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Platelet Serotonin Concentration at Term Pregnancy and after Birth: Physiologic Values for Croatian Population

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ABSTRACT

The aim of this study was to determine physiological value of platelet serotonin (5-HT) and its variations in the group of women in term pregnancy and after birth. Obtained results were compared to the platelet 5-HT level in nonpregnant women group. Determination of normal level of 5-HT in pregnancy and after could help in its further measurement and evaluation of different psychologic and psychiatric disorders related to pregnant and postpartal period, including better understanding of mood changes after the birth. A total of 137 healthy Croatian women were enrolled in the study – 82 of them were pregnant and 55 were not. Their blood was sampled and the platelet serotonin concentration was determined. In pregnant women the blood was sampled twice: at term pregnancy, and soon after birth. The mean value of 5-HT in pregnant women was 1.209 nmol/mgprotein, after the delivery 1.045 nmol/mgprotein, and in non pregnant 1.088 nmol/mgprotein. The concentrations were significantly different in those three groups. We did not find differences in 5-HT levels in groups divided by age.

Key words: platelet serotonin, pregnancy, mood changes after birth, postpartal mood changes

Introduction

Serotonin (5HT) is a biogenic amine that originates from the synthesis of essential amino acid tryptophan, widely distributed in plant and animal life. Due to discovery of similarity between some elements of the serotonergic system in the central nervous system (CNS) and platelets as peripheral representative^{9,10} more research of serotonergic system gradually followed in various fields of medicine^{2,7,18}, neurobiology⁹ and science^{4,12}. Similarity between central and peripheral serotonergic functioning enabled reliable information about physiology of serotonin and its variations in the CNS using minimally invasive procedures. Its role in regulation of mood, cognitive and motor functions, neuroendocrine regulation (breathing, sleep, cardiovascular function of metabolism, temperature, feeding, sexual activity) as well as the influence on circadian rhythms and other is established^{10,11,20,21}. Therefore, the disruption of physiological processes in serotonergic system can result with the disease of various systems^{12–14,20} and greatly hinder quality of life.

There are very few evidence based data about physiology of serotonergic system in pregnancy, its alteration after birth and consequently on the female adjustment to newly formed physiological condition. It would be valuable to determine the reasons why some of the child bearing women do not manifest physiological disorders after birth, or develop milder or heavier form of disease. We presumed that the starting point for understanding mood changes are the information about physiology of serotonergic system in pregnancy and after birth. Therefore, the aim of this study was to determine physiological value of platelet serotonin in term pregnancy and after birth. For better understanding of physiology of the serotonergic system in pregnancy the obtained values were compared to platelet serotonin level in non pregnant women of reproductive age. From the results we expected to define: 1. physiological concentrations of platelet serotonin level in healthy primigravidae at term and after the birth; 2. variation of serotonin level in the

studied group; 3. variation of serotonin level in pregnancy considering the level of 5-HT in non pregnant population of women.

The platelet serotonin concentration determined in the Croatian pregnant women population represent physiologic values of serotonin at term pregnancy. The research is performed and the researched values determined for the first time in the Croatian pregnant women population.

Materials and Methods

Health criteria for participating subjects were healthy primiparous women at term pregnancy with no prior medical history.

The research plan and its implementation was approved by the hospital Ethics Committee. Only when given the necessary information, signed consent for participation in the research was invested in her medical records.

The study included 100 pregnant women. Data for non pregnant women were used from the research of Pivac N³⁶ which involved 55 women of reproductive age. The concentration of 5-HT in both these groups was obtained by the same procedure, and expressed in the same measurement units, nmol/mg protein. Inclusion criteria were the same for all patients.

Their blood was sampled before and not later than two days after birth. Samples were collected in the Department of Gynecology and Obstetrics in the »Sestre milosrdnice« University Hospital from cubital vein. Samples were shipped within 24 hours to the »Ruđer Bosković« Institute, Department for molecular biology, Division for molecular neuropsychiatry, where the value of platelet 5-HT was determined. The information about the medical history, the course of childbirth and complications during labor and after birth was obtained from medical records of the patients.

Determination of platelet serotonin concentration

Platelet serotonin concentration was determined by the spectrofluorimetric method, as previously described¹⁷. Briefly, platelets were destroyed by sonication (20 kHz, amplitude 8 3 1023 mm for 30 sec). Specimens of standard, blank (water), and platelet sonicates were analyzed in duplicate. All samples were deproteinized with 1 mL of 10% ZnSO₄ and 0.5 mL of 1 N NaOH. For the preparation of fluorophore, 0.2 mL of L-cysteine (0.1%) and 1.2 mL of orthophthalaldehyde (0.05%) were added to deproteinized samples. The measurement of the serotonin fluorescence was performed on a Varian Cary Eclipse spectrofluorimeter. Platelet protein levels were measured by the spectrophotometric method¹⁶.

Statistical analysis

Due to the fact that the analysis using Kolmogorov-Smirnov test showed normal distribution of the results, the analysis was performed using untransformed data.

Physical-chemical factors were analyzed using parametric tests and t-test for testing the difference of significance between the two dependent and independent groups. Two-way analysis of variance (ANOVA) with the boundary value of $p < 0.01$ and $p < 0.05$ was used. The Pearson correlation coefficient is used for analysis of the correlation between certain parameters, with boundary significance $p < 0.05$. Results are presented as mean and standard deviation ($\bar{X} \pm SD$).

Results

The study was conducted on a sample of 100 nulliparous women. Eighteen patients were excluded from the study because of the inadequacy of the sample. The platelet serotonin level is expressed in nmol/mg protein.

The mean age in the pregnant women group is 27.3 ± 4 years. ($\bar{X} \pm SD$)

The mean age in the non pregnant group is 29.8 ± 5.5 . ($\bar{X} \pm SD$)

The mean value of platelet serotonin concentration in the pregnant women at term pregnancy is 1.209 ± 0.458 ($\bar{X} \pm SD$) and the mean value of platelet serotonin concentration in the researched group after the delivery is 1.045 ± 0.395 ($\bar{X} \pm SD$). The mean value of platelet serotonin level in non pregnant women group is 1.088 ± 0.280 ($\bar{X} \pm SD$) (Table 1). T-test for independent samples showed the significant difference in the platelet serotonin concentration between the researched groups. Comparing the platelet serotonin level in pregnant women at term pregnancy and in the same group after the delivery, significantly lower platelet serotonin level after the delivery is found ($t = 4.128$, $df = 81$, $p \leq 0.01$). Comparing the platelet serotonin level of pregnant women and non pregnant women statistically higher level of platelet serotonin is determined ($t = 1.57$, $df = 135$, $p \leq 0.01$). Comparing the platelet serotonin level in women after the delivery with non pregnant women we found significantly lower platelet serotonin concentration ($t = 0.96$, $df = 135$, $p \leq 0.01$) (Table 1).

The subjects were divided in two groups according to the age. The group aged 28 years or younger and older than 28 years. There were 38 women aged 28 years or younger and 44 women older than 28 years. The mean value of platelet serotonin concentration in the group of

TABLE 1
PLATELET SEROTONIN LEVEL IN THE GROUP OF NON PREGNANT WOMEN (1) PREGNANT WOMEN (2) AT TERM PREGNANCY AND AFTER DELIVERY (3), ARITHMETIC MEAN AND STANDARD DEVIATION ($\bar{X} \pm SD$)

5-HT	N	\bar{X}	SD
Non pregnant women (1)	55	1.088	0.280
After delivery (2)	82	1.045	0.395
Before delivery (3)	82	1.209	0.458

$p(1):(2) t = 0.96$, $df = 135$, $p \leq 0.01$

$p(1):(3) t = 1.57$, $df = 135$, $p \leq 0.01$

$p(2):(3) t = 4.128$, $df = 81$, $p \leq 0.01$

TABLE 2
THE DIFFERENCE IN PLATELET 5HT LEVEL BEFORE AND AFTER THE DELIVERY AND ITS CHANGE IN WOMEN ACCORDING TO THE AGE

5-HT platelet level	$\bar{X}\pm SD$ (1)	$\bar{X}\pm SD$ (2)	t	DF	p	N (1)	N (2)
Before the delivery	1.244±0.469	1.179±0.452	0.636	80	0.526	38	44
After the delivery	0.980±0.300	1.101±0.458	-1.383	80	0.1703	38	44
The difference in the 5-HT level change between the groups	0.263±0.352	0.078±0.347	2.393	80	0.019*	38	44

*p≤0.01

(1) – the group aged 28 years or younger

(2) – the group older than 28 years

women at term pregnancy in the group aged 28 years or less is 1.244±0.470 ($\bar{X}\pm SD$), and in the same group after delivery 0.980±0.300 ($\bar{X}\pm SD$) (Table 2). In the group of women older than 28 years the mean value of platelet serotonin concentration before delivery was 1.18±0.452 ($\bar{X}\pm SD$) and after delivery 1.10±0.458 ($\bar{X}\pm SD$) (Table 2).

Using T-test for independent samples we did not find significant difference in the platelet serotonin concentration between younger and older women neither at term pregnancy (t=0.636, df 80, p≥0.01; p≥0.05) nor after the delivery (t=-1.383, df 80, p≥0.01; p≥0.05). However, the variability of the concentration of platelet serotonin in the younger group was significantly higher than the variability of the concentration of platelet serotonin in the older group (t=2.39, df 80, p=0.02 Table 2).

Discussion

The results were carried out on a sample of 82 healthy primiparae.

Physiological concentrations of platelet serotonin were studied at term pregnancy and after birth.

The mean value of platelet serotonin at term pregnancy and after birth is established (Table 1).

Using t-test for dependent samples a statistically significant decrease of the platelet serotonin concentration after birth is determined (Table 1). Comparing the platelet serotonin concentrations of both groups with platelet serotonin level in the non pregnant women of reproductive age, we found significantly higher platelet serotonin level in the pregnant women at term pregnancy (Table 1) and significantly lower platelet serotonin concentration after delivery (Table 1).

Using t-test for independent samples we compared the concentration of platelet serotonin in the group of women aged 28 years or younger, and older than 28 years, statistically significant difference is not established (Table 2), but higher variability of the platelet serotonin level in the younger group was observed.

There are no studies in the current literature which compare precisely these parameters. According to some studies, the concentration of serotonin during pregnancy is variable, showing a gradual increase with the peak in the second quarter and a gradual tendency to fall on non

pregnant level during the period of term pregnancy¹⁵. The results obtained in this study do not corroborate the literature data of other studies. All the previously mentioned shows the need for determining precise normal range of serotonin in healthy pregnant women. In the literature is often cited study of serotonin concentrations in pregnant women whose pregnancy and delivery is complicated by an illness related to pregnancy such as preeclampsia or hypertension. The literature lists the various risk factors examined in the etiology of mood disorders occurrence^{8,11}.

A group of American authors have found younger age to be the risk factor for postpartum depression and in the population of black women^{14,15}. In Caucasian women the age is not recognized as a risk factor for developing postpartal mood disorders¹⁴. Also the results of the majority researches are based on questionnaires about postpartal mood change. Biological factors such as the concentration of serotonin is not measured. Other information about changing the concentration of serotonin before and after childbirth in the literature is very poor.

In conclusion we could say that mood changes after birth are the subject of numerous studies. Understanding the pathophysiology of the disorder is the key for its successful and prompt prevention. Attempts to identify the risk factors, prevention methods or mechanisms of the disease are unsuccessful. The results of almost all studies show the need for newer and multidisciplinary research approach, which in itself highlights the problem.

Long investigated interactions between serotonergic system, hormonal and other systems have been proven to be of a paramount importance. Postpartum mood disorders in the last decade have increased and are still increasing. Pregnancy is a very dynamic period for the women organism in a physiological/biological sense and in psychological terms. From the biological point the body passes through the maelstrom of hormonal changes and changes in other organ systems. From the psychological point, women are at a special state of adaptation to the organic and other changes. In these two complex systems the risk factors for puerperal mood disorders are not easily identifiable because of their variability and interactions.

Since the clear recognition of the symptom is difficult the prompt treatment is impossible. At this point, we can only treat the women who develop disease. There are no proven or recommended other preventive measures.

We believe that physiologic values of the serotonergic components for which is proven to be highly influenced in the time of pregnancy and puerperium are very important to determine. Knowing the baseline value every deviation can easily be detected which enables us for prompt reaction.

By measuring and determining physiologic values of platelet serotonin in healthy women at term pregnancy and after birth in Croatian women, we wanted to identify the risk factors with the emphasis on biological aspects of this problem.

Not only a significant change in the concentration of serotonin after birth is established but also a significant difference in the platelet 5-HT concentrations between the nonpregnant group and women before and after delivery. As previously mentioned, the data on the physiological concentrations of serotonin in the literature is very scarce, while the data in the same groups of pregnant women/new mothers with complications are numerous. It should be noted that the data in the literature was obtained in the research in other countries. According to our knowledge, this is so far the first study in Croatia, where we studied and obtained physiological concentrations of serotonin in healthy pregnant women.

We hope that the obtained values can and will be used as a starting point for further research in Croatia.

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TROMBOCITNI SEROTONIN U TERMINSKOJ TRUDNOĆI I NAKON PORODA – FIZIOLOŠKE VRIJEDNOSTI ZA HRVATSKU POPULACIJU

SAŽETAK

Svrha ovog istraživanja je bila utvrditi fiziološke vrijednosti trombocitnog serotonina (5-HT) i njegove promjene kod trudnica u terminu i nakon porod i usporediti dobivene vrijednosti 5-HT-a s vrijednostima kod žena koje nisu trudne. Utvrđivanje normalnih vrijednosti 5-HT-a u trudnoći i nakon bi moglo u budućnosti koristiti za daljnja mjerenja, te procjenu različitih psiholoških i psihijatarskih poremećaja vezanih s trudnoćom. Vrijednosti i varijacije 5-HT-a bi mogle pomoći i u boljem razumijevanju promjena raspoloženja nakon poroda. Ukupno 137 zdravih hrvatica je bilo uključeno u ispitivanje – 82 trudnica i 55 netrudnica. Uzimani su uzorci krvi u kojima je određivana koncentracija serotonina. U trudnica su uzeti uzorci u dva navrata, tijekom termineske trudnoće i uskoro nakon poroda. Srednja vrijednost 5-HT-a je u trudnica bila 1,209 nmol/mg proteina, nakon poroda 1,045 nmol/mg proteina, a u netrudnih žena 1,088 nmol/mg proteina. Razlike među skupinama su statistički značajne. Nismo našli statistički značajne razlike s obzirom na dob žena.