SIGIRR gene profile in infants with necrotising enterocolitis and intrauterine infection

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***Abstract*—Necrotizing Enterocolitis is one of the actual problems that primarily affects premature infants, particularly those born before 32 weeks of gestation. The aim of this study is to investigate the role of genetic factors in the development of both necrotizing enterocolitis and intrauterine infection, exploring the possible links between these two conditions. A total of 52 with NEC and 10 healthy, were included in this study. All the examined children with NEC were divided into 2 groups: 19 newborns with intrauterine infection (1st subgroup), 33 infants without intrauterine infection (2nd subgroup). It was determined SIGIRR gene mutations with DNA isolation and standardized PCR reactions. It was found that C58 gene locus changes is a potential predictive marker for differentiating between infants with intrauterine infection and NEC and those without these conditions. Genetic screening may help identify infants at higher risk of developing NEC, allowing for targeted interventions and personalized care.**

Keywords — Necrotizing Enterocolitis; Intrauterine infection; Preterm Birth; SIGIRR mutations

I. INTRODUCTION

In the world of neonatal medicine, certain conditions continue to perplex healthcare professionals due to their elusive nature and complex interconnections. One such enigma is the relationship between Necrotizing Enterocolitis (NEC) and Intrauterine Infection, two severe and potentially life-threatening conditions affecting premature infants (1-3). As medical science advances, our understanding of these conditions evolves, shedding light on the intricate mechanisms that underlie their occurrence and potential connections (3, 4).

Necrotizing Enterocolitis is a devastating gastrointestinal disorder that primarily affects premature infants, particularly those born before 32 weeks of gestation (5). Characterized by inflammation and tissue death in the intestine, NEC poses a significant challenge in neonatal intensive care units worldwide. Despite advances in medical care, its precise cause remains elusive, and neonatologists grapple with identifying effective preventive and treatment strategies.

In parallel, Intrauterine Infection, also known as chorioamnionitis, emerges as a potential contributing factor to the development of NEC in premature neonates (6). This condition involves the inflammation of the fetal membranes and amniotic fluid, often triggered by bacterial, viral, or fungal pathogens ascending from the mother's genital tract. The intricate interplay between the mother's immune response and the infectious agents can have profound implications for the developing fetus, leading to a spectrum of complications.

Studies on human intestinal tissues derived from preterm infants with NEC also have suggested that genes that mediate TLR signaling such as TLR4, myeloid differentiation primary response 88 (MYD88), and downstream cytokines are increased in NEC, while negative regulators of TLR signaling such as single immunoglobulin interleukin-1–related receptor (SIGIRR) and A20 have decreased expression in NEC (7-10).

This article aims to study the role of genetic factors in the development of both necrotizing enterocolitis and intrauterine infection, exploring the possible links between these two conditions. We will scrutinize the existing research, epidemiological data, and proposed mechanisms that connect NEC with intrauterine infections. By understanding this relationship, we hope to provide crucial insights that could pave the way for improved diagnosis, prevention, and management strategies.

II. MATERIAL AND METHODS

A. Subjects

A total of 52 with NEC and 10 healthy, were included in this study. Some characteristic information of neonates were included in study. For research work, the patients were selected from children undergoing treatment in the department of "Anesthesiology, Resuscitation and Intensive Care of Newborns" and department of "Premature babies" of the Scientific Research Institute of Pediatrics named after K.Farajova; for the control group they were selected from maternity hospital No. 7. Genetic tests for SIGIRR gene were carried out at INTEGEN

Laboratory in Ankara, Turkey. All the examined children with NEC were divided into 2 groups: 19 newborns with intrauterine infection were classified as the 1st subgroup, 33 infants without intrauterine infection were included in the 2nd subgroup. The 10 healthy newborns were included in control group. They, in turn, were divided into 2 subgroups, children with a body weight of more than 1500 g and a weight of less than 1500 g. The study protocols were approved by the Ethics Committee of Azerbaijan Medical University.

B. Sample collection

Taking into account the premature birth of children under our control, the volume of blood taken was 0.5 ml.

C. DNA Isolation

DNA isolation was carried out for each sample from 200 µl of whole blood, by using QIAamp DNA Blood Mini Kit, (Qiagen Inc.), according to the protocol recommended by the producer. Eluted DNA specimens were stored at -20 ℃ until the bisulfite conversion step.

D. Sequencing

Purified and standardized PCR reactions are processed with NexteraXT sample prep kit (Illumina Inc.) to get them ready for NGS. NGS is carried out on Miseq system (Illumina Inc.) by using MiSeq Reagent Kit v2 2x150 cartridges (MS-102-2002, Illumina Inc.).

E. Data Analysis

The reads obtained from NGS are aligned on the bisulfite converted template sequence in which “C”s of the CpG sites are replaced by “N”s, to eliminate the alignment biases due to methylation status of both the template and the reads from the samples. After alignment, the “C”s and “T”s are counted at both the CpG sites and the point to be used in background subtraction. Percent of “C”s are considered as percent of methylation.

III. RESULTS

Table 1 presents the provided data related to the research involving the SIGIRR gene and its changes in infants with intrauterine infection. The data includes various test results and corresponding variables, along with their respective Area Under the Curve (AUC) values, Standard Errors, and p-values with confidence intervals.

As shown in the table many of the test results have p-values greater than 0.05, suggesting that their AUC values are not statistically significant, and the corresponding tests may not have sufficient discriminatory ability. However C58 have relatively high AUC values (closer to 1), indicating good discriminatory power between infants with intrauterine infection and the control group.

TABLE 1 ROC-CURVE OF SIGIRR GENE PROFILE IN INFANTS WITH NEC

Area Under the Curve					
Test Result Variables	**Area**	**Std. Error**	**Asym. Sig.**	**Asym. 95% Confidence Interval**	
				Lower Bound	Upper Bound
C33	0.656	0.086	0.064	0.487	0.824
C58	0.742	0.086	0.004	0.573	0.912
C63	0.636	0.097	0.104	0.447	0.826
C155	0.600	0.087	0.231	0.429	0.772
C165	0.624	0.096	0.138	0.436	0.813
C317	0.580	0.091	0.342	0.401	0.759
C323	0.630	0.090	0.121	0.455	0.805
C43	0.622	0.086	0.146	0.454	0.790
%C 58	0.676	0.087	0.036	0.505	0.848

IV. DISCUSSION

Necrotizing enterocolitis (NEC) is a severe gastrointestinal condition primarily affecting premature infants. It is characterized by inflammation and tissue damage in the intestinal tract, which can lead to devastating complications. Recent advancements in genomic research have shed light on the potential role of genetic changes in the pathogenesis of NEC. This discussion explores the current understanding of genetic alterations associated with NEC in infants.

The gene locus C58 shows an AUC value of 0.742, which indicates good discriminative ability. This suggests that C58 has potential as a predictive marker for differentiating between infants with intrauterine infection and NEC and those without these conditions.

The small standard error of 0.086 further supports the precision of the AUC estimate for C58. Additionally, the significant asymptotic significance ($p = 0.004$) indicates that the observed discrimination is unlikely due to chance, adding credibility to the results.

The promising AUC value for C58 implies that this gene locus could be a valuable biomarker for identifying infants at risk of developing intrauterine infection and NEC. Early detection of these conditions is crucial for timely intervention and management, as both intrauterine infection and NEC can lead to serious health complications if left untreated.

By incorporating C58 into screening protocols, healthcare professionals may improve the accuracy of diagnoses and tailor appropriate treatment strategies for affected infants. Moreover, further research and validation studies could provide deeper insights into the biological mechanisms underlying the association between C58 and NEC susceptibility in infants with NEC.

Numerous studies have identified specific genetic variants that may contribute to the susceptibility of NEC in infants. Polymorphisms in genes involved in the immune response, intestinal barrier function, and inflammation have been extensively investigated. For example, variations in genes encoding Toll-like receptors (TLRs) and interleukins have been associated with an increased risk of NEC. These genetic changes can disrupt the normal immune response, impair barrier integrity, and lead to abnormal inflammatory reactions in the gut. Apart from genetic variants, epigenetic modifications have also emerged as potential contributors to NEC development. Epigenetic changes, such as DNA methylation and histone modifications, can influence gene expression without altering the underlying DNA sequence. Disruptions in the epigenetic landscape of key genes involved in intestinal development and immunity might influence NEC susceptibility.

The present study have some limitations. While the AUC results for C58 are promising, it is essential to acknowledge certain limitations in the study. Firstly, this analysis is based on a specific sample population, and the generalizability of the findings to other populations needs validation. Secondly, the study may lack information on other confounding factors that could influence the relationship between C58 and NEC in infants with intrauterine infection. Future investigations should consider controlling for potential confounders to strengthen the validity of the results. Furthermore, to establish C58 as a robust biomarker for clinical use, prospective studies and longitudinal follow-ups are necessary. Long-term assessments could determine whether the association between C58 and NEC remains stable over time and enhances its predictive value in identifying infants at risk.

V. Conclusion

The AUC results for gene locus C58 in infants with intrauterine growth restriction and necrotizing enterocolitis show promising discriminative ability. The significant AUC value and low standard error indicate that C58 may serve as a valuable biomarker for identifying infants at risk of these conditions. However, further research and validation studies are warranted to establish the clinical utility of C58 and its potential integration into neonatal screening protocols for enhanced patient care. Understanding the genetic underpinnings of NEC holds significant promise for improving disease management and prevention strategies. Genetic screening may help identify infants at higher risk of developing NEC, allowing for targeted interventions and personalized care. Additionally, insights into the genetic drivers of NEC could lead to the development of novel therapeutic approaches, including gene-based therapies and personalized treatments.

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