Plasma concentration of tryptophan and pregnancy-induced hypertension

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Abstract

Introduction. Pregnancy-induced hypertension (PIH) is one of the main clinical problems of unexplained etiopathogenesis. New factors involved in the pathogenesis of this disease are still being searched. The available literature lacks data regarding the differences in tryptophan concentrations in physiological and PIH-complicated pregnancy. Previous studies have shown that L-tryptophan treatment reduces blood pressure in hypertensive rats. The direct vascular effects of tryptophan have not been fully explored. In this study, the stimulating effect of tryptophan on the development of PIH was revealed. The aim of the present study was to assess the differences in plasma tryptophan concentrations in physiological pregnancies and pregnancies complicated with hypertension in the third trimester.

Material and methods. The study was carried on 105 complicated by PIH and 105 pregnant women with blood pressure within normal limits between 25 and 41 weeks of gestation. Tryptophan concentration was determined by the automated ion-exchange chromatography using an Amino Acid Analyser (AAA 400) by Ingos, Czech Republic. Tryptophan concentration was expressed in μmol/cm^3 plasma.

Results. The mean concentration of tryptophan in the third trimester of physiological pregnancy was found to be 0.035 ± 0.009 μmol/cm^3, whereas in PIH — 0.099 ± 0.007 μmol/cm^3.

Conclusions. The development of PIH in pregnant women is likely to be caused by increased concentrations of tryptophan, which is a substrate for production of serotonin and tryptamine. Further studies are needed to analyse the kinetics of tryptophan metabolism.

Key words: tryptophan, pregnant women, hypertension, blood plasma

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Introduction

Tryptophan is an exogenous aromatic amino acid, which undergoes multi-directional changes resulting in the formation of heterocyclic biogenic indoleamines, such as serotonin and tryptamine [1–3].

Mediated by tryptophan 5-hydroxylase, tryptophan is converted into 5-hydroxytryptophan catabolized by decarboxylase of aromatic amino acids into 5-hydroxytryptoamine (serotonin). Serotonin stimulates the contraction of vascular smooth muscles and small bronchi, induces vasoconstriction and increases blood pressure [1, 2, 4]. It is produced in the raphe nuclei, pineal body, intestinal mucosa and thrombocytes [5]. Moreover, serotonin is a neurotransmitter stimulating the central nervous system (CNS) responsible for memory, appetite, digestion, regulation of body temperature, sleep, mood and numerous cognitive functions [3, 6, 7].

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The human body needs about 10 mg of tryptophan to prevent symptoms associated with its deficiency [8]. Only 1% of dietary tryptophan is metabolized to serotonin [9].

Studies in rats did not explicitly confirm the effects of tryptophan on the development of arterial hypertension. According to Lark et al. [10] and Riesselmann et al. [11], tryptophan prevented its development; Wolf et al. [12] demonstrated that the effects of tryptophan on hypertension depended on its dose. The influence of tryptophan on pregnancy-induced hypertension is still unexplained.

Tryptamine is a biogenic amine produced in the human nervous and peripheral tissues, as well as by microflora of the gastrointestinal tract; moreover, it is an ingredient of various food products. Tryptamine is involved in the development of cardiovascular diseases, such as hypertension, myocardial infarction but also migraine [13, 14]. The serum concentration of tryptamine is correlated with the concentration of its precursor, L- tryptophan, which is metabolized to tryptamine by decarboxylase of aromatic amino acids [15]. Tryptamine has been demonstrated to constrict the rabbit aorta by direct stimulation of α-adrenergic and 5-HT (serotonin) receptors [16]. Tryptamine-induced vasoconstriction has also been observed in the rat mesenteric arteries [17], rat tail arteries [18, 19] and rat aorta [20].

Arterial hypertension in pregnant women is one of the essential clinical problems of unknown etiopathogenesis. It can induce severe obstetric complications, such as detachment of the placenta, cerebrovascular "episodes", organ failure, eclampsia, disseminated intravascular coagulation and many others [21].

Pregnancy-induced hypertension (PIH) is one of the most serious obstetric complications. PIH can occur without proteinuria or can be associated with significant proteinuria, called preeclampsia. When intravascular haemolysis, liver damage and thrombocytopaenia occur, the HELLP syndrome develops [22, 23]. Despite intensive studies carried out in many centres worldwide, the etiopathogenesis of PIH has not been explained. The literature data reveal that PIH is underlain by impaired placental blood flow [24]. At present, the main diagnostic criterion of hypertension in pregnancy is systolic blood pressure (SBP) equal to or higher than 140 mmHg measured at the interval of six hours or diastolic blood pressure (DBP) equal to or higher that 90 mmHg [21–23].

Objective

The aim of the present study was to assess the differences in plasma tryptophan concentrations in physiological pregnancies and pregnancies complicated with hypertension in the third trimester.

Material and methods

Patients

The study involved 210 pregnant women, 25–41 weeks of gestation, managed in the Chair and Department of Obstetrics and Pathology of Pregnancy Medical University of Lublin and Department of Gynaecology and Obstetrics with Admission Room of the Cardinal Wyszyński Regional Specialist Hospital in Lublin in the years 2010–2014. Experimental procedures were approved by the Bioethical Commission of the Medical University of Lublin, Poland (No KE-0254/223/2010). All women accepted the study protocol and gave written informed consent for participation.

The overall number of 210 women, who participated in the study, was divided into 2 groups: study and control.

In the study group (n = 105), elevated blood pressure (> 140/90 mmHg) was found, yet no concomitant diseases that could increase blood pressure (e.g. diabetes) were observed. According to the recommendations of the European Society of Hypertension (ESH) and the European Society of Cardiology (ESC), blood pressure was measured twice at a 6-hour interval in a sitting position using an electronic sphygmomanometer [22, 23]. In the control group (n = 105), blood pressure was within normal limits (120/80 mmHg); women in this group did not take any drugs that could affect the cardiovascular system. To obtain detailed data, women in the study and control groups were divided into 3 subgroups: 25–34, 35–38, 39–41 hbd, 35 cases each. Table I presents age, height, body weight, systolic (SBP) and diastolic (DBP) blood pressure of women participating in the study.

Biochemical analyses

The study material was venous blood collected in the fasting state from women with physiological pregnancies and pregnancies complicated with hypertension, 9 ml, to the test-tubes containing lithium heparin (aspiration-vacuum systems, Sarstedt, Germany).

Table I. Characteristics of women participated in this study

Parameters	Mean ± SD	
	Study group (n = 105)	Control group (n = 105)
Age (years)	32.11 ± 3.91	28.6 ± 3.87
Height [cm]	163.01 ± 5.3	165.93 ± 6.72
Body weight [kg]	96.48 ± 7.3	84.11 ± 10.33
SBP [mmHg]	162.98 ± 10.79	119.54 ± 6.76
DBP [mmHg]	97.67 ± 7.01	77.17 ± 4.53

The plasma from each blood sample was collected immediately after centrifugation at 4000 × g for 10 min and then stored at –80°C until analysis. For tryptophan concentration measurements, plasma was deproteinised with 6% sulphosalicylic acid in lithium — citrates buffer (pH = 2.6) and centrifuged at 12 000 × g for 20 min. Tryptophan was determined by the automated ion-exchange chromatography with five lithium-citrate buffers by Moore et al. [25] using an Amino Acid Analyser (AAA 400) by Ingos, Czech Republic.

Tryptophan concentration was expressed in µmol/cm^3 plasma.

Statistical analysis

Statistical analyses were performed using Statistica v. 10.0 software (StatSoft, USA).

Data were presented as a mean and standard deviation (SD). The distribution of quantitative parameters was assessed using the Shapiro-Wilk W test. All quantitative parameters were characterised by skew distribution; therefore, non-parametric tests were applied to evaluate inter-subgroup significant differences. Two independent groups were compared using the Mann-Whitney U test. Inter-quantitative parameter correlations were assessed by Spearman's rank correlation. The differences between mean values were considered as statistically significant at $p < 0.05$.

Results

The comparison of systolic and diastolic pressure between the control and study groups revealed significant differences. In the study group, both systolic and diastolic blood pressures were found to be higher (Fig. 1 and Fig. 2).

The mean tryptophan concentration and standard deviation in the third trimester of pregnancy were presented in Table II and Figure 3.

The mean concentration of tryptophan and standard deviation in the subgroups of the third trimester are listed in Table III.

Analysis of correlations between amino acid concentrations, systolic and diastolic pressures in hypertension-complicated pregnancies (Table IV).

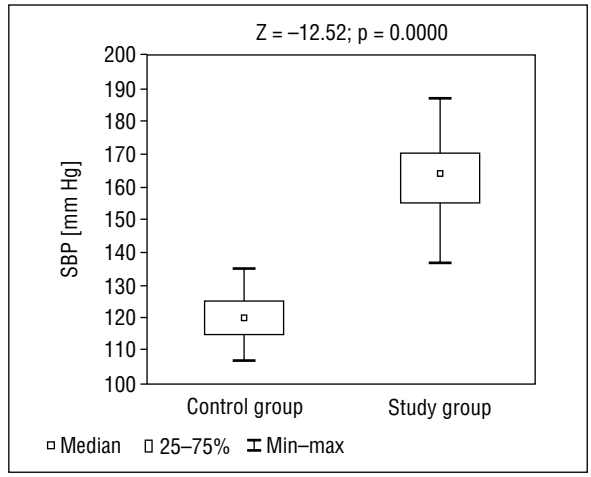

Figure 1. Differences in systolic pressures between physiological and hypertension-complicated pregnancies

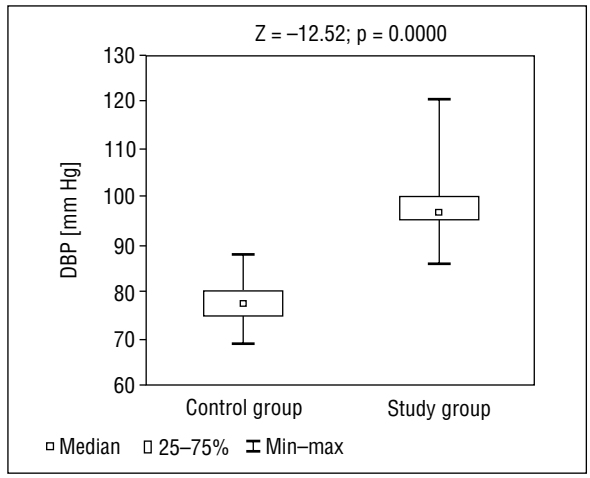

Figure 2. Differences in diastolic pressures between physiological and hypertension-complicated pregnancies

Table II. Plasma concentration of tryptophan in the control and study group with hypertension in the third trimester of pregnancy

Parameter	Mean ± SD		*P* value
	Study group (n = 105)	Control group (n = 105)	
TRP [µmol/cm^3]	0.099 ± 0.007	0.035 ± 0.009	< 0.00005

Discussion

Intracellular protein distribution induces a release of free amino acids, which are precursors for synthesis of other proteins. The intracellular pool of amino acids is in balance with the extracellular pool in plasma. This balance is regulated by membranous transport characteristic of individual amino acids. Another source of extracellular pool is amino acids derived from dietary proteins [26].

Plasma tryptophan concentrations in fasting normal humans vary between 55 and 65 µmol/cm^3. Concentrations depend on the individual's prior protein intake (i.e., higher after diet rich in

high-protein meals for a few days) [27], higher caloric intake [28], body mass index (lower in obesity) [29], age (lower in older men) [30] and gender (higher in males) [31]. Plasma tryptophan concentrations are readily increased by administering exogenous tryptophan. It can cause proportionate increases in brain tryptophan [32]. About 75–80% of the tryptophan in human plasma is loosely bound to albumin [33]. At first, it was anticipated that this binding would substantially retard the passage of tryptophan across the blood-brain barrier. Researchers considered measuring plasma free tryptophan (non-albumin bound), or the ratio of free tryptophan to the other large neutral amino acids, as the best predictor of brain tryptophan levels [34]. However, further studies often described treatment-induced changes in plasma free tryptophan, which were opposite in direction to those in brain tryptophan [35].

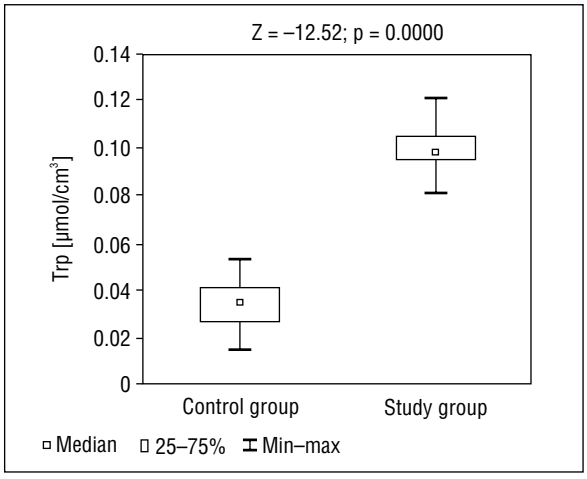

Figure 3. Concentration of TRP in the control and study group with hypertension in the third trimester of pregnancy

Studies on nitrogen metabolism demonstrated that women with physiological pregnancies develop hypoaminoacidaemia, i.e. a drop in blood amino acid concentration, which maintains until delivery [26, 36–38]. The condition results from the effects of gestational hormones and increased visceral absorption of amino acids [37–41].

Pregnancies complicated with hypertension are accompanied by numerous changes, e.g. increased peripheral and placental vascular resistance, reduced plasma volume, increased reactivity of maternal vascular bed to external pressors [21, 42]. However, there are no literature data regarding changes in tryptophan concentrations.

In this study, the stimulating effect of tryptophan on the development of pregnancy-induced hypertension was revealed. In pregnancies complicated with hypertension in the third trimester, the concentration of tryptophan increased by 0.064 µmol/cm^3, compared to physiological pregnancies. Similar results were found in the individual stages of the third trimester; the increase by 0.68 µmol/cm^3 was observed between 25–34 gestational week, by 0.066 µmol/cm^3 between 35–38 week and by 0.06 µmol/cm^3 between 39–41 week. However, the correlations between tryptophan concentration and systolic and diastolic pressure separately were not confirmed. The above demonstrates that increased concentration of this amino acid in pregnant women with PIH is associated with a simultaneous increase in systolic and diastolic pressures.

Table III. Plasma concentration of tryptophan in women with physiological pregnancies and pregnancies complicated with hypertension in the third trimester

TRP [µmol/cm^3]						
The third trimester range	**25–34 hbd**		**35–38 hbd**		**39–41 hbd**	
Group	Control (n = 35)	Study (n = 35)	Control (n = 35)	Study (n = 35)	Control (n = 35)	Study (n = 35)
Mean	0.031	0.099	0.032	0.098	0.04	0.1
SD	0.009	0.006	0.008	0.009	0.006	0.006
P value	p < 0.00005		p < 0.00005		p < 0.00005	

Table IV. Correlation between plasma amino acid concentration in the study group versus systolic and diastolic pressure

Group with hypertension (n = 105)					
	R	**p**		**R**	**p**
Trp & SBP	0.029	0.766	Trp & DBP	0.139	0.155

Amino acids are a group of compounds widely used by the human body. After their decarboxylation, biogenic amines are synthesised, i.e. factors indispensible for maintenance of cell viability and proper course of cellular processes [2, 3]. From tryptophan, heterocyclic biogenic indoleamines are synthesized, such as serotonin and tryptamine [1–3]. Serotonin induces an increase in blood pressure by vasoconstriction and stimulation of smooth muscle constriction [1, 3, 4]. Tryptamine is responsible for the development of such cardiovascular diseases as hypertension or myocardial infarction [13, 14]. Increased concentrations of the substrate (tryptophan) for production of the amines mentioned above are likely to result in their higher production. Once at higher concentrations, they induce more strongly an increase in blood pressure in pregnant women.

The literature lacks reports on the effects of tryptophan on the development of pregnancy-induced hypertension. The only data available regard its general impact on hypertension.

The studies by Cade et al. [43] did not confirm our results. The authors assessed the effects of tryptophan and 5-hydroxytryptophan on blood pressure in patients with slight and moderate hypertension and demonstrated that tryptophan reduced blood pressure. L-tryptophan administered in a dose of 4 g/day resulted in blood pressure decreases in 8 of 9 patients, whereas a dose of 800 mg/day improved the clinical condition of 5 of 8 patients. According to the authors, no adverse side effects of the treatment were observed and serotonin was responsible for at least a part of antihypertensive effect of L-tryptophan.

Feltkamp et al. [44] demonstrated that a single dose of L-tryptophan (50 mg/kg body weight) caused a reduction in blood pressure 90–120 minutes after administration in 14 patients with arterial hypertension yet resulted in no effects in normotensive patients. Moreover, they measured 5-HT uptake by platelets in patients not receiving L-tryptophan and did not find any differences between healthy and hypertensive patients. The administration of L-tryptophan changed the kinetics of uptake and increased 5-HT uptake in healthy individuals; in hypertensive patients, no such effects or only slight effects were noticed. Feltkamp et al. have suggested that essential hypertension central serotonergic mechanisms are involved in pathogenetic mechanisms and that the tryptophan-induced pressure-decreasing effect is caused by increased synthesis of central 5-HT.

The study by Wolf et al. [12] partially confirmed our findings. They administered L-tryptophan to rats and observed its first effects after 30 min.; the maximum response was detected after 60 min. The authors found that L-tryptophan in the doses of 25–100 mg/kg body weight increased blood pressure in normotensive rats by 10 to 15 mmHg. Lower doses also increased pressure in normotensive rats while higher doses reduced blood pressure even by 30–35 mmHg. However, according to them, effects of L-tryptophan on blood pressure in normotensive and spontaneously hypertensive rats cannot be explicitly explained by L-tryptophan effects on brain serotonin.

Riesselmann et al. [11] evaluated effects of tryptophan therapeutic diet on the development of cold-induced hypertension in rats. Continuous administration of lower doses of L-tryptophan (850 mg/ /day) prevented an increase in blood pressure, reduced hypertrophy of the heart and did not affect the body weight during exposure to cold. The use of higher doses (1.690 mg/day) reduced the rate of blood pressure increase without affecting hypertrophy of the heart, decreased the body weight gain and increased the excretion of epinephrine with urine. Therefore, it can be stated that higher doses are likely to be associated with some toxicity. Both doses of tryptophan did not prevent the other typical symptoms developing after exposure to cold, e.g. increased weight of kidneys, adrenal glands and brown fatty tissue, increased consumption of food and water, enhanced reactions to angiotensin II or increased concentrations of aldosterone in plasma. The results of studies in rats have demonstrated that continuous administration of L-tryptophan on a diet (850 mg/ /day) prevents the development of cold-induced hypertension.

Ardiansyah et al. [45] examined blood pressure and glucose metabolism induced by L-tryptophan in stroke-prone spontaneously hypertensive (SHRSP) rats. They demonstrated that oral administration of L-tryptophan increased blood pressure, glucose levels, insulin concentration, plasma amounts of nitric oxide and serotonin levels.

The level of serotonin (which increases after L-tryptophan) [46] is evidently associated with cardiovascular diseases and other side effects of tryptophan [47–51]. There is scientific evidence proving that serotonin formed from tryptophan results in side effects, such as vasoconstriction, that can lead to the development of hypertension [52].

Despite advances in perinatal medicine, arterial hypertension is still a serious risk for mothers and foetuses. The diagnostic procedures of pregnant women have numerous limitations. The methods using ionizing radiation, contrast or medicines are contraindicated and can be used only in life-threatening conditions [53–55]; therefore, early clinical

markers of pregnancy-induced hypertension are being searched for whose determinations would be safe for mothers and foetuses.

Our findings suggest that tryptophan could be such a marker. However, it cannot be explicitly stated that increased tryptophan concentrations result in an increase in serotonin or tryptamine and that blood pressure increases in pregnant women in such a way. In humans, only 1–5% of tryptophan delivered to the body is converted into serotonin [56]. Further studies are needed to analyse the kinetics of tryptophan metabolism in pregnant women with PIH.

Conclusions

Early diagnosis of pregnancy-induced hypertension is a challenge to modern maternal-foetal medicine. The identification of pregnant women at increased risk of this pathology will enable prophylactic treatment before the development of the first clinical symptoms, reducing the mortality of pregnant women. Since no single factor predisposing to PIH is known, many parameters have to be analysed simultaneously.

It is to be hoped that thanks to the popularisation of studies regarding early detection of PIH and the resultant reduced costs of intensive therapy, simple, cheap and easily available PIH tests will be designed in the nearest future.

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