| RESEARCH ARTICLE

Incidence of Necrotizing Enterocolitis and an Assessment of Related Risk Factors and Outcomes

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| **ABSTRACT**

Necrotizing Enterocolitis (NEC), defined as inflammation of the intestines most commonly due to a bacterial cause, is a relatively rare condition widely encountered in premature infants. Established risk factors for developing NEC include low gestational age, extremely low birth weight, sepsis, and hypotension. However, studies have investigated other causes that may alter the gut microbiome, such as blood transfusions, antibiotic use, and co-morbid conditions. The study design is a retrospective cohort study, and patient data were collected from King Hamad University Hospital (KHUH) after receiving the necessary approvals. Patients included in this study were from 2012 to 2020. The variables assessed included gestational age, birth weight, hemoglobin and hematocrit levels, prenatal and postnatal antibiotic use, blood transfusions, other transfusion products the patients received, and blood culture results. A total of 16 patients were identified as having NEC after thoroughly reviewing their files, and the data were analyzed at the KHUH Research Department. Although a small sample size was involved in this study, antibiotic use was a significant factor in the development of NEC as antibiotics such as ampicillin, gentamicin, and cefotaxime have been shown to alter the gut microbiome, which can predispose a patient to the development of NEC, as was reflected in our study. We also observed a 10.4-time relative risk for developing NEC in patients who had received blood transfusions. The use of antibiotics in patients with NEC has been shown to increase the likelihood of developing the condition, especially with the predominance of Enterobacteriaceae. Although controversial, blood transfusions have also been associated with the development of NEC. Due to the fragile vascular system in patients with NEC, constant phlebotomies and the introduction of packed red blood cells can alter the levels of nitric oxide present, predisposing premature infants to hypoxic conditions and, therefore, the development of NEC.

1. Introduction

Necrotizing enterocolitis (NEC) is considered one of the medical emergencies to be dealt with in the neonatal intensive care unit. Primarily an inflammatory condition of the intestines due to bacterial invasion and overgrowth (Ginglen et al. 2015), it can lead to multiple life-threading complications, such as intestinal perforation, with an overall mortality of approximately 32% (Singh et al. 2009). Currently, there has not been a study investigating the overall incidence of NEC globally; however, a recent systematic review showed an incidence of 2% to 7% in neonates born at 32 weeks of gestation and earlier (Murphy et al., 2013).

NEC manifests as a sequential response of the innate immune system that is triggered within the premature neonate's gastrointestinal tract when exposed to luminal microbiota. It can cause varying degrees of inflammation, eventually leading to necrosis of intestinal cells and perforation of the lumen (Tripathi et al., 2020). This occurs due to intestinal immaturity, resulting in a compromised epithelial barrier and an underdeveloped immune system (Tripathi et al., 2020). Moreover, NEC can lead to life-threatening complications, including peritonitis, systemic hypotension, and multisystem organ failure (Ginglen et al. 2015). Bell's Staging Criteria is often used to suggest appropriate management and treatment of NEC. Neonates are classified into one of three stages using systemic, intestinal, and radiographic signs (Thyoka et al. 2017):

- Stage 1 (mild).
- Stage 2 (moderate).
- Stage 3 (severe).

1.1 Risk Factors and their Correlation with Necrotizing Enterocolitis:

Risk factors of NEC include but are not limited to low gestational age, extremely low birth weight (ELBW), the use of ventilation devices, a low APGAR-score, treatment for patent ductus arteriosus, nosocomial infection, hypotension, and sepsis (Cassir et al. 2015). Recent studies have looked at correlations between NEC and the mode of neonate delivery and antibiotic exposure (Cassir et al. 2015) (which will be discussed in detail later on). Moreover, there has been an increased interest regarding the apparent association between receiving blood transfusions and the development of necrotizing enterocolitis in premature neonates.

1.2 Blood Transfusions and Necrotizing Enterocolitis Incidence:

Neonates born before 37 completed weeks of gestation tend to have an immature placental iron transport and fetal erythropoiesis. In addition, the reduced production and increased catabolism of erythropoietin, along with blood loss through phlebotomies, lead to low plasma levels of erythropoietin in neonates, which may result in anemia (Sasai-Takedatsu et al. 2020). Infants may display symptoms such as pale skin, tachycardia, rapid breathing, and fatigue. Consequently, there is a need for blood transfusions and the introduction of human recombinant erythropoietin (Sasai-Takedatsu et al., 2020). Therefore, neonates tend to be one of the most transfused patients in hospitals, with 90% of infants with a birth weight <1.0kg being given multiple red blood transfusions within the first few weeks of life.

Dangers of multiple, rapid blood transfusions include exposure to several donors, viruses, preservatives used in blood products, iron overload, and a potential increased risk of necrotizing enterocolitis (Tanaka et al., 2011). Transfusion-associated necrotizing enterocolitis (TANEC) is NEC that develops in a patient within 48 hours of receiving a blood transfusion (Tanaka et al. 2011). The mechanism of injury in TANEC has been theorized to be an abnormal response of mesenteric blood flow velocity that leads to a low perfusion state, leading to ischemia and vasoconstriction in the gut (8). Recent studies have shown TANEC to be associated with up to 35% of NEC cases (Tanaka et al. 2011) and are more prevalent in neonates with lower gestational age (<28 weeks), lower birth weight (<1,000 g), extreme anemia (hematocrit <25%), and transfusion of older stored blood (>10 days) (Widness et al. 2018). Additionally, formula feeding and prolonged parenteral feeding before and during transfusion were associated with an increased risk of NEC (10) due to the increased blood flow and velocity of the gut. In two separate reports, withholding feeding during transfusion and swapping formula with human milk resulted in a drastic reduction in the rate of development of NEC in the neonatal unit (Tanaka et al. 2011).

1.3 Antibiotics Usage and Necrotizing Enterocolitis Incidence:

The use of antibiotics is another important risk factor in the development of NEC. Research revolving around the pathogenesis of NEC indicates that antibiotic use can promote alterations in the microbiome of the gut flora. Although antibiotics target specific types of bacteria, they additionally tend to eliminate certain species that cause the overgrowth of opportunistic pathogens and expression of virulence factors that increase the likelihood of NEC (Watkins et al. 2016- Cervantes 2016). At present, antibiotics are readily prescribed, and an increase in multi-drug resistance microbes is observed; hence, antibiotics should be used and prescribed vigilantly. Overusing antibiotics prenatally and postnatally leads to gut flora disturbances in neonates (Cotton et al., 2015). Therefore, the potential link between antibiotic use and the pathogenesis of NEC should be further investigated due to the implications it may pose.

Some controversy about whether exposure to antibiotics is beneficial or harmful in the context of NEC. A 2009 retrospective cohort study suggested that infants who received prolonged antibiotics were less mature, had lower Apgar scores, and had an increased risk of developing NEC (Meister et al. 2018). However, other studies do not show that using antibiotics for NEC results in different

outcomes (Tanner et al. 2018). Hence, more extensive research is required into this niche feature of NEC to regulate the use of antibiotics and potentially update guidelines regarding further use in such cases.

2. Methods:

For this study, data from 16 infants admitted from January 2012 to January 2020 was considered. The data was collected after seeking approval from the Institutional Review Board at the King Hamad University Hospital (KHUH). Moreover, it was extrapolated with the aid of the Medical Records Department at KHUH. Once the data was received, the inclusion and exclusion criteria were implemented to acquire a sample of the target population: neonates diagnosed with NEC or those with a query diagnosis of NEC.

The inclusion criteria for this study were as follows:

- Neonates treated at King Hamad University Hospital
- Neonates transferred from other hospitals to King Hamad University Hospital
- Neonates that have either an NEC diagnosis or a query NEC diagnosis

The exclusion criteria for this study were as follows:

- Neonates are not treated at King Hamad University Hospital during illness.
- Neonates that did not have a clear indication of a diagnosis or query of a diagnosis of NEC

The patient's and mother's data were available from the hospital database. The following variables were taken into consideration:

- Gestational age of the infant (weeks).
- Birth weight (grams) of the infant at birth.
- List of Prenatal antibiotics used.
- List of Postnatal antibiotics used and on which day of life they were given.
- Initial Hemoglobin and Hematocrit reading.
- List of transfusion products and on which day of life they were given.

A total of 16 patients were identified as fitting the inclusion criteria. Once the variables mentioned earlier were collected for each patient, they were set in an Excel sheet and analyzed using SPSS v 25.0. Descriptive statistics were used to compute the frequencies and percentages for categorical variables and mean ± standard deviation for continuous variables. The Chi-square test was used to compare significant differences between two groups with categorical data. A non-parametric T-test was used to compute the differences between continuous scores. The relative risk for the cohort was calculated. All the statistical tests were 2-tailed, and a p-value of <0.05 was considered significant. A single member of this research study collected the data to maintain uniformity within the results, and the data was re-checked by a second member of this study.

3. Results:

Since establishing the Pediatrics department in King Hamad University Hospital (KHUH) in 2012, 16 cases of NEC have been reported to date. Three main variables were considered and investigated throughout this study: maternal prenatal antibiotic intake, neonatal blood transfusions, and neonatal antibiotic consumption. The variables mentioned above examined critical outcomes such as sepsis, perforation, and death.

Table 1: Correlation between Antibiotic Usage and NEC complications

		Mother receive antibiotics	Mother who did not receive antibiotics	P value
	Frequency	10	5	
Baby Characteristics	Gestational age (Weeks)	29.3 ± 4.0	33.8 ± 4.7	0.06
	Birth weight (Grams)	1331.3 ± 806.2	1901.8 ± 963.2	0.23
	Initial Hb (G\dL)	12.6 ± 3.2	18.3 ± 1.5	0.03
	Initial Hematocrit (%)	39.6 ± 6.6	57.2 ± 4.1	0.01

Comparison of gestational age, birth weight, initial hemoglobin, and initial hematocrit in NEC babies whose mothers received antibiotics vs mothers who did not receive antibiotics.

*Results are demonstrated as mean ± SD.

- Prenatal Antibiotic Intake (maternal): [variable=10, control=5] From the 16 mothers with NEC babies, only 15 had sufficient data on prenatal maternal intake of antibiotics. As evidenced by Table 1, mothers who had taken the antibiotics had babies with significantly lower hemoglobin (p=0.03) and lower hematocrit (p=0.01), both of which were statistically significant. It is also worth mentioning that babies born to mothers who had received antibiotics during pregnancy had a considerably lower gestational age and birth weight than those born to mothers who did not receive antibiotics during the gestational period; However, this finding is insignificant.

Table 2: Complications to neonates of mothers who received antibiotics and those who did not receive antibiotics.

	Mothers who received Antibiotic	Mothers who did not receive Antibiotic	P value
Death	50%	20%	0.3341
Sepsis	70%	40%	0.4623
Perforation	50%	40%	0.724

- In 10 out of 15 children, the mother has received antibiotics perinatally. Out of the 10, 50% of the babies had expired while the other half survived; the mothers who had not received antibiotics only had one case that expired (RR=2.5, 95% CI 0.3894 to 16.05, z score= 0.966, P=0.3341).
- Concerning sepsis, 7 out of 10 neonates whose mothers had received antibiotics eventually became septic at a given point during treatment. In comparison, the non-interventional group consisted of 5 NEC patients, where an additional 2 babies expired due to sepsis as an event during their hospitalization (RR=1.4, 95% CI 0.5708 to 3.4337, z score= 0.735, P=0.4623).
- In considering perforation as an outcome, 5 of the babies from the treatment group and an additional two from the control group had been afflicted (RR=1.25, 95% CI 0.362 to 4.318, z score= 0.353, P=0.724).

Table 3: Correlation between Blood Transfusions and NEC complications

	Received blood transfusions	Did note receive blood transfusions	P-Value
Death (%)	66.67		0.092
Sepsis (%)	66.67	57.14	0.542
Perforation (%)	55.56	57.14	0.542

- Comparison of death, sepsis, and perforation as outcomes for NEC patients who received blood transfusions and who did not receive a blood transfusion [Variable=9, Control=7].

- Among the entirety of the 16 NEC cases observed, 9 of those neonates had received blood transfusions. It was found that (66.67 %) of patients who had received transfusions had expired. Blood transfusions posed a significantly increased risk of death in this cohort. However, this finding was insignificant given the modest sample size (RR= 10.4, 95 % CI: 0.684 – 158.208, z-score= 1.69, p= 0.092).
- 6 (66.67%) of the babies who had received blood transfusions had become septic in comparison to the control group in which 4 (57.14%) of the remainder had become septic (RR= 1.3333, 95 % CI: 0.529 to 3.359, z- score=0.61, p= 0.542).
- 5 of the transfused babies had become susceptible to perforation, whereas 4 of the control group also suffered the same fate. (RR= 1.296, 95 % CI: 0.460 to 3.653, z- score=0.491, p= 0.623).

3.1 Usage of Antibiotics during Neonatal Period and NEC Complication Incidence

All 16 babies from the sample were on antibiotics immediately following birth to a few days after birth due to underlying clinical conditions. 10 babies, equating to 62.5 % of the study population, had started their antibiotic use before the diagnosis of NEC and had subsequently all developed the condition. However, 6 of them had their antibiotic course initiated after the diagnosis of NEC. Three of the six babies had blood transfusions after birth, or their mothers had been administered prenatal antibiotics. All six babies who were started on antibiotics after NEC was diagnosed survived except one baby who had received both blood transfusions and prenatal antibiotics (including Erythromycin).

Table 4: Predisposed Neonates to Antibiotics Perinatally, Postnatally, and Blood Transfusions

	Expired	Survived
All 3 Risk Factors Present	4	2
1 to 2 Risk Factors Present	2	7

- Comparison between mortality and several risk factors present.
- Out of the 16 babies, 6 babies died due to NEC. Four out of six were exposed to all three risk factors. The babies with all three pre-disposing factors were at a higher risk for fatality. (RR= 3, 95 % CI 0.78- 11.53, p=0.1099). One baby who suffered mortality was diagnosed with NEC on the 13th day of life and had received blood transfusions, and the mother had also been a part of the treatment group placed on prenatal antibiotics, including Erythromycin. The other baby who had died had co-morbid conditions such as "toxic liver disease with cholestasis" in addition to being preterm and having short bowel syndrome.

4. Discussion

Necrotizing Enterocolitis is an untoward medical condition that typically arises in premature formula-fed neonates and wreaks havoc on their intestinal system in the form of acute inflammation, infection, and necrosis, which can lead to mucosal injury, bowel perforation, pneumatosis intestinalis, peritonitis, sepsis, and even death. Established risk factors for developing NEC include low gestational age, extremely low birth weight, sepsis, and hypotension. However, studies have investigated other causes that may alter the gut microbiome, such as blood transfusions, antibiotic use, and co-morbid conditions (Cassir et al. 2015). This study focused on three elements and their effects and outcomes on NEC patients: maternal prenatal antibiotic intake, neonatal antibiotic consumption, and neonatal blood transfusions. Grave outcomes such as sepsis, bowel perforation, and death were considered.

The fetal gut tract (GIT) and an overgrowth of pathogenic enteric aerobic gram-negative rods (Watkins et al. 2016). A literature review conducted by Fjalstad et al. revealed that prolonged antibiotic therapy in neonates reduced gut microbial diversity. The decline in bifidobacteria not only led to NEC but also caused an increase in NEC with an exaggerated inflammatory response (Fjalstad et al. 1995).

In this retrospective study, all 16 babies received antibiotics during their hospitalization. 10 babies, equating to 62.5 % of the study population, had started their antibiotic use before the diagnosis of NEC and had subsequently all developed the condition. At the same time, the remaining 6 patients were commenced on antibiotics after a diagnosis of NEC had been established. Our results support a retrospective control case analysis conducted by Vanaja et al., where they matched 124 neonates with NEC to 248 controls and found nearly a 3-fold increase in the risk of developing NEC if the neonate was exposed to antibiotics for more than

10 days (Battersby et al. 2019). Even though our sample size was smaller, it was still observed that the increased duration of antibiotic exposure indicated a potential risk for NEC. However, an actual correlation between the type of antibiotic used and its culmination in NEC has not been established in this study. According to a study conducted by Tripathi et al., the most frequently used antibiotics for neonates were Ampicillin, Gentamicin, and Cefotaxime. The findings indicated that both prolonged exposures, as well as the use of broad-spectrum antibiotics, such as the above, were contributing factors to the development of NEC (Heida et al. 2012).

Intrapartum antibiotic prophylaxis is often used to prevent the onset of neonatal sepsis. In a study carried out in 2019, it was observed that maternal intrapartum antibiotic intake gave rise to a reduction in the number of Bacteroides phylum (during the 1st week of life), Bacteroides, and Parabacteroides (at 3 months), which are protective commensal bacteria while increasing the number of Enterobacteriaceae (at one week) (Banerjee et al. 2012). Enterobacteriaceae in the gut is known to be associated with the pathophysiology of NEC (Tapiainen et al. 2005). In this study, of the 16 mothers who eventually gave birth to babies afflicted with NEC, only 15 (10 had received antibiotics, whereas 5 had not) had sufficient data on their intrapartum antibiotic consumption. 70% of neonates whose mothers had received antibiotics eventually became septic at a given point during treatment as opposed to their control group counterparts, where 40% of the patients had succumbed to sepsis. 50% (5 out of 10) of the babies whose mothers were administered antibiotics in the intrapartum period had resulted in mortality, while the other half had survived; in comparison, the mothers who had not received antibiotics only had one case, which resulted in expiration of the neonate. Previous research has shown that narrow-spectrum beta-lactam antibiotics given to mothers during vaginal delivery have a long-term effect on the developing microbiome of the infant's gut (Agwu et al., 2020). Maternal use of antibiotics before delivery (ampicillin and azithromycin) resulted in 7.5% of neonates developing NEC (Samuels et al. 2017). Infants who developed NEC were more likely to have a history of in-utero exposure to ampicillin than those who did not develop NEC (Strauss et al., 2010).

Although it remains a controversial subject with varying results, the ongoing inquiry about whether receiving blood transfusions in preterm neonates increases the risk of NEC raises many questions. The primary reason for packed red blood cell (PRBC) transfusion in neonates is anemia. As previously mentioned, indications for transfusion include symptomatic anemia (signs such as tachycardia, bradycardia, and oxygen desaturations) (Gephart 2012), hematocrit less than 25% (LaGamma 2018), and acute blood loss (most commonly due to intraventricular hemorrhage due to prematurity). Blood transfusions may also be primarily required for extremely low birth weight infants in the early weeks after birth as per a study conducted by (Widness JA et al. in 2005), which stated that due to the extremely fragile condition of these patients and the severity of illness that they may develop, the number of phlebotomies done per week is increased and can result in 10 to 30% blood loss during the procedure per week (Berkhout 2016). Moreover, neonatal exchange transfusions and intrauterine transfusions through umbilical vessels have shown an increase in the incidence of NEC, according to a review article published in the Archives of Disease in Childhood by Agwu JC (Dierikx et al. 2020).

Understanding the pathophysiology of PRBC's relation to NEC can aid in the understanding of this controversial yet paramount subject. The normal physiological response to anemia includes multiple mechanisms, but one of interest in the post-transfusion setting is the release of hemoglobin-bound nitric oxide (Widness et al., 2018). In packed red blood cells, there are lower levels of RBC nitric oxide, leading to increased nitric oxide being recruited by the free hemoglobin introduced to the recipient. The free hemoglobin has a much higher affinity for nitric oxide. This leads to hypoxic nitric oxide-dependent vasodilation, which results in reduced blood flow (Widness et al., 2018). This process is important in neonatal gastrointestinal blood flow, primarily intestinal microcirculation. The intestinal microcirculation is a complex network of vessels where the arteriolar plexuses present primarily regulate the intestinal blood flow as they are the branch between the intestinal macrocirculation and microcirculation.

Endothelin-1 is an amino acid peptide, mainly secreted by endothelial cells, that acts as a potent endogenous vasoconstrictor (Reed 2005). Plasma endothelin-1 (ET-1) levels were seen to be elevated after a blood transfusion (30). This surge could suggest that PRBC transfusion could lead to endothelin-induced oxygen radical formation, which may lead to tissue injury and eventually necrotizing enterocolitis (Kowalczyk n.d). It has been hypothesized and tested those vasoconstrictive properties, especially Endothelin-1 (ET-1), and vasodilator properties, largely nitric oxide when in imbalance, lead to dysregulation of intestinal blood flow (Kawano et al. 2018). In a study conducted by Kawano Y et al. in 1995 that studied the effects of blood volume expansion and depletion of levels of ET-1 in rats, it was reported that there were increased levels with volume depletion. In contrast, decreased levels were observed with volume expansion (Silverman n.d). When taking into consideration that free hemoglobin from PRBC transfusions has a higher affinity for nitric oxide, a potent vasodilator, and decreased levels of ET-1, a vasoconstrictor, after a blood

transfusion, this can potentially indicate that due to hypoxic vasodilation, PRBC transfusions increase the incidence of NEC in neonates.

In this study, there was a 10.4-time relative risk of death in neonates who had received blood transfusion compared to those who did not. 66.7 % (Chi-square p=0.01) of our patients who had received blood transfusions had resulted in a fatality. There was almost a 10% increase in the incidence of sepsis between the patients who had received transfusions (66.67%) vs. those who did not (57.14%). Several studies show that infants who developed Transfusion Associated Necrotizing Enterocolitis (TANEC) were considerably more likely to develop it at later postnatal ages, more prone to experience severe intraventricular hemorrhage, and were also nearly twice as likely to die when compared to those who developed NEC that was not linked with transfusion (Tanaka et al. 2011).

Although a link between neonatal exposure to antibiotics and the increased likelihood of the development of NEC was established, further studies in the future must be undertaken to determine whether the increase in the incidence of cases is attributed to a causal relationship or a spurious relationship between the risk factor and the diagnosis. Additionally, another limitation of the study was that it did not contain a control group to compare gender, gestational age, birth weight, the incidence of sepsis, and perforation. It would also be ideal for the study to be conducted in a larger or multicenter hospital to reach a larger target population. Moreover, focusing on the different types of antibiotics usually prescribed to both mothers and neonates and cross-examining them with the incidence rate of NEC for each antibiotic would be grossly beneficial in influencing future prescribing protocols of antibiotics in expectant mothers and premature babies. Despite the association between blood transfusions and NEC, there is limited evidence to support a causal relationship between the two. All of the studies analyzed to support our research were retrospective, observational, and systematic reviews. There is an immense call for more prospective studies for neonatal antibiotic intake and blood transfusions. If a relationship were to be established and proven, we would better control factors during pre- and post-transfusion routines and alter guidelines regarding recommended doses and types of transfusions delivered to reduce the number of neonates developing necrotizing enterocolitis.

5. Conclusion

NEC is a rare condition predominantly found in premature infants that has significant morbidity and mortality. Risk factors for NEC include low gestational age, low birth weight, antibiotics use prenatally and postnatally, blood transfusions, and co-morbid conditions such as sepsis. Our study demonstrated that, albeit with a small sample size, the aforementioned risk factors, which can be controlled, play a part in the pathogenesis of NEC. The limitation of this study is the low number of patients and population; a wider range of hospitals should be involved to further solidify the results. Therefore, it is vital to conduct further, more extensive studies to assess the impact of different risk factors and their related outcomes.

6. Statements and Declarations

This research received no external funding, and the authors declare no conflict of interest. All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organization or those of the publisher, the editors, and the reviewers. Authors are required to state whether ethical approval was sought or not for the present study, especially if the study is a clinical trial or animal experiment. The authors took verbal consent from all participants to use the data.

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