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Epigenetic modification of SIGIRR genes ın necrotizing enterocolitis and a prognostic model of peritonitis

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Necrotizing enterocolitis (NEC) is a non-inflammatory disease caused by infectious factors against a background of immature innate defense mechanisms and hypoxic-ischemic injury of the intestinal mucosa.

Aim – to investigate the relationship between epigenetic alterations in the SIGIRR gene profile and the development of peritonitis in complicated cases of NEC.

Materials and methods. A total of 44 preterm infants diagnosed with necrotizing enterocolitis were included in the study. The results from 8 practically healthy preterm infants were used as a control group. Epigenetic changes caused by the methylation of 36 gene loci of the SIGIRR gene were examined in the blood samples of the infants. A comparative analysis of the SIGIRR gene profile changes was performed between 10 newborns with NEC who developed peritonitis and 42 newborns without peritonitis (34 with NEC and 8 practically healthy).

Results. The most significant changes were observed in loci %C35, %C58, and %C292. The Spearman correlation coefficients (ρ) for %C35 and %C58 were positive (0.319 and 0.332, respectively), indicating a potential direct association between variations in the SIGIRR gene and the development of peritonitis ($p<0.05$). The methylation of loci %C35 and %C58 of the SIGIRR gene was found to be a valuable biomarker for identifying infants with NEC who are at risk of developing peritonitis.

Conclusion. In preterm infants with SIGIRR gene modifications, early antibiotic therapy guided by clinical signs such as abdominal distension and feeding intolerance, along with laboratory, bacteriological, and radiological monitoring, as well as timely surgical consultation and early surgical intervention, may reduce the incidence of sepsis, peritonitis, and neonatal mortality.

The study was conducted in accordance with the principles of the Declaration of Helsinki. The study protocol was approved by the institution's local ethics committee. The informed consent was obtained from patients.

The authors declare no conflict of interest.

Keywords: preterm, necrotizing enterocolitis, peritonitis, SIGIRR gene, epigenetic modification.

Епігенетична модифікація генів SIGIRR при некротичному ентероколіті та прогностична модель перитоніту

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Некротичний ентероколіт (НЕК) – це незапальне захворювання, спричинене інфекційними факторами на тлі незрілих вроджених захисних механізмів та гіпоксично-ішемічного пошкодження слизової оболонки кишечника.

Мета – дослідити зв'язок між епігенетичними змінами у профілі гена SIGIRR та розвитком перитоніту у складних випадках НЕК.

Матеріали та методи. Дослідження охопило 44 недоношених дітей із діагнозом НЕК. Результати 8 практично здорових недоношених дітей використано як контрольну групу. Епігенетичні зміни, спричинені метилюванням 36 локусів гена SIGIRR, досліджено у зразках крові немовлят. Порівняльний аналіз змін профілю гена SIGIRR було проведено між 10 новонародженими з НЕК, в яких розвинувся перитоніт, та 42 новонародженими без перитоніту (34 з НЕК та 8 практично здорових).

Результати. Найбільш значні зміни спостерігалися в локусах %C35, %C58 та %C292. Коефіцієнти кореляції Спірмена (ρ) для %C35 та %C58 були позитивними (0,319 та 0,332 відповідно), що вказує на потенційний прямий зв'язок між варіаціями гена SIGIRR та розвитком перитоніту ($p<0,05$). Метилювання локусів %C35 та %C58 гена SIGIRR виявилося цінним біомаркером для виявлення немовлят із НЕК, які мають ризик розвитку перитоніту.

Висновок. У недоношених дітей із модифікаціями гена SIGIRR рання антибіотикотерапія, що керується такими клінічними ознаками, як здуття живота та харчова непереносимість, разом із лабораторним, бактеріологічним та радіологічним моніторингом, а також своєчасною хірургічною консультацією та раннім хірургічним втручанням, може зменшити частоту сепсису, перитоніту та неонатальної смертності.

Дослідження проведено відповідно до принципів Гельсінської декларації. Протокол дослідження було схвалено місцевим етичним комітетом установи. Від пацієнтів було отримано інформовану згоду.

Автори заявляють про відсутність конфлікту інтересів.

Ключові слова: недоношеність, некротичний ентероколіт, перитоніт, ген SIGIRR, епігенетична модифікація.

Necrotizing enterocolitis (NEC) is a non-inflammatory disease caused by infectious factors against a background of immature innate defense mechanisms and hypoxic-ischemic injury of the intestinal mucosa. It is characterized by a tendency for the process to become generalized and progress into a systemic inflammatory response [6,10].

During the neonatal period, NEC occurs in 3–5 out of every 1,000 newborns and accounts for approximately 4% of all infants admitted to neonatal

intensive care units. About 80–90% of affected infants are low-birth-weight preterm neonates. According to the results of 27 cohort studies, NEC develops in 7 out of every 100 very-low-birth-weight infants admitted to intensive care units [1,10]. Despite advances in the care and treatment of preterm infants, the incidence of NEC has not declined [2,3].

Conservative treatment methods such as bowel rest, parenteral nutrition, infusion therapy, antibiotic treatment, and maintenance of acid-base balance may alleviate the clinical symptoms of NEC; however, in approximately 10% of cases, the disease progresses to a surgical stage, making timely surgical intervention critically important [5,12]. Intestinal perforation results in death in 76% of cases [8,18], while postoperative mortality rates range from 60% to 80% [14].

In recent years, a unified hypothesis explaining the pathogenesis of NEC has been proposed. It is suggested that, in preterm infants, an imbalance in enhanced pro-inflammatory signaling within the intestinal mucosa leads to mucosal injury and subsequent development of NEC [7,9].

Observations have shown that, despite activation of Toll-like receptor 4 (TLR4) signaling, NEC does not develop in the majority of preterm infants. This is because, in these neonates, TLR4 signaling is attenuated by counter-regulatory genes. A gene inhibiting TLR4 activity in the intestine, SIGIRR (Single Immunoglobulin IL-1-Related Receptor; Gene ID: 59307), has been identified.

The TIR-8 receptor (Toll/interleukin-1 receptor 8) acts as a negative regulator of the activity of the cytokines IL-1α and IL-1β and as an antagonist of TLR signaling. Under normal conditions, SIGIRR is expressed by cells of the kidneys, lungs, and gastrointestinal tract; under conditions of infection- and hypoxia-induced stress, it is also expressed by monocytes and dendritic cells.

SIGIRR regulates postnatal intestinal adaptation, inhibits inflammation induced by lipopolysaccharides of gram-negative bacteria involved in the development of NEC, and serves as a «brake» by preventing excessive activation of TLR4 and uncontrolled inflammation [16,17]. In NEC, mutations in the SIGIRR gene lead to disruption of postnatal immune tolerance in the intestine. NEC develops as a result of enhanced lipopolysaccharide-driven inflammatory responses caused by loss of function of SIGIRR genetic variants identified in preterm infants [19].

The ***aim*** of the study was to investigate the relationship between epigenetic alterations in the SIGIRR gene profile and the development of peritonitis in complicated cases of NEC.

Material and methods of the study

The study analyzed epigenetic changes resulting from methylation across 36 gene loci of the SIGIRR gene in blood samples obtained from 52 preterm infants (44 with NEC and 8 clinically healthy) who were treated in the Department of Neonatal Anesthesiology, Resuscitation and Intensive Care and the Department of Pathology of Preterm Infants at the Research Institute of Pediatrics. A comparative analysis of SIGIRR gene profile changes was performed between 10 newborns with NEC who developed peritonitis (P+) and 42 newborns without peritonitis: 34 with NEC and 8 practically healthy (P-).

Alterations at the %C35, %C58, and %C292 loci were found to be of particular significance.

Analysis of the SIGIRR gene in blood samples was performed at the INTERGEN Laboratory in Ankara, Republic of Türkiye, using the Sanger sequencing method.

The numerical parameters obtained from the study were analyzed using the non-parametric Mann–Whitney test, with $p<0.05$ considered statistically significant. Spearman's correlation method was used to assess the strength and significance of correlations between variables, while the Fisher–Snedecor test was applied for qualitative variables. To evaluate the prognostic informativeness of the obtained results, ROC analysis was performed; ROC curves for sensitivity and specificity were constructed, and optimal cutoff points were calculated. Statistical analysis was carried out using IBM SPSS Statistics version 26.

The study was conducted in accordance with the principles of the Declaration of Helsinki. The study protocol was approved by the institution's Local Ethics Committee. The informed consent was obtained from patients.

Results of the study and discussion

Table 1 presents a comparison of statistically significant differences in various parameters of the SIGIRR gene loci between the two subgroups of infants (with and without peritonitis). Statistically significant differences in the %C35 and %C58 loci were observed between the two groups.

Table 1

SIGIRR gene locus parameters in infants with NEC with and without peritonitis

SIGIRR	Groups	n	M	Me	Q1	Q3	PU
%C35	P-	42	41.45	40.83	36.67	47.11	0.023*
	P+	10	34.92	34.40	29.35	39.67	
%C58	P-	42	14.57	13.16	10,92	15,15	0.018*
	P+	10	10.77	10.15	7.85	11.9	
%C292	P-	42	0.83	0.69	0.38	1.27	0.140
	P+	10	1.73	1.39	0.83	1.78	

Notes: n – sample size; M (mean) – mean value; Me – median; Q1 – first quartile (25th percentile); Q3 – third quartile (75th percentile); Pu – statistical significance of differences between two independent groups according to the Mann–Whitney test; * – null hypothesis rejected.

Table 2

Area under the curve

Test Result Variable(s)	Area	Std. Error	Asymptotic Sig.	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
%C35	0.267	0.079	0.023	0.111	0.422
%C58	0.257	0.086	0.018	0.089	0.426
%C292	0.723	0.087	0.030	0.551	0.894

As shown in Figure, the area under the ROC curve (AUC) for the %C58 gene locus was 0.257±0.086 (p=0.018), indicating statistical significance. The AUC for methylation at the %C35 gene locus was 0.267±0.079 (p=0.023). In addition, although methylation changes at the %C292 gene locus demonstrated a relatively high ROC curve area, no statistically significant difference was detected (p=0.140).

The promising area under the curve (AUC) values for %C35 and %C58 indicate that these gene loci represent valuable biomarkers for identifying infants with NEC who are at risk of developing peritonitis (Table 2).

The informativeness values for the %C35, %C58, and %C292 gene loci of the SIGIRR gene in infants with NEC are presented in Table 3. As shown, the %C35, %C58, and %C292 loci demonstrate higher informativeness, with a cutoff value for %C58 below 12.6. Its sensitivity (Sn), specificity (Sp), and overall diagnostic value (ODV) were 90.0±9.5%, 61.9±7.5%, and 67.3±6.5%, respectively.

The effectiveness of positive and negative result evaluation was 36.0±9.6 and 96.3±3.6, respectively.

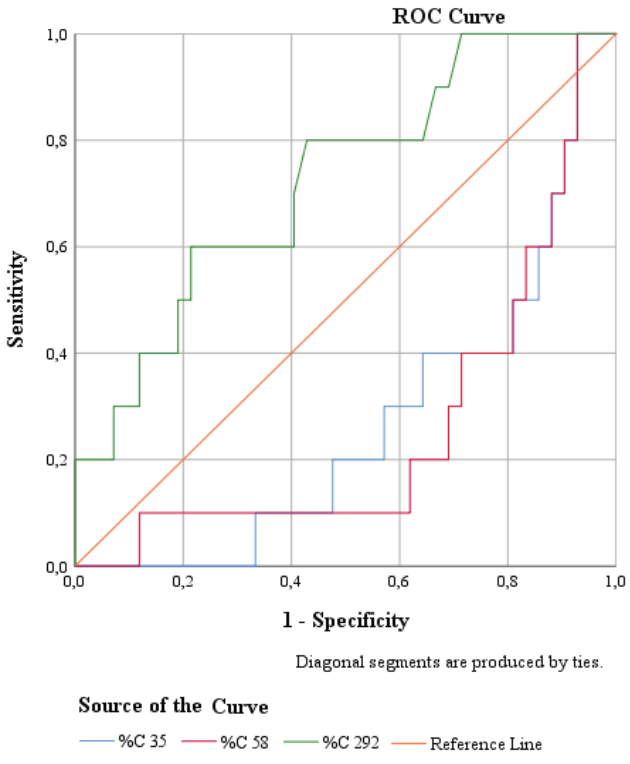

Fig. ROC curve of regression analysis for the SIGIRR gene loci in infants with NEC who developed peritonitis

Table 3

Informativeness values of the SIGIRR gene loci in infants with NEC complicated by peritonitis

Statistical parameters	Indicator		
	%C35	%C58	%C292
Cut off point	<35	<12.6	>1.14
Sn%	60.0±15.5	90.0±9.5	60.0±15.5
Sp%	81.0±6.1	61.9±7.5	69.0±7.1
ODV %	76.9±5.8	67.3±6.5	67.3±6.5
pPV	42.9±13.2	36.0±9.6	31.6±10.7
nPV	89.5±5.0	96.3±3.6	87.9±5.7
LR+	3.15 sufficient	2.36 sufficient	1.94 insufficient
LR-	0.49 sufficient	0.16 good	0.58 insufficient

Table 4

Results of the correlation analysis between the SIGIRR gene loci and infants with NEC complicated by peritonitis

Indicator		%C 35	%C 58	%C 292
Peritonitis	ρ (Rho)	0.319	0.332	0.304
	p	0.021*	0.016*	0.028*
	N	52	52	52

Notes: ρ (rho) – Spearman correlation coefficient; p – statistical significance of the correlation coefficient; * – null hypothesis rejected, p<0.05.

The positive likelihood ratio (LR+) was 2.36, and the negative likelihood ratio (LR-) was 0.16, indicating good diagnostic performance. This marker demonstrates high clinical relevance in predicting peritonitis in patients with NEC due to its high sensitivity, negative predictive value (NPV), and LR- indices.

For the %C35 locus, sensitivity was 60.0±15.5%, specificity 81.0±6.1%, and overall diagnostic value 76.9±5.8%. The LR+ value of 3.15 indicates that a positive test result increases the probability of disease. These high values reflect strong test performance. The LR- value was 0.49, confirming the diagnostic significance of negative test results, as values below 1 support test usefulness.

For the %C292 locus, the LR+ and LR- values were considered insufficient.

To predict the impact of epigenetic changes resulting from SIGIRR gene methylation on disease severity in infants with NEC, a prognostic model was developed based on three gene loci that demonstrated statistically significant differences and high informativeness.

For the %C58 locus, the odds ratio (OR) was 14.6, indicating that the likelihood of developing peritonitis in infants with NEC is approximately 14.6 times higher than in the comparison group. The 95% confidence interval (CI) ranged from 1.7 to 126.5, suggesting that the true OR lies within this interval. The difference was statistically significant ($p<0.05$). The effect size (ES) was 20.1, with a 95% CI of 13.7-26.6, further strengthening confidence in the magnitude of the association and indicating a highly significant result.

For the %C35 locus, the OR was 6.4, meaning that the probability of peritonitis in this group was approximately 6.4 times higher than in the control group. The 95% CI (1.4-28.0) confirms the statistical significance of this finding, although the upper limit is 4.5 times lower compared with the %C58 locus. The result was supported by $p<0.05$.

Thus, two gene loci (%C35 and %C58) exerted a statistically significant effect on the investigated outcome. The %C58 locus demonstrated the highest odds ratio, identifying it as the strongest predictor of peritonitis development in infants with NEC. The statistical significance of all findings indicates that these results are unlikely to be due to chance.

Table 4 presents the results of the correlation analysis conducted between the SIGIRR gene loci and peritonitis indicators in infants with NEC. The Spearman correlation coefficients (ρ) for %C35 and %C58 were positive (0.319 and 0.332, respectively), indicating a potential direct association between variations in the SIGIRR gene and the development of peritonitis ($p<0.05$). A positive correlation suggests that methylation of the SIGIRR gene may be associated with an increased incidence of peritonitis in infants affected by NEC. This association is likely related to the role of the gene in regulating inflammatory responses in the intestine. A positive correlation was also observed for the %C292 locus, and this result was statistically significant ($p<0.05$).

Thus, the high mortality rate associated with NEC is a major driving factor for extensive investigations into its pathogenesis. To date, no «gold standard» biomarker or predictor for this severe disease has been identified. Diagnosis is based on a combination of clinical and laboratory findings that are evaluated dynamically [11,15]. Studies conducted at the molecular and cellular levels to elucidate the pathogenesis of NEC are particularly noteworthy [13]. A better understanding of the molecular mechanisms underlying this disease may facilitate the development of life-saving and preventive therapeutic strategies [4].

Based on the results of the present study, the promising methylation indicators of the %C35 and %C58 loci of the SIGIRR gene support their consideration as valuable biomarkers for identifying infants at risk of developing NEC and peritonitis. In preterm infants with SIGIRR gene modifications, early antibiotic therapy guided by clinical signs such as abdominal distension and feeding intolerance, along with laboratory, bacteriological, and radiological monitoring, as well as timely surgical consultation and early surgical intervention, may reduce the incidence of sepsis, peritonitis, and neonatal mortality.

The authors declare no conflict of interest.

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