superimposed preeclampsia than in the remaining patients from the CH group without proteinuria (p<0.05). There was no correlation between the values of enzymes derived from the urine and the concentration of uric acid in the blood, which is an indicator of PE severity.

Discussion

In our study, no differences in the brush border enzyme activity in urine between groups of pregnant women with hypertensive disorders and controls have been found. To the best of our knowledge, only a few recent studies evaluated the brush border enzyme excretion in pregnancy.

Table I. Clinical characteristics of the groups.

Group	GH N=41	PE N=28	CH N=21	HC N=30
Maternal age (years) X ±SD	28.4±6.1 ⁺	30.5±6.7	32.76±4.3 ⁺	28.8 ±5.5 [†]
Primiparous n (%)	23 (56.1)	22 (78.57)	13 (61.9)	20 (66.67)
Gestational age at sampling (weeks) X±SD	35.66±3.89 ⁺	34.11±3.89	32.48±5.3 ⁺	33.9±5.5
Mean systolic blood pressure (mmHg) X±SD	151.4±7.9 ^Δ	153.3±7.48 [#]	154.16±12.27 [†]	121.67±14.5 ^{Δ#†}
Mean diastolic blood pressure (mmHg) X±SD	95.58±5.4 ^Δ	98.18±4.89 [#]	97.99±7.4 [†]	74.68±8.3 ^{Δ#†}
Daily urinary protein loss (g/L) X±SD	0	3.88±3.48 [¶]	1.23±1.3 [¶]	0
Gestational age at delivery (weeks) X±SD	37.39±3.05 ^{Δ†}	34.7±3.83 ^{#†}	35.38±3.6 ⁺	39.23±1.65 ^{#Δ}
Caesarean section delivery n (%)	20 (48.78) [†]	21 (75.0) ^{#†}	17 (80.9) ⁺	11 (36.6) [#]
Birth weight (g) X ±SD	3086±770 [†]	2188±880 ^{#†}	2478±1040 ⁺	3343±440 ^{#†}
5 minutes Apgar score (points) X±SD	9.44±1.34 ⁺	8.92±1.62 [#]	8.85±1.42 ⁺	9.83±0.38 ^{#†}

PIH – Pregnancy Induced Hypertension; PE –Preeclampsia; CH – Chronic Hypertension; HC – Healthy controls; X – mean; SD – Standard Deviation
p < 0.05: [†]CH vs HC; ⁺GH vs CH; [#]PE vs HC; ^ΔGH vs HC; [†]GH vs PE; [¶]PE vs CH

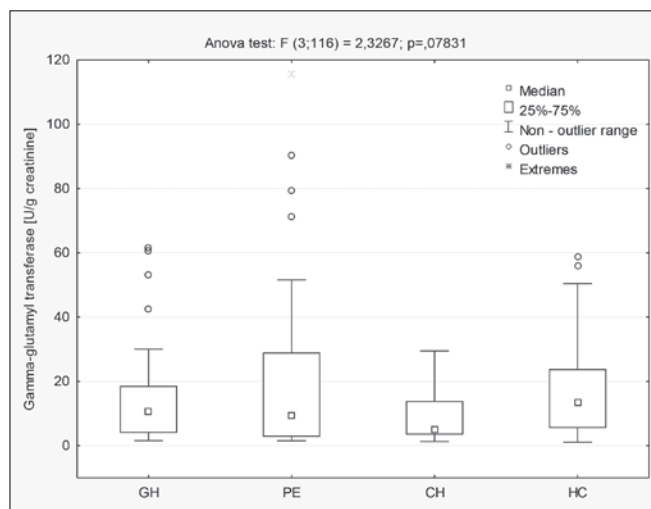
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Figure 1. Box and whiskers plot of gamma-glutamyl transferase (GGT) concentrations in the urine of pregnant women.

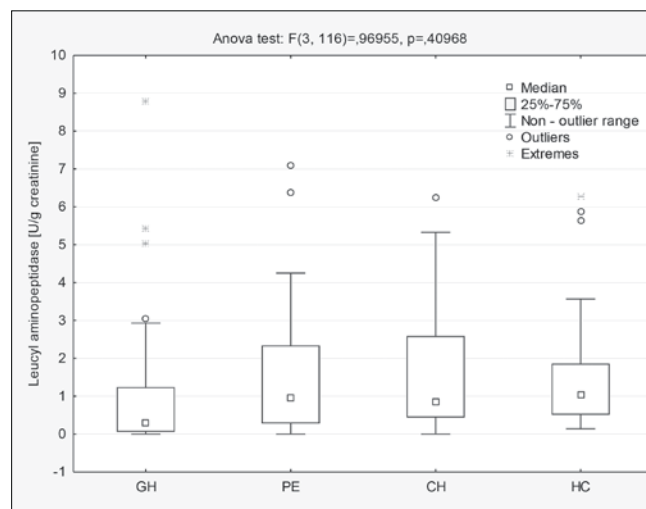


Figure 3. Box and whiskers plot of leucyl aminopeptidase (LAP) concentrations in the urine of pregnant women.

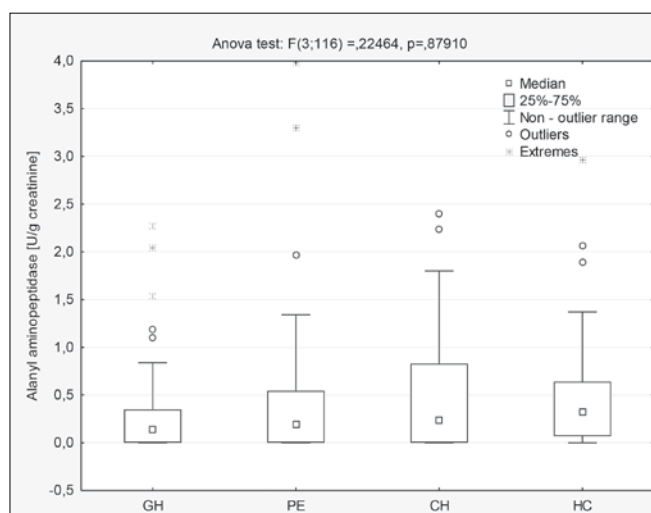


Figure 2. Box and whiskers plot of alanyl aminopeptidase (AAP) concentrations in the urine of pregnant women.

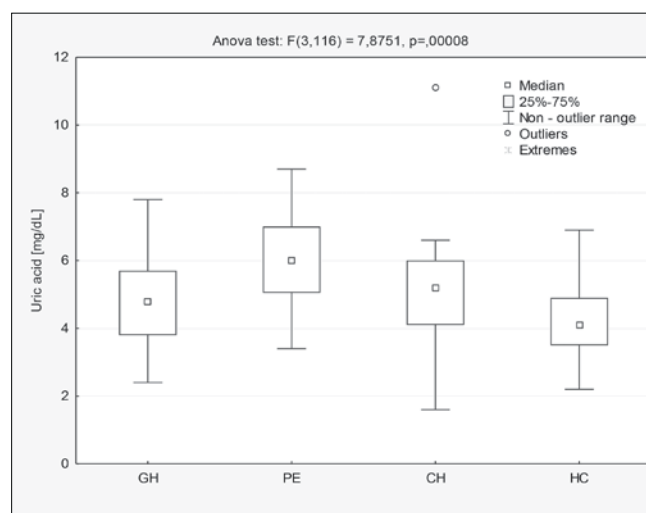


Figure 4. Box and whiskers plot of uric acid (UA) concentrations in the blood of pregnant women.

Noble et al., showed that GGT concentration was higher in the urine of pregnant women in comparison to their non-pregnant peers. The process of GGT excretion starts around 13 weeks of gestation and progresses steadily until completion of pregnancy, what the authors explained as the occurrence of adaptive changes in kidneys [11]. Similar observations were made by Cheung et al., who showed a higher GGT activity in the urine of pregnant women, particularly in the third trimester of pregnancy, in relation to non-pregnant subjects, as a result of decreased reabsorption in the proximal tubules of the enzyme with the duration of pregnancy [12]. The current literature lacks papers on a GGT excretion in the urine of women during pregnancy complicated by PE and hypertension. Our study showed no difference in this aspect between healthy pregnant controls and pregnant women with preeclampsia, gestational hypertension, and chronic hypertension.

In a small material, Mullan showed that LAP excretion in the urine in a normal pregnancy is maintained at a constant, low

level. Additionally, in relation to a normal pregnancy, higher values of the enzyme have been found in multiple pregnancies and in pregnancies complicated by severe preeclampsia. The level of LAP in the urine remained low in women with GH, while in the group with a chronic hypertension it increased notably [13]. Also, Borestein et al., studied the concentration of LAP in the urine of healthy pregnant women and women whose pregnancy was complicated by preeclampsia. They showed the level of LAP in the urine of healthy pregnant women to be independent of gestational age. Also, in case of severe PE, the excretion of that enzyme in urine reached a significant increase [14]. Our study did not confirm the above mentioned observations, revealing no differences in the urinary excretion of LAP between healthy controls and pregnant women with preeclampsia, gestational hypertension, or chronic hypertension.

Cheung et al., evaluated AAP excretion in the urine of non-pregnant and pregnant women (during each trimester of a pregnancy). They found the AAP quantities to be significantly higher

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in the second and the third trimester as compared to concentrations in the urine of non-pregnant women, which is caused by a decrease in enzyme reabsorption in renal proximal tubules [12]. Jacob and Balasubramaniam also evaluated the concentrations of AAP in the urine of healthy pregnant women, confirming a significant increase in its excretion with the duration of pregnancy, and with a subsequent decrease after 36 weeks [6]. Goren et al., also marked the concentration of AAP in the urine of non-pregnant women, healthy pregnant women (in each trimester of pregnancy), and pregnant women with a mild and a severe PE. Their results confirmed an increase in the excretion of AAP with the duration of physiological pregnancy. A significant increase was also found in enzyme concentrations in mild and severe PE when compared to its value in the urine in the third trimester of normal pregnancies. In their opinion, an increase in the level of that enzyme in the urine along with the duration of pregnancy reflects the adaptive changes in kidneys during pregnancy. The striking increase in AAP secretion in the course of EP suggests a coexisting renal tubular damage with contraction of arterioles and microinfarcts [15]. Our findings showed no differences between AAP values in the urine of healthy pregnant women and pregnant women with preeclampsia, gestational hypertension, and chronic hypertension.

The obtained results suggest that there is no functional damage to the brush border of the proximal kidney tubules in the early stages of disorders associated with increased blood pressure during pregnancy. In our previous study, we observed elevated urinary excretion of another group of enzymes (lysosomal enzymes) in patients with preeclampsia, which may be indicative of selective organelle damage within the proximal tubules [16]. No significant correlations were found between urinary excretion of the three studied brush border enzymes and the systolic or diastolic pressure values. Moreover, no correlation between the excretion of GGT, LAP and AAP in the urine and the level of uric acid in the blood serum (which is an indicator of EP severity) was found, indicating that these enzymes are not suitable as markers of the severity of PE or hypertension in pregnancy.

Conclusions

The obtained results suggest no damage to the brush border of the proximal kidney tubules in the early stages of disorders associated with increased blood pressure during pregnancy.

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