Оригинальное исследование

Original article

NOD-like receptors in pathogenesis of missed and early spontaneous abortions

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Abstract

Nucleotide-binding oligomerization domain-like receptors (NOD-like receptors) are cytosolic signaling receptors of innate immune cells recognizing ligands derived from bacteria, viruses, fungi and protozoa. They can initiate apoptosis and pro-inflammatory cytokines production. Meanwhile, the role of decidual NOD-like receptors in pathogenesis of early miscarriages remains unknown. **Aim:** to study NOD-like receptor (NOD1, NOD2, NLRP1, NLRP3, NLRC4) messenger ribonucleic acid (mRNA) expression in decidual tissue from patients with missed and spontaneous abortions compared to progressive pregnancy.

Materials and Methods. NOD1, NOD2, NLRP1, NLRP3, NLRC4 and pathway protein receptor-interacting-serine/threonine-protein kinase 2 (RIP-2) mRNA expression in decidua from 34 patients with missed abortions (group I), 34 patients with spontaneous abortions (group II) and 57 women with progressive pregnancy admitted for artificial abortion (group III, control group) were analyzed by reverse transcription quantitative polymerase chain reaction (PCR) at gestational age of 6–10 weeks. Exclusion criteria were as follows: endocrine disorders, severe extragenital diseases, antiphospholipid syndrome, inherited thrombophilia, uterine malformations and fetal chromosomal abnormalities. Samples were collected by uterine abrasion.

Results. It was found that mRNA expression of NOD2 was significantly higher in decidua from patients with missed and spontaneous abortions, whereas for RIP-2 (related to relevant signaling pathway) – in women with missed abortions. A moderate positive correlation between gestational age and mRNA expression for NOD2 (R = 0.48; p = 0.01) and RIP-2 (R = 0.41; p = 0.007) was observed in women with progressive pregnancy. In contrast, women with missed abortions showed a moderate negative correlation between body weight and mRNA expression for NOD2 (R = –0.46; p = 0.03) and RIP-2 (R = –0.51; p = 0.02) whereas spontaneous abortions was associated with moderate negative correlation between RIP-2 mRNA expression and body weight (R = –0.47; p = 0.04) as well as body mass index (R= –0.48; p = 0.04) along with moderate positive correlation with age of menarche (R = 0.46; p = 0.04). However, compared with progressive pregnancy no significant differences were found in expression level form NOD1, NLRP1, NLRP3 and NLRC4 mRNA in decidua from patients with missed and spontaneous abortions.

Conclusion. Elevated NOD2 mRNA expression was observed in decidua from patients with missed and spontaneous abortions compared to progressive pregnancy paralleled with upregulated RIP-2 mRNA expression in missed abortions. Finally, it was found that NOD1, NLRP1, NLRP3 and NLRC4 were not involved in pathogenesis of miscarriages.

Keywords: NOD-like receptors, NOD1, NOD2, RIP-2, NLRP1, NLRP3, NLRC4, missed abortion, spontaneous abortion, miscarriage, decidual tissue

NOD-подобные рецепторы в патогенезе неразвивающейся беременности и самопроизвольных выкидышей ранних сроков

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Резюме

Введение. NOD-подобные рецепторы – рецепторы, содержащие нуклеотид-связывающий домен олигомеризации (англ. nucleotide-binding oligomerization domain-like receptors), – это цитоплазматические сигнальные рецепторы клеток врожденного иммунитета, распознающие лиганды вирусов, бактерий, грибов и простейших. Они могут индуцировать апоптоз и синтез провоспалительных цитокинов. Однако роль NOD-подобных рецепторов децидуальной ткани в патогенезе невынашивания беременности ранних сроков не изучалась.

Цель: изучить экспрессию матричной рибонуклеиновой кислоты (мРНК) NOD-подобных рецепторов (NOD1, NOD2, NLRP1, NLRP3, NLRC4) в децидуальной ткани пациенток с замершей беременностью и самопроизвольными абортами в сравнении с прогрессирующей беременностью.

Материалы и методы. Экспрессия мРНК NOD1, NOD2, NLRP1, NLRP3, NLRC4 и белка их сигнальных путей, рецептора, взаимодействующего с серин/треонинкиназой 2 (англ. receptor-interacting-serine/threonine-protein kinase 2, RIP-2), в децидуальной ткани 34 пациенток с неразвивающеся беременностью (группа I), 34 пациенток с самопроизвольными выкидышами (группа II) и 57 женщин с прогрессирующей беременностью, поступивших для проведения медицинского аборта (группа III, контрольная группа), была изучена методом обратнотранскриптазной количественной полимеразной цепной реакции (ПЦР) на сроке 6–10 нед беременности. Критериями исключения были эндокринные заболевания, тяжелые экстрагенитальные заболевания, антифосфолипидный синдром, наследственная тромбофилия, аномалии матки и хромосомные аномалии плода. Материал получали путем выскабливания полости матки.

Результаты. Экспрессия мРНК NOD2 была достоверно выше в децидуальной ткани пациенток с неразвивающейся беременностью и самопроизвольными выкидышами ранних сроков, а RIP-2 (белка его сигнальных путей) – у женщин с неразвивающейся беременностью. У пациенток с прогрессирующей беременностью наблюдалась умеренная положительная корреляция между сроком беременности и экспрессией NOD2 (R = 0,48; p = 0,01) и RIP-2 (R = 0,41; p = 0,007). У женщин с неразвивающейся беременностью была выявлена умеренная отрицательная корреляция между массой тела и экспрессией мРНК NOD2 (R = –0,46; p = 0,03) и RIP-2 (R = –0,51; p = 0,02). У пациенток с самопроизвольными выкидышами ранних сроков наблюдалась умеренная отрицательная корреляция экспрессии мРНК RIP-2 с массой тела (R = –0,47; p = 0,04) и индексом массы тела (R = –0,48; p = 0,04) и умеренная положительная корреляция с возрастом менархе (R = 0,46; p = 0,04). Экспрессия мРНК NOD1, NLRP1, NLRP3 и NLRC4 в децидуальной ткани пациенток с неразвивающейся беременностью и самопроизвольными выкидышами ранних сроков не имела достоверных различий с прогрессирующей беременностью.

Заключение. У пациенток с неразвивающейся беременностью и самопроизвольными выкидышами ранних сроков в децидуальной ткани наблюдается увеличение экспрессии мРНК NOD2 по сравнению с прогрессирующей беременностью, у пациенток с неразвивающейся беременностью это сопровождается увеличением экспрессии мРНК RIP-2. Не выявлено влияние NOD1, NLRP1, NLRP3 и NLRC4 на патогенез невынашивания беременности ранних сроков.

Ключевые слова: NOD-подобные рецепторы, NOD1, NOD2, RIP-2, NLRP1, NLRP3, NLRC4, неразвивающаяся беременность, самопроизвольный аборт, выкидыш, децидуальная ткань

Highlights

What is already known about this subject?

► NOD-like receptors comprise a family of intracellular pattern-recognition receptors sensing microbe-derived ligands and eliciting inflammation and apoptosis. Meanwhile, the data regarding a role of NOD-like receptors in pathogenesis of obstetrical complications are sparse.

► The expression of NOD-like receptors is assessed predominantly in the trophoblast, placenta and amniotic membranes, but not in decidual tissue.

► Virtually, no data on a role for NOD-like receptors in the pathogenesis of miscarriages are available.

What are the new findings?

► Expression of NOD1, NLRP1, NLRP3 and NLRC4 mRNA in decidua from patients with missed and early spontaneous miscarriages vs. ongoing pregnancy did not significantly differ.

► Meanwhile, NOD2 mRNA expression is significantly higher in missed and early spontaneous miscarriages, whereas expression of RIP-2 involved in related signaling cascade – solely in missed abortions.

How might it impact on clinical practice in the foreseeable future?

► NOD2 may be considered as a potential target for prophylaxis and treatment of early miscarriage.

Основные моменты

Что уже известно об этой теме?

► NOD-подобные рецепторы – это класс образраспознаю-щих рецепторов, способных распознавать лиганды микро-организмов и инициировать воспаление и апоптоз. Однако роль NOD-подобных рецепторов в патогенезе акушерских осложнений изучена недостаточно.

► Экспрессия NOD-подобных рецепторов изучена преиму-щественно в трофобласте, плаценте и амниотических оболочках, но не в децидуальной ткани.

► Данные о роли NOD-подобных рецепторов в патогенезе невынашивания практически отсутствуют.

Что нового дает статья?

► Экспрессия мРНК NOD1, NLRP1, NLRP3 и NLRC4 в дециду-альной ткани пациенток с неразвивающейся беременно-стью и самопроизвольными выкидышами ранних сроков не имеет значимых различий с прогрессирующей бере-менностью.

► Однако экспрессия мРНК NOD2 достоверно выше у паци-енток с неразвивающейся беременностью и самопроиз-вольными выкидышами ранних сроков, а экспрессия его сигнального белка RIP-2 – только у пациенток с неразви-вающейся беременностью.

Как это может повлиять на клиническую практику в обозримом будущем?

► Рецептор NOD2 может быть потенциальной мишенью для профилактики и лечения невынашивания беременности ранних сроков.

Introduction / Введение

The unique feature of female reproductive tract is its ability to provide defense against pathogens and immune tolerance to semiallogeneic fetus. It is known that excessive immune response to exogenous and endogenous stimuli can lead to miscarriage [1].

Initial pathogen recognition is provided by innate immune signalling receptors, among which the role of Toll-like receptors in the pathogenesis of early miscarriages has been largely studied [2–6]. NOD-like receptors are positioned in the cellular cytoplasm and can recognize viruses, bacteria, protozoa and fungi in the female genital tract as well as participate in inducing apoptosis and inflammation (**Fig. 1**) [7]. At the same time, no data on the role of NOD-like receptors in decidual tissue in pathogenesis of early miscarriages are currently available.

Aim: to study NOD-like receptor (NOD1, NOD2, NLRP1, NLRP3, NLRC4) messenger ribonucleic acid (mRNA) expression in decidual tissue from patients with missed and spontaneous abortions compared to progressive pregnancy.

Materials and Methods / Материалы и методы

Study design / Дизайн исследования

Observational prospective cross-sectional compara-tive cohort study was performed in the Perinatal Center of Belgorod Regional Clinical Hospital of St. Joasaph. Quan-titative reverse-transcriptase polymerase chain reaction (PCR) was carried out in the Laboratory of Human Molecu-lar Genetics, Belgorod National Research University. Sam-ples were collected from January 2017 till January 2018. Currently, artificial abortions in early pregnancy are provi-ded only by administered medications (mifepristone + miso-prostol), therefore not allowing to collect endometrial samples by curettage of the uterine cavity at this gesta-tional age. Hence, there were used complementary DNA (cDNA) from previously collected samples. After extraction and reverse transcription, cDNA was stored at –80 °C (to store cDNA for unlimited time) until further investigated.

Comparison groups / Группы сравнения

Total 125 women (aged 20–35 years old) were exa-mined at a single time point (6–10 weeks of gestation). Women were divided into three groups: group I – 34 pa-tients with missed abortions, 27.88 ± 1.07 years old, 7.50 ± 0.39 weeks of gestation; group II – 34 patients with sponta-neous abortions, 27.05 ± 0.86 years old, 7.44 ± 0.37 weeks of gestation; group III (control group) – 57 wo-men with ongoing (progressive) pregnancy, admitted to the Perinatal Center for artificial abortion, 26.46 ± 0.52 years old, 8.23 ± 0.18 weeks of gestation. Baseline clinical cha-racteristics were recorded for all subject groups.

Inclusion and exclusion criteria / Критерии включения и исключения

Inclusion criteria for group I: gestational age – from 6 to 10 weeks; clinical picture of missed abortion (crown-
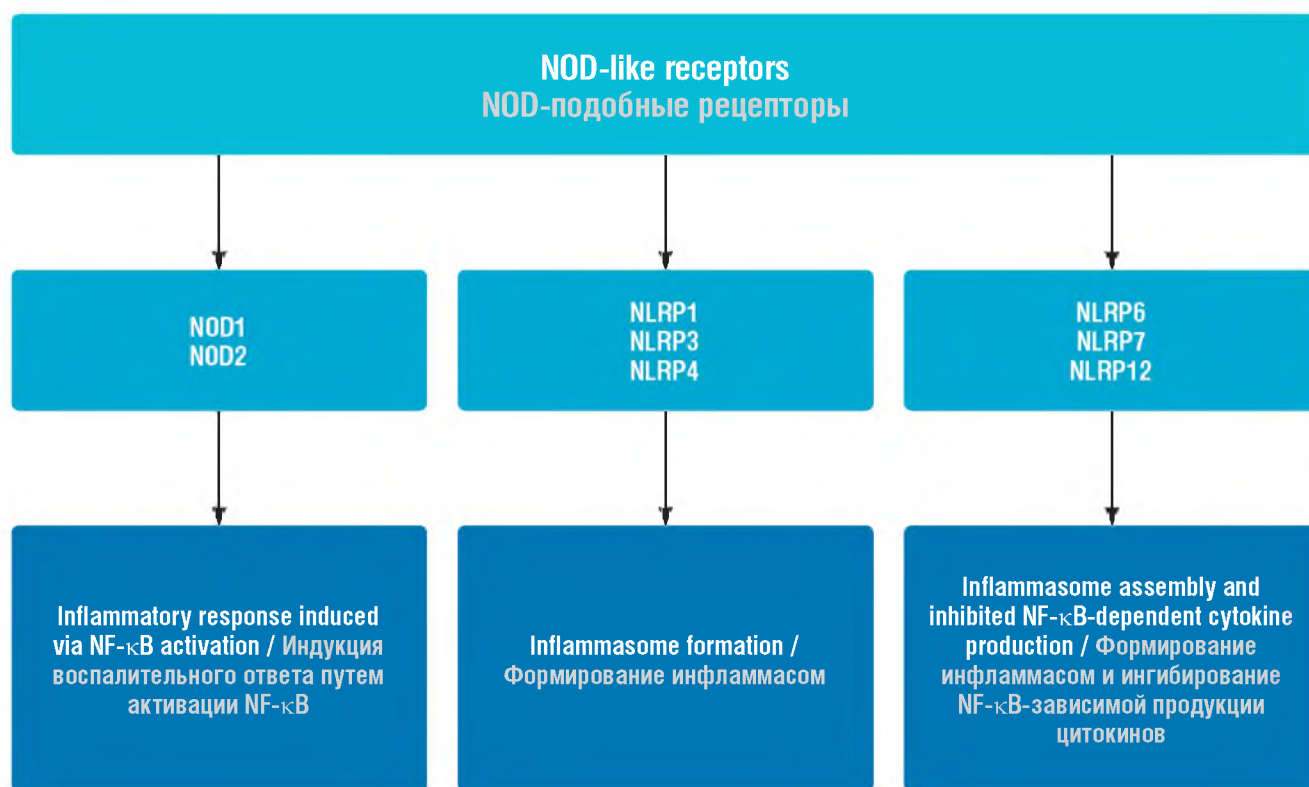
Figure 1. Functions of NOD-like receptors.

Note: NOD – nucleotide-binding oligomerization domain; NLRP – nucleotide-binding oligomerization domain, leucine rich repeat and pyrin domain containing; NLRC – NLR family, CARD domain containing; NF-κB –nuclear factor kappa-B.

Рисунок 1. Функции NOD-подобных рецепторов.

Примечание: NOD – нуклеотид-связывающий домен олигомеризации; NLRP – нуклеотид-связывающий домен олигомеризации, содержащий обогащенные лейцином повторы и пириновый домен; NLRC – рецептор, относящийся к семейству NOD-подобных рецепторов, содержащий домен CARD; NF-κB – нуклеарный фактор каппа-B.

rump length exceeds 7 mm, in case no embryo heart-beat, or mean gestational sac diameter is more than 25 mm in absence of embryo, or embryo with heartbeat is absent for more than 14 days after ultrasound detection of gestational sac without yolk sac, or embryo with heartbeat is absent 10 days after ultrasound examination detecting gestational sac with yolk sac).

Inclusion criteria for group II: gestational age – from 6 to 10 weeks; clinical picture of spontaneous abortion (presence of the gestational sac completely detached from uterine wall, after previously detected progressive pregnancy with embryo heartbeat, regular contractions of shortened cervix myometrium, dilation of internal and internal cervical os, expulsion of gestational sac from uterine cavity).

Inclusion criteria for group III: gestational age – from 6 to 10 weeks; ongoing uterine pregnancy, detection of embryo heartbeat; a woman admitted for artificial abortion by uterine abrasion, written and signed consent for uterine abrasion was obtained.

Exclusion criteria: fetal chromosomal abnormalities; uterine malformations; severe extragenital diseases; endocrine disorders; inherited thrombophilia; antiphospholipid syndrome; refuse to sign patient's consent.

The diagnosis was made based on clinical data and ultrasound examination data to be further confirmed by histopathological examination.

Study methods / Методы исследования

Messenger RNA expression for NOD1, NOD2, NLRP1, NLRP3, NLRC4 as well as related signalling pathway protein RIP-2 (receptor-interacting-serine/threonine-protein kinase 2) in decidual samples was assessed by PCR according to MIQE (Minimum Information for Publication of Quantitative Real-Time PCR Experiments) guidelines [8].

Epithelial tissue samples were obtained by uterine abrasion to be stored in RNAlater solution (Thermo Fisher Scientific, Madison, WI, USA).

Trisol (Invitrogen, Waltham, MA, USA) was used for RNA isolation followed by running ethidium bromide-containing agarose gel electrophoresis for quality control. Next, RNA samples were treated with by DNase I (RNase free) kit (Thermo Fisher Scientific, Madison, WI, USA) to avoid genomic DNA contamination. Reverse transcriptase Mint kit was used to run reverse transcription added with 20 µM of oligoDT (Evrogen, Moscow, Russia) and 500 ng of RNA using PCR Thermal Cycler "Tercyc" (DNA Technology, Moscow, Russia). cDNA quality was

analysed in ethidium bromide-containing agarose gel electrophoresis.

PCR primers were designed using NCBI (National Center for Biotechnology Information) database (Bethesda, MD, USA) (**Table 1**).

qPCR-mix HS SYBR kit (Evrogen, Moscow, Russia) was used for amplification reaction using amplifier CFX96 (Bio-Rad, Hercules, CA, USA) as follows: 14 μL of sterile water, 2 μL of forward and reverse primers each, 5 μL of qPCRmix HS SYBR, and 2 μL of cDNA sample (in triplicates). The amplification cycle included: 5 minutes at 95 °C, followed by 45 three-step cycles (15 seconds at 95 °C, 30 seconds at annealing temperature according, see **Table 1**, and 30 seconds at 68 °C). Two housekeeping genes – peptidylprolylisomerase A (PPIA) and β-actin were used as reference genes.

The results were calculated and presented in relative units as $2^{-\Delta\Delta Cq}$ using the formula:

$$R = 2^{-(Cq\ target - (Cq\ ref1 + Cq\ ref2)/2)},$$

where R – normalized mRNA expression of study gene; Cq ref1 and Cq ref2 – Cq of housekeeping genes; Cq target – Cq of study gene [9].

Ethical aspects / Этические аспекты

The study was designed in accordance with the ethical standards of the Declaration of Helsinki of the World Medical Association (1964) and its subsequent amendments and comparable ethical standards. All patients signed written consent for collection of samples and clinical data, including permission for publication without personal data. The study was approved by Ethics Committee of Belgorod Regional Clinical Hospital of St. Joasaph, Belgorod, Russia (Protocol No. 15 dated of December 21, 2016).

Statistical analysis / Статистический анализ

Statistical analysis was performed by using Statistica 13.2 (Statsoft Inc., Tulsa, OK, USA) and GraphPad Prism 8.0 (Dotmatics, Boston, MA, USA) software. Normality of distribution was estimated by Kolmogorov–Smirnov test. If normal distribution was found, data were presented as M ± SEM and inter-group differences were estimated by t-test for independent samples. If non-normal distribution was detected, result values were represented as median with 95 % confidence interval (CI) and inter-group differences were estimated by Mann–Whitney U-test. Correlation analysis was performed using Spearman's rank criterion. Differences were considered significant at p-value ≤ 0.05.

Results / Результаты

Clinical and anamnestic characteristics of the groups examined / Клинико-анамнестическая характеристика обследованных групп

All studied groups were compatible by age, gestational age, and morphometric parameters – weight, height, and body mass index (BMI). No significant differences in anamnesis data were found between all studied groups, except pregnancy outcomes (**Table 2**).

NOD-like receptor mRNA expression in decidual tissue / Экспрессия мРНК NOD-подобных рецепторов в децидуальной ткани

NOD2 mRNA expression in decidual tissue from patients with spontaneous abortions and missed

Table 1. Primers for quantitative polymerase chain reaction.

Таблица 1. Праймеры для количественной полимеразной цепной реакции.

Gene Ген	Accession number Код доступа	Product length, basic pairs Длина продукта, пары оснований	Forward primer 5'-3' Прямой праймер 5'-3'	Reverse primer 5'-3' Обратный праймер5'-3'	Annealing temperature, ℃ Температура отжига, ℃
NOD1	NM_006092.4	821	CCTGGTGGCCAAGTGATTGT	ACCGAAGGAAATTGCCATCAAAG	55
NOD2	NM_022162.2	240	CTAATGGGCTTTGATGGGGGAA	AGGTGGAAGCCCTCGTAGT	55
NLRP1	NM_033004.4	942	TACCGGTGGAACTCTTGTGC	GGGCTGGAGGGATCAGAGTA	55
NLRP3	NM_004895.4	570	CTGAGCTGACCGTCGTCTTT	AACCAGCTACAAAAAGCATGGAT	64
NLRC4	NM_021209.4	949	CCTGCTGACTGAGAGAACACA	GGCAGTTCTGGGGCTTGAAT	55
RIP-2	NM_003821.5	374	TGCTCGACAGTGAAAGAAAGGA	TCGTGACTGTGAGAGGGACA	55
β-actin	NM_001101.5	994	CAGGCACCAGGGCGTGATGG	GATGGAGGGGCCGGACTCGT	64
PPIA	NM_021130.5	327	CCGCCGAGGAAAACCGTGTACT	TGGACAAGATGCCAGGACCCGT	64

Note: *NOD – nucleotide-binding oligomerization domain; NLRP – nucleotide-binding oligomerization domain, leucine rich repeat and pyrin domain containing; NLRC4 – NLR family, CARD domain containing 4; RIP-2 – receptor-interacting-serine/threonine-protein kinase 2 (RIPK2 or RIP2); PPIA – peptidylprolylisomerase A.*

Примечание: *NOD – нуклеотид-связывающий домен олигомеризации; NLRP – нуклеотид-связывающий домен олигомеризации, содержащий обогащенные лейцином повторы и пириновый домен; NLRC4 – рецептор, относящийся к семейству NOD-подобных рецепторов, содержащий домен CARD4; RIP-2 – рецептор, взаимодействующий с серин/треонинкиназой 2; PPIA – пептидилпролилизомераза A.*

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Table 2. Clinical and anamnestic characteristics of the women examined (M ± SEM).

Таблица 2. Клинико-анамнестическая характеристика обследованных женщин (M ± SEM).

Parameter Показатель	Group I Группа I n = 34	Group II Группа II n = 34	Group III Группа III n = 57	p_{1-3}	p_{2-3}	p_{1-2}
Demographic characteristics / Демографические характеристики						
Age, years Возраст, лет	27.88 ± 1.07	27.05 ± 0.86	26.46 ± 0.52	0.19	0.56	0.55
Gestational age, weeks Срок беременности, недель	7.50 ± 0.39	7.50 ± 0.39	8.23 ± 0.18	0.06	0.06	0.99
Height, cm Рост, см	162.96 ± 1.27	165.95 ± 1.31	163.24 ± 1.42	0.90	0.26	0.11
Weight, kg Масса тела, кг	61.16 ± 2.00	65.36 ± 3.40	63.69 ± 1.61	0.36	0.62	0.29
Body mass index, kg/m² Индекс массы тела, кг/м²	23.01 ± 0.75	23.64 ± 1.10	24.21 ± 0.80	0.38	0.70	0.66
Anamnesis data / Данные анамнеза						
Age of menarche, years Возраст менархе, лет	13.00 ± 0.32	12.77 ± 0.30	12.68 ± 0.14	0.29	0.76	0.61
Duration of menstrual cycle, days Длительность менструального цикла, дней	28.00 ± 0.40	27.71 ± 0.39	28.04 ± 0.19	0.92	0.71	0.61
Duration of menstruation, days Длительность менструации, дней	4.74 ± 0.13	4.89 ± 0.13	4.89 ± 0.09	0.33	0.41	0.25
Total number of pregnancies, including: Общее количество беременностей, включая:	2.83 ± 0.41	2.77 ± 0.35	2.84 ± 0.22	0.92	0.87	0.96
childbirth / роды	0.96 ± 0.26	0.77 ± 0.17	1.10 ± 0.10	0.58	0.12	0.56
medical abortions / медицинские аборты	1.08 ± 0.19	1.00 ± 0.20	1.71 ± 0.16	**0.02**	**0.01**	0.77
spontaneous abortions / самопроизвольные выкидыши	0.16 ± 0.09	0.50 ± 0.13	0.03 ± 0.02	0.11	**0.001**	**0.03**
missed abortions / неразвивающаяся беременность	0.63 ± 0.13	0.50 ± 0.13	–	**0.001**	**0.001**	0.50

Note: *p_{1-3} – significant differences between group I and the control group; p_{2-3} – significant difference between group II and the control group; p_{1-2} – significant difference between group I and group II; significant differences are highlighted in bold.*

Примечание: *p_{1-3} – статистическая значимость различий между группой I и контрольной группой; p_{2-3} – статистическая значимость различий между группой II и контрольной группой; p_{1-2} – статистическая значимость различий между группами I и II; выделены значимые различия.*

abortions was significantly higher compared to subjects with ongoing pregnancy (**Fig. 2**). In women with missed abortions vs. ongoing pregnancy, it was accompanied by elevated RIP-2 mRNA expression.

mRNA expression of NOD1 and NOD-like receptors involved in inflammasome assembly revealed no significant differences between all studied groups.

We estimated a correlation between decidual mRNA expression level for NOD2 and RIP-2 and clinical data (**Table 2**).

It was found, that patients with progressive pregnancy (control group) showed only moderate positive correlation between gestational age and expression level for NOD2 (R = 0.48; p = 0.01) and RIP-2 (R = 0.41; p = 0.007) mRNA.

Patients with missed abortions displayed a moderate negative correlation between body weight and mRNA expression for NOD2 (R = –0.46; p = 0.03) and RIP-2 (R = –0.51; p = 0.02).

Patients with spontaneous abortion showed no correlation between expression level for NOD2 mRNA and presented clinical characteristics. However, expression of RIP-2 mRNA had a moderate negative correlation with the body weight (R = –0.47; p = 0.04), but demonstrated a moderate positive correlation with age of menarche (R = 0.46; p = 0.04).

Discussion / Обсуждение

According to the recent microbiome studies, bacterial pathogens represent the major causative cues in early-stage miscarriages [10, 11]. Therefore, a decidual inflammation induced via NOD-like receptors is of the great interest.

NOD2 is a cytosolic receptor becoming activated after binding to muramyl dipeptide, the major component of peptidoglycan (the part of Gram-negative and Gram-positive bacteria) [12]. The main pathway protein for
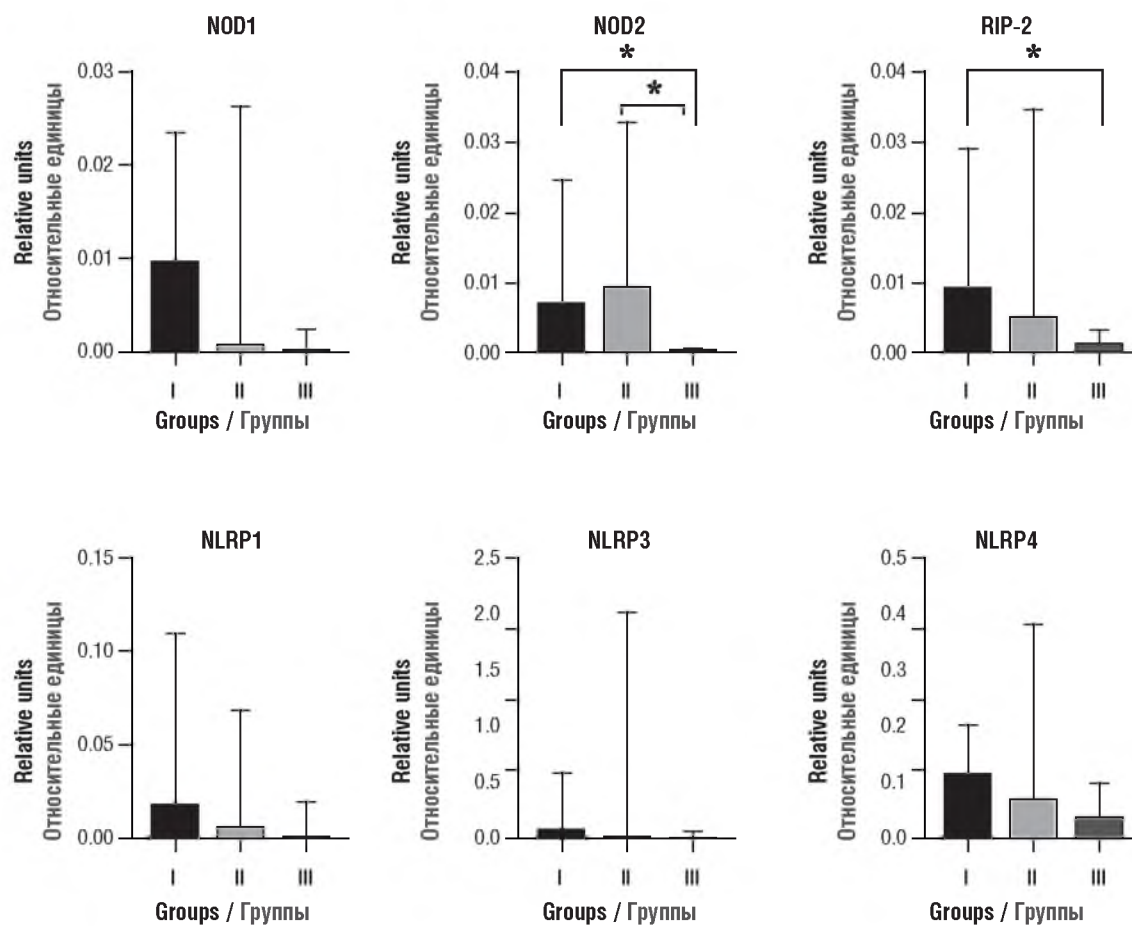
Figure 2. Comparison of NLRs mRNA expression in decidual tissue from patients with missed abortions (group I, n = 34), spontaneous abortions (group II, n = 34), and women with ongoing pregnancy admitted for artificial abortions (group III, n = 57). Data are presented as median with 95 % confidence interval.

Note: *$p < 0.05$ – significant inter-group differences; NOD1 – nucleotide-binding oligomerization domain 1; NOD2 – nucleotide-binding oligomerization domain 2; RIP-2 – receptor-interacting-serine/threonine-protein kinase 2; NLRP 1 – nucleotide-binding oligomerization domain, leucine rich repeat and pyrin domain containing 1; NLRP 3 – nucleotide-binding oligomerization domain, leucine rich repeat and pyrin domain containing 3; NLRC4 – NLR family, CARD domain containing 4.*

Рисунок 2. Сравнение экспрессии мРНК в децидуальной ткани у пациенток с неразвивающейся беременностью (группа I, n = 34), самопроизвольными выкидышами, (группа II, n = 34) и женщинами с прогрессирующей беременностью, поступившими для медицинского аборта (группа III, n = 57). Данные представлены как медиана и 95 % доверительный интервал.

Примечание: *$p< 0.05$ – значимые различия между группами; NOD1 – нуклеотид-связывающий домен олигомеризации 1; NOD2 – нуклеотид-связывающий домен олигомеризации 2; RIP-2 – рецептор, взаимодействующий с серин/треонинкиназой 2; NLRP1 – нуклеотид-связывающий домен олигомеризации, содержащий обогащенные лейцином повторы и пириновый домен; NLRP3 – нуклеотид-связывающий домен олигомеризации, содержащий обогащенные лейцином повторы и пириновый домен 3; NLRC4 – рецептор, относящийся к семейству NOD-подобных рецепторов, содержащий домен CARD4.*

NOD2 intracellular signal transduction is presented by RIP-2 capable to activate NF-κB and mitogen-activated protein kinase (MAPK) and resulting in production of pro-inflammatory cytokines, chemokines and antimicrobial peptides [13].

NOD2 also recognizes viral RNA leading to production of interferon-β and activation of antiviral adapter protein mitochondrial antiviral-signaling protein (MAVS). NOD2 also triggers 2'-5'-oligoadenylate synthetase 2 (OAS2), activating RNAse L leading to degradation of viral RNA [14]. NOD2 ensures autophagy of infected cells, which is important for elimination of viruses, bacteria, and protozoa [15] as well as for antigen presentation [16].

The expression of NOD2 mRNA and protein was detected in the decidualized stroma and glandular epithelium in early ongoing pregnancy [17, 18]. It was found that *in vitro* stimulation of decidualized endometrial stromal cells by muramyl dipeptide upregulates expression of NOD2 mRNA and protein as well as further promotes secretion of monocyte chemoattractant protein 1 (MCP-1) and interleukin-1β (IL-1β). IL-1β, in turn, can stimulate NOD2, which might potentiate inflammation

via a positive feedback loop. Stimulation by muramyl dipeptide also results in dose-dependent induction of apoptosis in decidualized stroma cells [18].

Currently, no data regarding NOD2 expression in decidual tissue from patients with spontaneous abortions and missed abortions are available. Patients with vs. without history of unexplained recurrent miscarriages had downregulated NOD2 expression at the level of mRNA and protein [19]. However, the exclusion criteria in the study included concomitant infection. In addition, patients in both main and control group were admitted for medical abortion inferring that during sample collection all of them had a progressive (ongoing) pregnancy.

In another study, NOD1 and NOD2 mRNA and protein expression was elevated in trophoblast from patients with recurrent miscarriages compared to normal ongoing pregnancy [20]. Meanwhile, no data on decidual NOD1 expression in patients with miscarriage were published. It is known that activation of NOD1 occurs upon binding to diaminopimelic acid (iE-DAP), a part of the peptidoglycan found in some Gram-positive and all Gram-negative bacteria [12].

It can be suggested that upregulated decidual mRNA expression for NOD2, but not NOD1, in patients with spontaneous abortions and missed abortions might be due to Gram-positive, rather than Gram-negative bacteria present in the decidual tissue.

In addition, no data regarding a role for NLRP1 and NLRC4 in pathogenesis of early miscarriages are found. We described no significant differences in decidual expression level for NLRP1 and NLRC4 mRNA in patients with spontaneous abortions and missed abortions.

Ligands for NLRP1 are presented by muramyl dipeptide and anthrax lethal toxin. Expression of NLRP1 mRNA is regulated by protein SREBF1 regardless of pro-inflammatory cytokine levels [21]. It is known, that NLRP1 binding to apoptosis-regulating proteins B-cell lymphoma 2 apoptosis regulator (BCL-2) and B-cell lymphoma-extra large apoptosis regulator (BCL-XL) attenuates inflammasome formation [22, 23]. Earlier, normal or elevated decidual expression of BCL-2 mRNA was identified in patients with spontaneous abortions or missed abortions, respectively [24]. Apparently, this may prevent the formation of NLRP1 inflammasome and induction of apoptosis in the decidual tissue.

The main ligands for human NLRC4 are presented by bacterial flagellin and structural proteins of bacterial type III secretion system [25, 26].

In our study, no significant differences were found in NLRP3 mRNA expression in both patient groups compared to ongoing pregnancy group. NLRP3 detects the whole spectrum of bacterial, fungal, protozoan, and viral ligands, as well as a number of host damage-associated molecular patterns [26]. In nonpregnant patients with vs. without history of recurrent miscarriages of unknown origin, during the secretory phase of menstrual cycle endometrium was discovered to have upregulated expression of NLRP3 protein [1], but not mRNA [27]. Higher NLRP3 mRNA expression was found in peripheral blood mononuclear cells from patients with history of recurrent miscarriage compared to healthy women [28]. Further research is required to assess a role of NLRP3 in the pathogenesis of miscarriages.

Patients with progressive pregnancy we found to show a significant positive correlation between decidual expression level of NOD2 and RIP-2 mRNA and gestational age suggesting that NOD2 level normally escalates in parallel with increasing gestational age.

Patients with missed abortions had significant negative correlation between NOD2 and RIP-2 mRNA expression and body weight, whereas patients with spontaneous abortions – between RIP-2 mRNA expression and body weight as well as body mass index. No data regarding features of NOD2 and RIP-2 expression in human decidua with regard to body weight were found. However, NOD2-deficient mice were shown to display insulin resistance and higher level of adipose tissue [29].

Patients with spontaneous miscarriages had also a positive correlation observed between decidual RIP-2 mRNA expression and age of menarche onset. It is known that higher BMI is associated with earlier menarche [30]. Therefore, patients with low body weight usually have menarche onset at older age. Thus, it is consistent to find a negative correlation between RIP-2 mRNA expression and body weight as well as BMI, and its positive correlation with age of menarche onset.

Study limitations / Ограничения исследования

Limitations of the study were accounted for by a relatively small quantity of samples and a single-center study design conducted only in a single region of Russian Federation. Multicenter studies, especially those involving multiple countries, can provide more comprehensive insights into a role of NOD-like receptors in pathogenesis of miscarriages.

Conclusion / Заключение

In summary, upregulated NOD2 mRNA expression is observed in decidua from patients with spontaneous abortions and missed abortions compared to progressive pregnancy resulting in increased RIP-2 mRNA expression level in missed abortions. NOD1, NLRP1, NLRP3 and NLRC4 were found not to be involved in miscarriage pathogenesis.

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