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Diagnostic Markers of Primary Infertility in Women of Reproductive Age with Hypothalamic Dysfunction in the Pubertal Period

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Abstract

The aim of the study was to assess fertility in women of reproductive age with hypothalamic dysfunction (HD) in the pubertal period and to determine the diagnostic significance of pro-inflammatory (TNF-α and IL-1β), anti-inflammatory cytokine (IL-10) and NF-kB activity in the diagnosis of primary infertility in these women.

Materials and Methods: Fertility was assessed in 86 women of reproductive age with HD in the pubertal period. A comparative characteristic of fertile women (Group 1, n=46) and primary infertility women (Group 2, n=21) with HD in the pubertal period was performed. FPG and FPI were determined after 8 to 12 hours of fasting. Serum IRI concentrations were measured using an ELISA kit. The levels of TNF-α, IL-1β and IL-10 were determined in the venous blood serum after a 12-hour fasting, as well as in uterine aspirate (UA) on the 21st day of the menstrual cycle using ELISA kits. The activity of NF-kB was determined in UA on the 21st day of the menstrual cycle using an enzyme immunoassay kit.

Results: BMI in Group 1 was significantly lower than in Group 2: 22.63±2.68 kg/m^2 versus 27.05±4.03 kg/m^2 (P=0.000). WC in women of Group 1 was 66.11±5.66 cm versus 78.52±10.54 cm in Group 2 (P=0.000); WC >80 cm was found in 2(4.4%) and 14(66.7%) women, respectively (P=0.000). The average levels of FPG and FPI were significantly higher in Group 2. Serum levels of TNF-α and IL-1β in Group 2 were significantly higher than in Group 1. The serum level of anti-inflammatory cytokine IL-10 was significantly lower in Group 2; accordingly, the TNF-α/IL-10 ratio in Group 2 was 1.8 times higher than in Group 1. The IL-1β level in UA (P=0.000) and the TNF-α/IL-10 ratio (P=0.02) were significantly higher in women of Group 2 than Group 1, which indicated the pronounced inflammatory effects of TNF-α in the endometrium. In women of Group 2, the NF-kB level in UA was 1.4 times higher than in Group 1 (P=0.000).

Conclusion: Every fourth woman of reproductive age with HD in the puberty period has primary infertility. The results obtained indicate the activation of the Th-1 immune response with the formation of the inflammatory reactions at the systemic level and in the endometrium. Diagnostically significant markers of primary infertility are the serum TNF-α level and the UA levels of IL-1β and NF-kB. **(International Journal of Biomedicine. 2017;7(3):213-217.)**

Key Words: hypothalamic dysfunction • primary infertility • endometrium • cytokines

Abbreviations

BMI, body mass index; **FPG**, fasting plasma glucose; **FPI**, fasting plasma insulin; **HD**, hypothalamic dysfunction; **IRI**, immunoreactive insulin; **IR**, insulin resistance; **UA**, uterine aspirate (an aspirate from the uterine cavity); **WC**, waist circumference.

Introduction

In recent years, more attention has been paid to the development of reproductive health and the reproductive potential of adolescent girls, as future mothers. It has been noted that in the Russian Federation in the 16-17 age group, the prevalence of endocrine pathology is 5 times higher than in the whole population.[1] According to the data of a number of researchers, the prevalence of HD in the pubertal period among girls is from 7.1% to 25%.[2,3] On the background of HD, obesity has been diagnosed in 61.1% of adolescent girls.[4] Analysis of prospective studies shows that HD in the pubertal period leads to excess body weight in the reproductive age [5,6] and increases the frequency of reproductive disorders by 2

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times.[3,7,8] According to the data of a number of researchers, a slight inflammatory process is formed on the background of obesity.[7,9-11] Pro-inflammatory cytokines secreted by fat tissue affect the state of the endometrium and ovulation.[11-13] In this regard, studying a number of proinflammatory and anti-inflammatory cytokines at the systemic and local levels in women of reproductive age with HD in the pubertal period is the most promising way of determining the diagnostic markers of primary infertility.

The aim of the study was to assess fertility in women of reproductive age with HD in the pubertal period and to determine the diagnostic significance of pro-inflammatory (TNF-α and IL-1β), anti-inflammatory cytokine (IL-10) and NF-kB activity in the diagnosis of primary infertility in these women.

A prospective study was conducted between 2000 and 2013. The mean follow-up was 4.7±1.7 years. Of the 170 potential participants in the pubertal period, 86 were enrolled in the study at the reproductive age, and 84 were excluded from the study.

The study protocol was reviewed and approved by the Ethics Committee of Amur State Medical Academy. All participants provided the written informed consent. Inclusion criteria were age over 18 years, HD (ICD-10-CM E23.3) manifested by the neuroendocrine (excessive body weight or obesity) and neurotrophic (pink or white striae) disorders according to electroencephalography (EEG) in the pubertal period. Exclusion criteria were tubal infertility (ICD-10-CM N 97.1), previous pelvic inflammatory diseases in history (ICD-10-CM N70 - N75), women of reproductive age who do not plan pregnancy.

In order to identify diagnostic markers of primary infertility in women of reproductive age, we performed a comparative characteristic of fertile women (Group 1, n=46) and primary infertility women (Group 2, n=21) with HD in the pubertal period. In the reproductive age, fertility categories were assessed in accordance with the standardized WHO protocol No.88093.

BMI is calculated using Quetelet's formula (kg/m²). The nature of the distribution of adipose tissue was determined by WC. WC >0.80 cm showed an abdominal type of obesity according to IDF. FPG and FPI were determined after 8 to 12 hours of fasting. Serum IRI concentrations were measured using an ELISA kit (Monobind Inc., USA). To determine IR, Caro index was used (FPG(mmol/L)/IRI(μIU/ml)); Caro index <0.33 indicates IR.

Blood samples (5 ml) for serological tests were obtained from the ulnar vein on an empty stomach at 8:00 a.m. Studies were carried out in paired sera. Samples of the sera were stored at -20°C. The levels of TNF-α, IL-1β and IL-10 were determined in the venous blood serum after a 12-hour fasting, as well as in UA on the 21st day of the menstrual cycle using ELISA kits ("Interleukin 1-ELISA-BEST", "alpha"-TNF-IFA-BEST (ZAO Vector-Best, Novosibirsk), Interleukin-10 (BenderMed Systems, USA)) according to the manufacturer's recommendations. The activity of NF-kB was determined in UA on the 21st day of the menstrual cycle using an enzyme immunoassay kit (Cayman Chemical Co., USA) according to the manufacturer's recommendations.

Statistical analysis was performed using StatSoft Statistica v6.0. The mean (M) and standard deviation (SD) were calculated. Differences of continuous variables departing from the normal distribution, even after transformation, were tested by the Mann-Whitney *U*-test. Categorical variables were analyzed using the Chi-square test with the Yates' correction. Pearson's Correlation Coefficient (r) was used to determine the strength of the relationship between the two continuous variables. A probability value of $P<0.05$ was considered statistically significant.

Results and Discussion

Fertility was assessed in 86 women of reproductive age with HD in the pubertal period: 46(53.5%) women were found to be fertile, 21(24.4%) women were with primary infertility and 14(16.3%) with secondary infertility; 5(5.8%) women were with unknown fertility and a male factor of infertility. According to the goal of the study, a comparative analysis was performed between the fertile women (Group 1, n=46)) and women with primary infertility (Group 2, n=21).

The average age of women in the study groups did not differ significantly: 21.91±1.11 years and 21.85±0.97 years. BMI in Group 1 was significantly lower than in Group 2: 22.63±2.68 kg/m^2 versus 27.05±4.03 kg/m^2 (P=0.000). WC in women of Group 1 was 66.11±5.66 cm versus 78.52±10.54 cm in Group 2 (P=0.000); WC >80 cm was found in 2(4.4%) and 14(66.7%) women, respectively (P=0.000).

The average age at menarche for women in Group 1 and Group 2 was 11.67±0.76 years and 11.52±0.92 years, respectively, and did not differ significantly. Gynecological history in women of Groups 1 and 2 was complicated by oligomenorrhea (N91.3) in 2(4.35%) and 9(42.9%), respectively (P=0.000), and by excessive and frequent menstruation (N92.0) in 2(4.35%) and 8(38.1%), respectively (P=0.001). Secondary amenorrhea (N91.1) in the reproductive age was diagnosed only in 1(2.2%) woman of Group 1. Abortion in the reproductive age with complications was found in 1(2.2%) woman of Group 1. There were no spontaneous abortions in women of both groups. Two (4.3%) women of Group 1 had a delivery in their gynecologic history, and 11(23.9%) women of reproductive age in Group 1 used combined oral contraceptives to regulate the menstrual cycle and contraception.

The levels of FPG and FPI were determined in all women of Groups 1 and 2 (Table 1).

Analysis of carbohydrate metabolism showed that the average level of FPG was significantly higher in Group 2. The FPI level in women of Group 1 was 2.2 times lower than in Group 2. A Caro index <0.33 was found in 15(71.4%) women of Group 2 and in 6(13.3%) women of Group 1 (P=0.000).

To identify systemic immune disorders, serum levels of pro-inflammatory cytokines were determined. Serum levels of TNF-α and IL-1β in Group 2 were significantly higher than in Group 1 (Table 2). The serum level of anti-inflammatory cytokine IL-10 was significantly lower in Group 2; accordingly, the TNF-α/IL-10 ratio in Group 2 was 1.8 times higher than in Group 1.

Table 1.

Parameters of carbohydrate metabolism in women with HD in the pubertal period

Variable	Group 1 (n=46)	Group 2 (n=21)	*P*
FPG, mmol/l (>6.1 mmol/l)	4.27±0.51	4.55±0.52	0.03
FPI, μIU/ml (>20.0 μIU/ml)	9.62±1.99	20.97±10.75	0.000
Caro index (<0.33)	0.46±014	0.28±0.16	0.000

Table 2.

Serum cytokine levels in women with HD in the pubertal period

Variable	Group 1 (n=34)	Group 2 (n=16)	*P*
TNF-α, pg/ml	24.09±2.11	28.63±3.85	0.000
IL-1β, pg/ml	39.77±3.27	43.08±3.39	0.001
IL-10, pg/ml	13.56±1.05	10.06±2.74	0.000
TNF-a/IL-10	1.78±0.22	3.17±1.35	0.000

We determined in UA the local immune disorders, the levels of the main Th-1 and Th-2 cytokines involved in the regulation of the inflammatory process and NF-kB, as one of the regulators controlling the cascade of reactions associated with the cytokine activations. Thus, the serum level of TNF-α did not differ between the two groups, but the IL-1β level in UA was significantly higher in women of Group 2 than Group 1 (P=0.000) (Table 3).

Table 3.

UA cytokine levels in women with HD in the pubertal period

Variable	Group 1 (n=34)	Group 2 (n=16)	*P*
TNF-α, pg/ml	18.95±1.05	19.73±3.91	0.28
IL-1β, pg/ml	28.24±1.41	30.59±2.79	0.000
IL-10, pg/ml	5.87±1.88	4.88±1.87	0.08
TNF-a/IL-10	3.57±1.18	4.59±1.88	0.02

The IL-10 levels in UA did not differ significantly between Groups 1 and 2, but the TNF-α/IL-10 ratio was 1.3 times higher in Group 2 than in Group 1 (3.57±1.18 and 4.59±1.88, respectively, *P*=0.02), which indicated the pronounced inflammatory effects of TNF-α in the endometrium. Imbalance in the production of pro-inflammatory and anti-inflammatory factors indicated the activation of the Th-1 immune response with the formation of the inflammatory reactions at the systemic level and in the endometrium, which is a factor in reducing its implantation ability. In women of Group 2, the NF-kB level in UA was 1.4 times higher than in Group 1: 8.76±1.74 pg/ml versus 6.33±1.0 pg/ml (*P*=0.000).

In Group 2, we found significant positive correlations between BMI and serum TNF-α level (*r*=-0.63), BMI and IL-1β level in UA (*r*=-0.60), BMI and NF-kB level in UA (*r*=-0.81), as well as negative correlation between BMI and IL-10 level in UA (*r*=-0.62) (Table 4).

Table 4.

Correlations between BMI and cytokine levels in the blood serum and UA

Variable	Group 1 (n=34)		Group 2 (n=16)	
	r	*P*	*r*	*P*
TNF-α (serum)	-0.1	0.549	0.63	0.009
IL-1 β (serum)	0.18	0.273	0.40	0.115
IL-10 (serum)	0.06	0.737	-0.40	0.121
TNF-α (UA)	-0.14	0.393	0.18	0.494
IL-1 β (UA)	0.19	0.259	0.60	0.013
IL-10 (UA)	0.14	0.393	-0.62	0.009
NF-kB	0.23	0.149	0.81	0.0001

Thus, according to our data, an increase in BMI (1.2 times) and WC >80 cm (66.7%) against a background of an increase in PFF and insulin (2.2 times) indicated the formation of abdominal obesity in 66.7% of women and insulin resistance in 71.4% of women with primary infertility in the reproductive age with HD in the pubertal period.

As is well known, insulin stimulates the production of gonadotropic hormones in the hypothalamus, disrupting the cirroral rhythm, which affects the functioning of the hypothalamo-pituitary-ovarian axis.[3,12,14] The association between obesity and insulin resistance is largely due to changes in the function of adipose tissue. According to many authors, obesity plays a significant role in reproductive disorders, leading to fertility decline.[11,14] Obesity may impair reproductive functions by affecting both the ovaries and endometrium.[15] In several studies, it is found that the risk of infertility is threefold higher in obese women than in non-obese women[16] and their fertility seems to be impaired in both natural and assisted conception cycles.[17,18] It has been shown that the probability of pregnancy is reduced by 5% per unit of BMI exceeding 29 kg/m^2.[19] It has been unequivocally proven that fat is metabolically active; as a result of lipolysis, the release and production of a number of proinflammatory cytokines occur,[5,9] which is also confirmed by our research. In primarily infertile women of reproductive age, such immune disorders as increasing the proinflammatory cytokines TNF-α and IL-1β and reducing the anti-inflammatory cytokine IL-10 with an increase in serum TNF-α/IL-10 ratio reflect the predominant Th1-type inflammatory response with the formation of systemic inflammatory reactions. Correlation analysis showed a direct relationship between BMI and serum TNF-α level. TNFα was the first cytokine to be implicated in the pathogenesis of obesity and insulin resistance.[20]Adipose tissue expression of TNFα is positively correlated with adiposity and insulin resistance.[20,-22] Chronic exposure to TNFα induces insulin resistance both in vitro and in vivo .[22,23].

Many reports have shown that TNF-α may have an important role in the IR pathogenesis by multiple mechanisms, such as downregulation of genes that are required for normal insulin action, direct effects on insulin signaling, induction of elevated free fatty acids via stimulation of lipolysis, and negative regulation of peroxisome proliferator-activated receptor-γ (PPARγ), an important insulin-sensitizing nuclear receptor.[24-27] In addition, TNF-α functions as a modulator of gonadotropin release in the hypothalamus[28] and an activator of intravascular coagulation.[10] TNF-α is a pro-angiogenic factor and contributes to impaired vascularization of the endometrium.[28]

Investigation of the level of pro-inflammatory cytokines in UA showed the diagnostic significance of an increase in IL-1β level and the TNF-α/IL-10 ratio in women with primary infertility. We found a direct correlation between BMI and IL-1β level and an inverse correlation between BMI and IL-10. Based on the results of other authors, IL-1β increases the production of prostaglandins, causing uterine contractions, which may be important in miscarriages.[13] IL-10, which is involved in the process of hemopoiesis and angiogenesis, has a powerful anti-inflammatory and immunomodulatory effect and plays a major role in suppressing the excessive production of pro-inflammatory mediators.[29] The decrease in IL-10 level and the predominance of pro-inflammatory cytokines over anti-inflammatory cytokines can alter the direction of the mother's immune response with the formation of the inflammatory reactions in the endometrium, which is a factor in reducing its implantation ability.

The correlation analysis also showed a direct, strong correlation between BMI and NF-kB in UA. NF-kB as a transcription factor is involved in the control of a large number of normal cellular and organismal processes, such as immune and inflammatory responses, developmental processes, cellular growth, and apoptosis.[30] These capacities of NF-kB can be used in the diagnosis of the implant ability of the endometrium.

In conclusion, every fourth woman of reproductive age with HD in the puberty period has primary infertility. The results obtained indicate the activation of the Th-1 immune response with the formation of the inflammatory reactions at the systemic level and in the endometrium. Diagnostically significant markers of primary infertility are the serum TNF-α level and the UA levels of IL-1β and NF-kB.

Competing interests

The authors declare that they have no competing interests.

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