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EVALUATION OF PROGESTERONE AND THE PROGINS PROGESTERONE RECEPTOR GENE POLYMORPHISM IN THE DEVELOPMENT OF SOME FORMS OF PREMENSTRUAL SYNDROME

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ABSTRACT

One of the most common neuroendocrine syndromes in gynecology is premenstrual syndrome. The article is devoted to the study of progesterone level and frequency of polymorphic variants T1T2 of progesterone receptor gene PROGINS, as well as their possible relationship in patients with various forms of PMS. The study involved 50 women of reproductive age with PMS diagnosis, including 25 women with edematous form of the disease, 25 - with neuropsychic form. Of the same 50 patients twenty five women had mild form of the disease, and 25 women had severe form. Control group consisted of 25 women without PMS diagnosis. Progesterone level was determined in blood serum twice in the first and second phase of a menstrual cycle. Polymorphism of T1T2 of PROGINS progesterone receptor gene was studied using polymerase chain reaction.

We found hypoprogesteronemia of the luteal phase of a menstrual cycle in women with PMS, especially pronounced in persons with edematous and severe forms of the disease ($p<0.05$).

Polymorphic variant T1T2 of PROGINS gene can be considered as a marker of PMS development. T1T1 genotype was significantly associated with development of edematous form ($\chi^2=4.50$, $p=0.03$) and its presence may indicate a tendency to develop into severe form of PMS ($\chi^2=3.21$, $p=0.07$). T2 allele can be considered as a protective mechanism for the appearance of PMS, particularly its edematous and severe forms, in which the presence of T2 allele reduces the risk of PMS, respectively, by 3.0 ($\chi^2=4.50$, $p=0.03$, $OR=0.20$, 95% CI 0.05-0.78, $p=0.02$) and by 1.71 times ($\chi^2=3.21$, $p=0.07$, $OR=0.27$, 95% CI 0.08-0.95, $p=0.04$) compared with the control group.

Reduced blood level of progesterone in women with PMS correlated with the presence of T1T1 genotype of PROGINS gene. PMS patients with T1T2 genotype exhibited significantly low levels of this hormone in the second phase of menstrual cycle ($p<0.05$) compared to women with the same genotype in the control group. Furthermore, women with this neuroendocrine syndrome and T1T2 genotype showed no statistically significant difference in progesterone level in these groups compared with healthy women with the same genotype ($p>0.05$).

KEYWORDS: *premenstrual syndrome, progesterone, progesterone receptor gene PROGINS, polymorphism.*

Introduction

Premenstrual syndrome (PMS) is one of the most common neuroendocrine syndromes in gynecology. It affects mostly women of reproductive age, regardless of their country of residence [*Direkvand-Moghadam A et al., 2014*]. More than 150 symptoms of physical and psychological nature may occur for 2-14 days before menstruation. This condition significantly disturbs the lifestyle of women [*Choi D et al., 2010; Dennerstein L et al., 2010*]. In the development of PMS significant role belongs to reproductive hormones. Progesterone is a hormone, specifically involved in the luteal phase of a menstrual cycle. Its study raises many questions about its role in the development of this syndrome. Progesterone, and most likely its metabolites allopregnanolone and pregnanolone, have some importance in genesis of various symptoms of PMS in the luteal phase (mood changes, metabolic

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disorders) [*Ziomkiewicz A et al., 2012; Bäckström T et al., 2014*]. But the results of studies of its level in women with PMS are controversial. Scientists have found an increase [*Lekareva TM, 2007*], decrease [*Qiao M et al., 2008*], and even a stable level of progesterone in the luteal phase of a menstrual cycle in women with this syndrome [*Yakovleva EB, Loskutova OV, 2009; Ismailov SI et al., 2010*].

Progesterone exerts its effects on cells through receptors A and B. Progesterone gene is located in the chromosome 11q22-23. One of the polymorphic variants of progesterone gene is PROGINS [*Romano A et al., 2007*]. To date, the role of genetic factors in the development of gynecological pathology is not well studied. Is an open question about the meaning of gene PROGINS in the development of breast cancer [*Giacomazzi J et al., 2012; Rockwell LC et al., 2012*], genesis of recurrent abortion [*Su MT et al., 2011; Traina E et al., 2011*]. PROGINS polymorphism of progesterone receptor may be associated with endometriosis and infertility on its background [*Christofolini DM et al., 2011; Costa IR et al., 2011*].

The contribution of genetic factors in pathogenesis of PMS is least studied. In the study of polymorphism of S/L of PROGINS progesterone receptor gene, its role in the emergence of this disease was not found. Scientists believe that certain meaning of combination of "increased functional activity of the corpus luteum in the second phase of a menstrual cycle and presence of L allele of PROGINS progesterone receptor gene" takes place in the development of PMS [*Aganezova NV, 2011*].

The objective of this study was to determine the level of progesterone and frequency of polymorphic variants T1T2 of PROGINS progesterone receptor gene, as well as their possible relationship in patients with various forms of PMS.

Material and Methods

The clinical study was conducted at Ivano-Frankivsk Clinical Maternity Hospital (Ivano-Frankivsk, Ukraine).

A total of 75 patients of reproductive age were involved in the study. The control group included 25 without PMS diagnosis, and the test group included 50 women with PMS diagnosis. Diagnosis of PMS was determined on the basis of the presence of cyclical manifestations of the disease in the luteal phase of a menstrual cycle and the patient's self-observation diary for 2-3 menstrual cycles (R. Moos Menstrual Distress Questionnaire). It should be noted that there are several classifications of PMS, including clinical form, severity of the disease, etc. These 50 patients had edematous and neuropsychic forms in accordance with V.P. Smetnik et al (1999) classification. Severity of the disease was determined in the same 50 patients in mild and severe forms according to the Order No. 676 of the Ministry of Health of Ukraine, 31.12.2004 [*Ministry of Health of Ukraine, 2004*]. The criteria for selection of patients in research are presented in Table1

Progesterone level was determined in blood serum twice at 5-7th and 18-22nd days of a menstrual cycle using immunoenzymometric analysis with a reagent set Progesterone EIA (XEMA Co., Ltd., Russia) and the analyzer Stat Fax 303 Plus (USA).

T1T2 polymorphism of the PROGINS progesterone receptor gene was studied in a research laboratory, Department of Medical Genetics, Shupyk National Medical Academy of Postgraduate Education (Kiev, Ukraine). Material for the study was peripheral blood, which was collected into tubes with EDTA in the amount of 2.7 *ml*. Then DNA was isolated using a commercial set DNA-sorb-B (Institute of Epidemiology of the Ministry of Health of Russian Federation). Polymerase chain reaction was performed using the reagents of Fermentas Company (Lithuania) in thermocycler FlexCycler (Analytik Jena, Germany). DNA amplification products were separated according to their molecular weight by electrophoresis in 2% agarose gel with addition of ethidium bromide. Imaging was performed using a computer system Vitran.

Statistical analysis was conducted using Statistica 6.0 software. We calculated arithmetic mean value (M), average standard deviation (m), significance of differences of research findings (p). Comparison of two independent groups by a single feature employed nonparametric Mann-Whitney test, comparison of two dependent groups was performed with a Wilcoxon test. All calculations were run with reliable probability (1-P) 0.95. We also used criterion χ^2, odds ratio (OR) and confidence interval (CI). The difference between the values to be compared were considered significant by $p<0.05$.

TABLE 1.

The criteria for selection of patients in research

Inclusion criteria:	Exclusion criteria:
• reproductive age (18-44 years) • regular menstrual cycles • presence of PMS • written consent of the patient.	• women who had pregnancy or lactation at the time of the study • disorders of menstrual cycle • focal lesions of breast • dysfunctional uterine bleeding of unknown etiology • acute inflammation of pelvic organs • tumors of uterus and ovaries of unknown etiology • endometrial hyperplasia • genital endometriosis • severe somatic pathology in the history • organic pathology of the central nervous system • mental illness • hormonal tumors • diabetes • adrenal diseases • malignant tumors in the present or in the past • premenstrual dysphoric disorder • women who took psychotropic medications or hormonal therapy within the last 3 months.

Results and Discussion

Progesterone levels in the follicular phase of a menstrual cycle were similar in control (4.96±0.68 *ng/ml*) and test groups (5.16±0.47 *ng/ml*). In both groups of patients we found a significant increase of serum progesterone in phase II compared to phase I (Fig. 1). In the control group its concentration increased by 12.17 times to 60.37±6.18 *ng/ml* ($p<0.001$). Increase of progesterone in the luteal phase in women with PMS was considerably lower (7.61 times) than in healthy women reaching 39.29±4.33 *ng/ml* ($p<0.001$) and amounted to 65.08% of the level in control group ($p<0.004$).

Concentration of progesterone in the phase I did not differ in women with various forms of PMS and was consistent with the level of healthy women (table 2). The smallest increase of progesterone was observed in patients with edematous form of PMS by 5.43 times reaching 29.16±3.48 *ng/ml* ($p<0.001$), which accounted for only 48.30% of that of the control group ($p<0.001$), and with severe form by 7.06 times reaching 37.12±5.55 *ng/ml* and amounted to 61.49% of the level of healthy women ($p<0.001$). In patients with neuropsychic form progesterone increased 10.00 times reaching 49.42±7.49 *ng/ml* ($p<0.001$) and was 81.86%

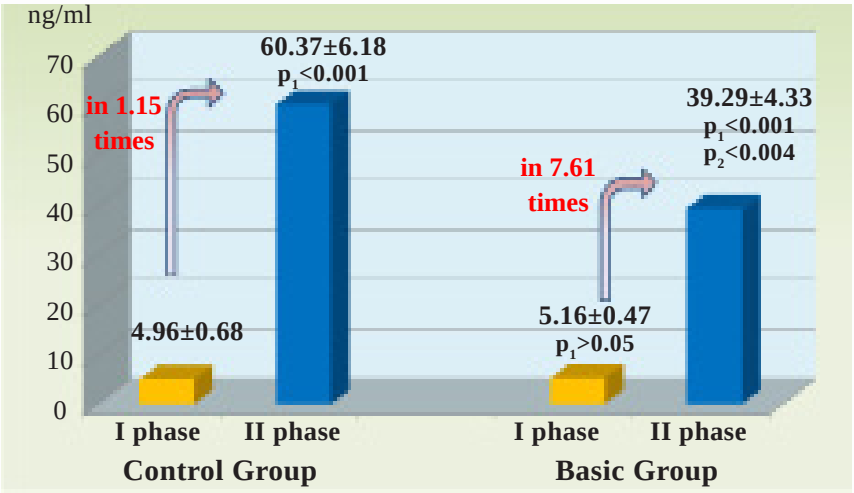

FIGURE 1. *Progesterone level in blood serum in women under study in two phases of a menstrual cycle.*
NOTES: *p_1 – probability of difference of index of the second phase of menstrual cycle relative to the first one, p_2 – probability of difference of index relative to control group*

Table 2.

Progesterone levels in women's blood serum, depending on the form of PMS, *ng/ml*

Mark	Control group n=25		Test group with PMS forms: Edematous n=25		Neuropsychic n=25		Mild n=25		Severe n=25	
Phase of cycle	I	II	I	II	I	II	I	II	I	II
M	4.96	60.37	5.37	29.16	4.94	49.42	5.05	42.97	5.26	37.12
±m	0.68	6.18	0.45	3.48	0.82	7.49	0.80	6.75	0.50	5.55
p_1		<0.001		<0.001		<0.001		<0.001		<0.001
p_2				<0.001		0.08		0.02		0.007

Notes: p_1 – index of probability relative to index of I phase
p_2 – index of probability relative to index of control group

(p=0.08) of the index of women in the control group. Mild PMS form was characterized by 8.51 times increase of the hormone level reaching 42.97±6.75 *ng/ml* (71.18% progesterone level of healthy women).

Evaluation of results of the distribution of T1T2 polymorphic variants of the PROGINS progesterone receptor gene showed that T2T2 pathological variant was quite rare in test and control groups, amounting to 2.0% of the women with PMS diagnosis and 8.0% without PMS (Fig. 2).

T1T1 genotype was 1.38 times more common in women with PMS (72.0%) compared with healthy women (52.0%). Analysis of the forms and severity of the disease determined significant differences in

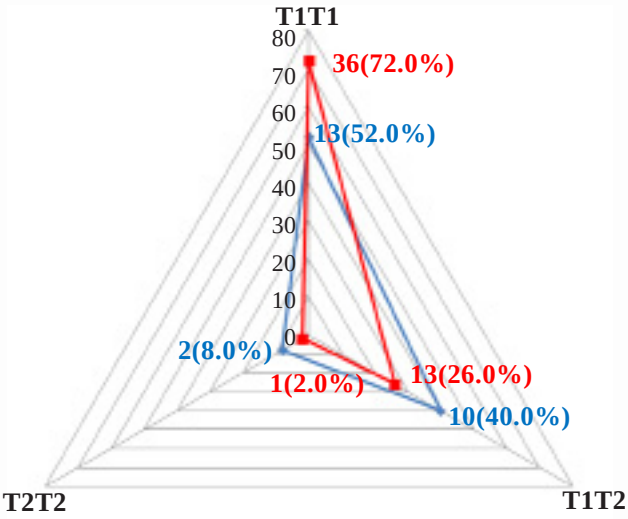

Figure 2. *The frequency of T1T2 polymorphic variants of the progesterone receptor gene PROGINS in women under study.*
Note: *Control group (Blu Curve), Test group (Red curve)*

the prevalence of the genotypes among patients with edematous form of PMS and those of control group (table 3). T1T1 genotype was determined to be 1.61 times more frequent in women with edematous form (χ^2=4.50, p=0.03), and 1.54 times more frequent in women with severe form (χ^2=3.21, p=0.07) compared to healthy ones (table 4).

Analysis of the T1T2 genotype distribution demonstrates a tendency to greater prevalence (1.54 times) in women of control group (40.0%) compared to the test group (26.0%). In patients with edematous and severe forms of PMS, T1T2 genotype was found to be 2.5 and 2.0 times less frequent than that in healthy women (p>0.05).

Validity of the significance of T1T1 genotype in the development of PMS is also supported by the fact that the value of odds ratio in the case of this disease in total (in test group) and its various forms and severity is greater than one (1.39-4.85). While in patients with edematous and severe forms, p is less than 0.05 relative to healthy women (table 5). This indicates to the association of T1T1 genotype of PROGINS gene with increased risk of PMS.

T1 allele was the most common in women of both groups. Its prevalence in patients with PMS amounted to 98.0% (49 patients), and 92.0% (23 women) in healthy women. While 100.0% of women with edematous and severe forms of the neuroendocrine syndrome were carriers of this allele, those with neuropsychic and mild PMS forms comprised 96.0% (24 patients).

We found that the T2 allele of the PROGINS gene was determined in women of the control group 1.70 times more frequent (12 women,

TABLE 3.

Frequency of T1T2 polymorphism of the PROGINS progesterone receptor gene in women under study

Groups	n	T2T2		T1T2		T1T1	
		Abs.	%	Abs.	%	Abs.	%
Control	25	2	8.0	10	40.0	13	52.0
Edematous form	25	-	-	4	16.0	21	84.0
Neuropsychic form	25	1	4.0	9	36.0	15	60.0
Mild form	25	1	4.0	8	32.0	16	64.0
Severe form	25	-	-	5	20.0	20	80.0
Test group, total	50	1	2.0	13	26.0	36	72.0

TABLE 4.

Frequency of T1T2 polymorphism of the PROGINS progesterone receptor gene in women under study depending on form and severity of PMS compared with control group

Groups		n	T2T2			T1T2			T1T1		
			%	χ^2	p	%	χ^2	p	%	χ^2	p
Control		25	8.0			40.0			52.0		
PMS form	Edematous	25	-	-	-	16.0	2.48	0.12	84.0	4.50	0.03
	Neuropsychic	25	4.0	0.00	1.00	36.0	0.00	1.00	60.0	0.08	0.78
	Mild	25	4.0	0.00	1.00	32.0	0.09	0.77	64.0	0.33	0.57
	Severe	25	-	-	-	20.0	1.52	0.21	80.0	3.21	0.07
Test group, total		50	2.0	0.39	0.53	26.0	0.32	0.57	72.0	2.13	0.14

NOTE: p – probability of difference of index relative to control group

TABLE 5.

T1T2 genotypes of the PROGINS progesterone receptor gene polymorphism as markers of the PMS risk

PMS forms	Indicator	T2T2	T1T2	T1T1
Edematous	OR	0.18	0.29	4.85
	CI	0.01-4.04	0.08-1,09	1.29-18.26
	p	0.28	0.07	0.02
Neuropsychic	OR	0.48	0.84	1.39
	CI	0.04-5.65	0.27-2.65	0.45-4.25
	p	0.56	0.77	0.57
Mild	OR	0.48	0.71	1,64
	CI	0.04-5.65	0.22-2.25	0.53-5.09
	p	0.56	0.56	0.39
Severe	OR	0.18	0.38	3.69
	CI	0.01-4.04	0.11-1.30	1.05-12.96
	p	0.28	0.13	0.04
Test group, total	OR	0.23	0.53	2.37
	CI	0.02-2.72	0.19-1.46	0.87-6.44
	p	0.25	0.22	0.09

48.0%) than in persons of the test group (14 patients, 28.0%; χ^2=2.13, p=0.14, OR=0.42, 95% CI 0.16-1.14, p=0.09). T2 allele was 3.0 and 1.71 times less frequent in patients with edematous and severe PMS forms as compared to the controls, respectively, in 16.0% (χ^2=4.50, p=0.03, OR=0.20 95% CI 0.05-0.78, p=0.02) and 20.0% (χ^2=3.21, p=0.07, OR=0.27, 95% CI 0.08-0.95, p=0.04). In patients with neuropsychic form of the disease the number of T2 allele carriers did not differ from that of healthy women and reached 40.0% (χ^2=0.08, p=0.77, OR=0.72, 95% CI 0.24-2.22 p=0.57).

Such a statistical accuracy of T2 allele distribution in study groups may indicate its importance in the development of the disease as a protective element against the emergence of PMS.

We further attempted to determine whether there is a relationship between progesterone blood level and a form of PROGINS gene genotype. Analysis of the research findings demonstrated significant differences between the presence of various genotypes of the PROGINS progesterone gene T1/T2 polymorphism in various PMS forms and levels of serum progesterone in the luteal phase of a menstrual cycle (table 6). Women of the test group with T1T1 genotype had progesterone concentration in the luteal phase of a menstrual cycle 1.94 times lower than women with T1T1 genotype in the control group (p<0.001), amounting to 51.49% of the control level. The lowest content of progesterone in persons under study with T1T1 genotype was found in women with edematous and severe forms of the disease. In edematous PMS form and the presence of T1T1 genotype, the level of this hormone was 2.34 times less (p<0.001), in severe form 2.04 times less (p=0.001), as compared to healthy women with T1T1 genotype, and reached 42.69% and 49.06% of the controls' level. In persons with neuropsychic and mild PMS forms and with T1T1 genotype significantly low levels of progesterone were found, amounting to 63.81% (p=0.03) and 54.45% (p=0.002) of the levels of the control group patients with T1T1 genotype.

In women with PMS who had T1T1 genotype, the concentration of progesterone was lower by 19.37% than in the T1T2 genotype carriers of the same group. In particular, the patients with T1T1 genotype had lower progesterone level than those with T1T2 genotype: in edematous form by 9.44%, in neuropsychic form by 10.48% lower, and in severe PMS by 38.67%. In healthy women with T1T1 genotype the level of progesterone was, conversely, higher than that in T1T2 or T2T2 genotypes carriers. Furthermore, women with T1T2 genotype of the test group showed no statistically significant difference in progesterone levels compared to women with the same genotype in the control group (p>0.05).

Table 6.

Progesterone levels of women in the luteal phase of a menstrual cycle depending on T1/T2 polymorphism of PROGINS progesterone receptor gene, *ng/ml*

Groups	T1T1		T1T2		T2T2	
	n	M±m	n	M±m	n	M±m
Control	11	67.18 ± 4.59	7	56.11 ± 13.95	2	37.75±34.55
Edematous form	21	28.68 ± 4.05 p<0.001	4	31.67 ± 5.26 p=0.26	-	-
Neuropsychic form	15	42.87 ± 7.10 p=0.02	9	47.89 ± 11.70 p=0.71	1	161.30
Mild form	16	36.58 ± 5.45 p=0.004	8	40.95 ± 9.89 p=0.42	1	161.30
Severe form	20	32.96 ± 5.46 p=0.001	5	53.74 ±14.13 p=0.68	-	-
Test group, total	36	34.59 ±3.91 p<0.001	13	42.90 ± 8.37 p=0.45	1	161.30

***Note**: p – index of probability in comparison with control group*

Conclusions

The changes identified point to a deep and complex nature of PMS that is specific to each form of the disease. PMS is characterized by hypoprogesteronemia in the luteal phase of a menstrual cycle, especially pronounced in edematous and severe forms of the disease ($p<0.05$).

Polymorphic variant T1T2 of the PROGINS gene can be considered as a marker of PMS development. T1T1 genotype in women with edematous form was determined to be 1.61 times more frequent ($\chi^2=4.50$, $p=0.03$, OR=4.85, 95% CI 1.29-18.26, $p=0.02$), and 1.54 times more frequent in women with severe form ($\chi^2=3.21$, $p=0.07$, OR=3.69, 95% CI 1.05-12.96, $p=0.04$) compared to the control group. Thus, T1T1 genotype was significantly associated with the development of edematous forms of the disease and its presence may indicate a tendency to develop severe form of PMS.

T2 allele can be considered as a protective mechanism for the appearance of PMS, particularly its edematous and severe forms, in which the presence of T2 allele reduces the risk of PMS, respectively by 3.0 times ($\chi^2=4.50$, $p=0.03$, OR=0.20 95% CI 0.05-0.78, $p=0.02$) and by 1.71 times ($\chi^2=3.21$, $p=0.07$, OR=0.27, 95% CI 0.08-0.95, $p=0.04$) compared to the control group.

Reduced blood level of progesterone in women with PMS correlated with the presence of T1T1 genotype of the PROGINS gene. In the presence of edematous, neuropsychic, severe and mild forms of PMS and the T1T1 genotype there were significantly low levels of this hormone in the second phase of a menstrual cycle ($p<0.05$) relative to women with the same genotype in the control group. Furthermore, there was no statistically significant difference in progesterone levels in women with T1T2 genotype of these groups as compared to the indicators of the control group ($p>0.05$).

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