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RESEARCH

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NECROTIZING ENTEROCOLITIS IN LOW WEIGHT NEWBORNS: HIERARCHIZED ANALYSIS OF ASSOCIATED FACTORS

Enterocolite necrosante em recém-nascidos de baixo peso: análise hierarquizada dos fatores associados

Enterocolitis necrotizante en recién nacidos de bajo peso: análisis jerárquico de factores asociados

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ABSTRACT

Objective: To analyze the occurrence of necrotizing enterocolitis and the factors associated with its development in infants of low birth weight. **Methods:** Case-control study , 1:3, 95% confidence level and 80% study power, total of 236 newborns with low weight, 59 infants with diagnosis of necrotizing enterocolitis and 177 RN without. Maternal and neonatal variables were organized into blocks and levels (distal, intermediate and proximal). To verify association with the response variable NEC, hierarchical logistic regression was used. **Results:** Statistically significant association with NEC, antenatal corticosteroid use (OR = 2.90; p <0.001), reduced amniotic fluid (OR = 2.03; p <0.001), resuscitation at birth (OR = 1). , 35; p = 0.010), birth weight ≤1500g (OR = 3.32; p <0.001), transfusion (OR = 2.11; p = 0.040) and surfactant use (OR = 2.41; p = 0.020). **Conclusion:** Factors related to gestational period, birth and hospitalization may be influencing the onset of NEC. Intervention in these variables may decrease the chance of NEC.

DESCRIPTORS: Necrotizing enterocolitis; Premature; Neonatal Intensive Care Unit; Infant Premature Diseases; Premature Birth.

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RESUMO

Objetivo: Analisar a ocorrência de enterocolite necrosante e fatores associados ao seu desenvolvimento em recém-nascidos de baixo peso. **Métodos**: Estudo caso controle, 1:3, nível de confiança de 95% e poder do estudo de 80%, total de 236 RN de baixo peso, sendo 59 RN com ECN e 177 RN sem ECN. As variáveis maternas e neonatais foram organizadas em blocos e em níveis (distal, intermediário e proximal). Para verificar associação com a variável resposta ECN empregou-se a regressão logística hierarquizada. **Resultados:** Observou-se associação estatisticamente significante com ECN, o uso de corticóide antenatal (OR=2,90; p<0,001), líquido amniótico reduzido (OR=2,03; p<0,001), reanimação ao nascimento (OR=1,35; p=0,010), peso ao nascimento ≤1500g (OR=3,32; p<0,001), transfusão (OR=2,11; p=0,040) e uso de surfactante (OR=2,41; p=0,020). **Conclusão: F**atores relacionados ao período gestacional, ao nascimento e hospitalização podem estar influenciando no aparecimento da ECN. Intervenção nestas variáveis pode diminuir a chance de ECN.

DESCRITORES: Enterocolite necrosante; Prematuro; Unidade de Terapia Intensiva Neonatal; Doenças do Prematuro; Nascimento Prematuro.

RESUMEN

Objetivo: Analizar la aparición de enterocolitis necrotizante y los factores asociados con su desarrollo en lactantes de bajo peso al nacer. **Métodos:** Estudio de casos y controles, 1: 3, nivel de confianza del 95% y 80% de poder de estudio, un total de 236 recién nacidos con bajo peso al nacer, 59 recién nacidos con ECN y 177 recién nacidos sin ECN. Las variables maternas y neonatales se organizaron en bloques y niveles (distal, intermedio y proximal). Para verificar la asociación con la variable de respuesta NEC, se utilizó la regresión logística jerárquica. **Resultados:** Asociación estadísticamente significativa con NEC, uso prenatal de corticosteroides (OR = 2.90; p <0.001), reducción del líquido amniótico (OR = 2.03; p <0.001), reanimación al nacer (OR = 1). , 35; p = 0.010), peso al nacer ≤1500g (OR = 3.32; p <0.001), transfusión (OR = 2.11; p = 0.040) y uso de surfactante (OR = 2.41; p = 0,020). **Conclusión:** Los factores relacionados con el período gestacional, el nacimiento y la hospitalización pueden estar influyendo en la aparición de NEC. La intervención en estas variables puede disminuir la posibilidad de NEC.

DESCRIPTORES: Enterocolitis Necrotizante; Recien Nacido Prematuro; Unidades de Cuidado Intensivo Neonatal; Enfermedades del Prematuro; Nacimiento Prematuro.

INTRODUCTION

Necrotizing Enterocolitis (NEC), a serious gastrointestinal disease, is among the main causes of neonatal mortality in the neonatal intensive care unit (NICU) environment, particularly affecting the population of low birth weight newborns (NB). Its incidence is inversely proportional to gestational age and birth weight, affecting 12% of children weighing less than 1500g and causing death in 30% of cases. It is considered a clinical emergency, due to the rapid evolution of signs and symptoms such as abdominal distension, bilious vomiting and hematochezia for severe cases of peritonitis, pneumoperitoneum and septic shock, thus, early diagnosis and treatment is essential.[1-3]

Several efforts have been expended in an attempt to elucidate the cause for the appearance of NEC, however, so far it is understood that it is a disease of multifactorial cause. Several factors can predispose this disease such as prematurity, polycythemia, use of umbilical catheter, twinning, respiratory disorders, persistence of the ductus arteriosus, rapid progression of the diet and use of artificial milk in enteral nutrition.[3]

Despite all the assistance provided by a multidisciplinary team and the available technological apparatus, there is still an increase in the frequency of NEC cases in neonatal ICUs.[1-3] Thus, as there are several factors associated with NEC, it was observed in the literature[2-3] that, above all, neonatal factors are pointed out as involved in the causality of this pathology, however, the results presented did not obey a hierarchical analysis causing a possible existence of confounding variables in the final result presented.

Thus, the hierarchical analysis will provide subsidies for understanding the mechanisms responsible for the appearance of NEC in low birth weight newborns, and the question is: what are the factors associated with NEC in this population? In a hierarchical analysis, what are the maternal and neonatal aspects involved in the appearance of this pathology?

In this context, the objective of the present study is to analyze the occurrence of necrotizing enterocolitis and the factors associated with its development in low birth weight newborns.

METHODOLOGY

Case-control study on ECN in low birth weight newborns, carried out from March 1, 2014 to June 20, 2015, in two NICUs in São Luís - MA, one of the federal public network comprising 20 beds and the other another from the state public network consisting of 30 beds.

The sample size was constituted in the proportion of 1: 3 (1 case for 3 controls), establishing a confidence level of 95% and study power of 80%, sufficient to detect an OR = 2.5, with an exposure factor , maternal infection among NEC cases of 67.8% based on the literature4; totaling 59 cases of NEC and 177 controls without NEC.

Any newborn with a weight equal to or less than 2500g, with confirmed diagnosis of NEC and classified as stage II or III according to the BELL criterion modified by Walsh and Kleigman4 was considered as a case of NEC and as a control, all NB admitted to the NICU, weighing 2500 g or less, without a diagnosis of NEC, nor a radiograph with intestinal changes such as paralytic ileus, distension or perforation of the intestinal loop.

Low birth weight newborns were considered all newborns weighing 2500 g or less at birth. NBs with gastrointestinal malformations or who died within 24 hours after birth were used as non-inclusion criteria.

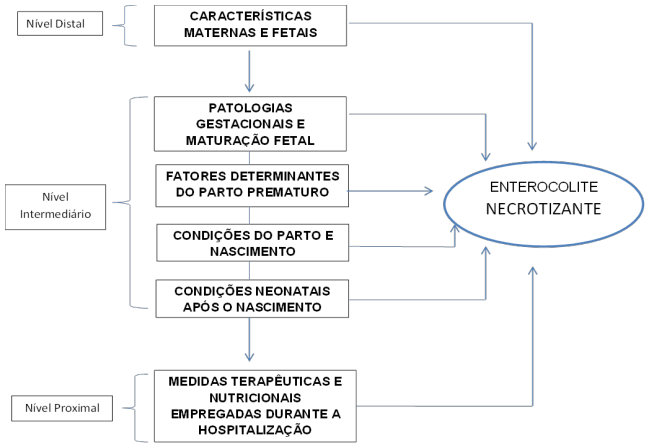
Data collection was initiated by NEC cases (case group), from the neonatal unit's death record book and in the database of the Hospital Infection Control Commission-CCIH, for location in the Medical Archive Service (SAME). Control group NBs (without ECN) were selected according to the sequential order of the NICU admission record book.

Maternal and neonatal variables were grouped into a block: first block constituted by maternal characteristics: age, number of pregnancies, number of births, number of abortions, twinning, prenatal care (less than 7 consultations; 7 consultations or more); and fetal characteristics: sex; second block consisting of gestational pathologies: broken bag time (up to 12 hours; over 12 hours), arterial hypertension (blood pressure equal or above 140x90mmHg), amniotic fluid with fetal odor and maturation: corticotherapy; third block consisting of the determinants of premature birth: placental flow (confirmed by doppler obstetric ultrasonography and categorized as normal / altered), amount of amniotic fluid (identified by obstetric evolution and confirmed by ultrasound examination and categorized as normal/ reduced) and amniotic fluid with meconium; fourth block constituted by the conditions of delivery: type of delivery and birth: Apgar score of the 5th minute, anoxia, resuscitation, birth weight, gestational age, and classification of the newborn5; fifth block consisting of neonatal conditions after birth: respiratory disorder, congenital heart disease, apnea and seizure; sixth block consisting of therapeutic measures: venous umbilical catheterization; arterial umbilical catheterization; surfactant; transfusion; use of indomethacin; use of ventilatory support; length of stay on invasive mechanical ventilation in days (median); and nutritional during hospitalization: type of enteral nutrition (breast milk (LM) or pasteurized human milk (LHP); artificial formula), days of life to start the diet, and speed of progression of the diet (categorized as less than or equal to 20ml/Kg/day and greater than 20ml/Kg /day).

The data were analyzed using the Stata 11.0 program. Necrotizing Enterocolitis was considered a dependent variable and maternal and neonatal variables were independent. To assess the normality of the quantitative variables, the Shapiro-Wilk test was used. Qualitative variables were expressed in proportion. The differences between the means, when compared according to the groups (case or control), were evaluated by the T-Student tests, while the differences between the medians, according to the groups were evaluated by the Man Whitney test.

An unadjusted analysis was performed where the association of all maternal and neonatal variables was tested in relation to the outcome. Those with a p-value <0.20 were maintained for the second phase. For the second phase, a hierarchical type analysis was used, which proposes the grouping of variables into levels according to the influence on the outcome, classifying them in distal variables in the first block, in intermediate level 1 those in the second block; at intermediate level 2 those of the third block; at intermediate level 3 those of the fourth block; at intermediate level 4 those of the fifth block, and proximal level those of the sixth block, according to the flowchart (Figure 1).

Figure 1 - Flowchart of the hierarchical relationship between the variables and their relationship to the outcome



Variables at the distal level were introduced at a single time, which in the unadjusted analysis showed p <0.20; only the variables that maintained p-value <0.10 remained at this level. The variables of the distal level were maintained, the next step was the study of the variables of the intermediate level 1. The variables of the distal and intermediate level 1 were maintained, the variables of the intermediate level 2 were introduced, keeping the variables with p-value < 0.10 regardless of possible changes in the statistical significance of the distal and intermediate levels 1. Following this analysis, the intermediate level 3 variables were introduced, keeping the variables with p-value <0.10. Subsequently, the intermediate level 4 variables were added, and those with a p-value less than 0.10 remained independent of possible changes in the statistical significance of the variables of the distal, intermediate levels 1, 2 and 3. Finally, the variables were inserted of the proximal level in a similar way to the previous levels and remaining in the final model, the variables that maintained p-value <0.10. The OR values were estimated, with the reference category OR = 1, the 95% confidence intervals were constructed and the p values were determined. In terms of statistical relevance, variables with p-value <0.05 were considered. The study complies with Resolution 466/2012, being approved by the Research Ethics Committee of the Federal University of Maranhão, under the opinions of number 698,693 and 927,908.

RESULTS

Of the 59 cases of NEC, 61.02% were female, the median hospital stay was 45 days, and 40.68% died. Among the 177 control NBs, more than half (54.55%) were male, the median hospital stay was 19.5 days, and 14.12% died.

In the unadjusted analysis, the maternal variables that showed statistical significance with the appearance of NEC were: use of antenatal steroids and reduced amniotic fluid. Mothers with abnormal placental flow during pregnancy were twice as likely to develop NEC in low birth weight newborns (Table 1).

Table 1 - Univariate analysis of maternal variables of low birth weight newborns in cases of NEC and controls. São Luís-MA, 2015.

Variables	Cases (n=59) n (%)	Controls (n=177) n (%)	OR	IC 95%	p
Twinning					
No	56 (94,92)	156 (88,14)			
Yes	03 (5,08)	21 (11,86)	0,40	0,11-1,39	0,15
Antenatal corticotherapy					
No	17 (28,81)	96 (54,23)			
Yes	42 (71,18)	81 (45,76)	2,93	1,55-5,53	<0,001
Placental Flow					
Normal	22 (37,28)	96 (54,23)			
Changed	24 (40,67)	81 (45,76)	2,07	1,06-4,06	0,03
Amniotic fluid					
Normal	25 (43,86)	135 (78,49)			
Reduced	30 (52,63)	34 (19,77)	2,19	1,58-3,03	<0,001

Among the neonatal variables, a statistically significant NEC was observed: resuscitation at birth, birth weight less than or equal to 1500g, presence of respiratory disorder, use of venous and arterial umbilical catheter, use of surfactant, blood transfusion and use of support ventilation (Table 2).

Table 2 - Univariate analysis of neonatal variables of low birth weight NBs in cases of NEC and controls. São Luís-MA, 2015.

Variables	Cases (n=59) n (%)	Controls (n=177) n (%)	OR	IC 95%	p
Gender					
Female	36 (61,02)	80 (45,45)			
Male	23 (38,98)	96 (54,55)	0,53	0,29-0,97	0,04
Anoxia					
No	49 (83,05)	156 (88,13)			
Yes	08 (13,55)	20 (11,29)	1,85	0,97-3,55	0,06
Resuscitation					
No	13 (22,03)	110 (62,14)			
Yes	46 (77,96)	65 (36,72)	1,51	1,26-1,79	<0,001
Birth weight (grams)					
>1500	15 (25,42)	116 (65,54)			
≤1500	44 (74,58)	61 (34,46)	5,58	2,87-10,82	<0,001
Respiratory disorder					
No	01 (1,69)	31 (17,51)			
Yes	58 (98,31)	146 (82,49)	1,35	1,16-1,56	<0,001
Apnea					
No	32 (54,23)	160 (90,39)			
Yes	27 (45,76)	17 (9,60)	2,62	1,31-5,23	0,01
Convulsion					
No	48 (81,35)	166 (93,78)			
Yes	11 (18,64)	11 (6,21)	3,46	1,41-8,47	0,01
Venous umbilical catheter					
No	26 (44,06)	138 (77,96)			
Yes	33 (55,93)	39 (22,03)	4,49	2,40-8,39	<0,001

Variables	Cases (n=59) n (%)	Controls (n=177) n (%)	OR	IC 95%	p
Arterial umbilical catheter					
No	25 (42,37)	137 (77,40)			
Yes	34 (57,62)	40 (22,59)	4,03	1,97-8,21	<0,001
Surfactant					
No	17 (28,81)	128 (72,31)			
Yes	41 (69,49)	49 (27,68)	3,41	2,03-5,74	<0,001
Transfusion					
No	19 (32,20)	139 (78,53)			
Yes	40 (67,80)	38 (21,47)	4,89	2,66-8,97	<0,001
Type of enteral nutrition					
LM/LHP***	46 (77,96)	156 (88,13)			
Artificial formula	08(13,55)	10 (5,64)	2,71	1,01-7,27	0,05
Speed of diet progression					
≤20ml/Kg/day		124 (70,05)			
>20ml/Kg/day		42 (23,72)	0,51	0,22-1,18	0,12
Apgar 5th minute		09 (1)**	0,71	0,54-0,94	0,02
Gestational age		33 (3,04)*	0,79	0,71-0,88	<0,001
Duration of invasive mechanical ventilation		02(3)**	4,76	2,64-8,60	<0,001
Beginning of diet (days of life)		02 (2) **	1,16	1,02-1,32	0,03

* Average (standard deviation) ** Median (interquartile range)

LM / LHP *** - breast milk / pasteurized human milk

The results also revealed that being a male NB reduces the chance of having NEC by 47%, as well as episodes of anoxia, apnea and seizure. As for nutritional aspects, it was found that the use of artificial formula for enteral nutrition almost triples the chance of low birth weight newborns to have NEC (Table 2).

When the mean gestational age was compared between the case and control groups, it was observed that the mean gestational age was lower (31 weeks) among the newborns in the case group, as well as a longer time spent on invasive mechanical ventilation (median 6 days), with a chance of developing NEC almost 5 times higher, that is, for each additional day in this type of ventilation, the chance of developing this disease increases by 376%. On the other hand, for each increase of one unit of the APGAR index in the 5th minute, the chance of the newborn being affected by NEC decreases by 29% (Table 2).

In the adjusted analysis, among the variables of the distal level, only the male gender remained in the model. (Table 3).

Table 3 - Adjusted analysis of the distal level variables associated with NEC. São Luís-MA, 2015.

Variable	OR*	IC** 95%	P
Gender			
Female			
Male	0,55	0,30-1,02	0,06

*OR- *Oddsratio* **IC- confidence interval

On the other hand, in the adjusted analysis of the variables of the distal + intermediate levels, the male gender remained without statistical significance. Regarding the use of antenatal corticosteroids, statistical significance was observed during the analysis of the intermediate levels 1, 2 and 4, losing significance during the analysis with the variables of the intermediate level 3. The variables maintained a significant association at all levels: reduced amniotic fluid, resuscitation at birth, and birth weight ≤1500g (Table 4).

Table 4 - Adjusted analysis of the variables of the distal level + intermediate level associated with the NEC. São Luís-MA. 2015

Variables	OR*	IC** 95%	p
Intermediate 1			
Male	0,55	0,30-1,02	0,06
Use of antenatal corticosteroids	2,90	1,53-5,51	<0,001
Intermediate 2			
Male	0,60	0,29-1,25	0,17
Use of antenatal corticosteroids	2,45	1,12-5,39	0,03
Reduced Amniotic Fluid	2,03	1,38-2,98	<0,001
Intermediate 3			
Use of antenatal corticosteroids	1,91	0,87-4,23	0,11
Reduced Amniotic Fluid	2,04	1,40-2,96	<0,001
Resuscitation at birth	1,35	1,09-1,66	0,01
Birth weigh ≤1500g	3,32	1,52-7,24	<0,001

Variables	OR*	IC** 95%	p
Intermediate 4			
Use of antenatal corticosteroids	2,26	1,00-5,13	0,05
Reduced Amniotic Fluid	1,97	1,34-2,92	<0,001
Birth weigh ≤1500g	3,22	1,42-7,28	0,01
Resuscitation at birth	1,33	1,07-1,65	0,01
Apnea	1,67	0,91-3,05	0,09

OR- *Oddsratio* IC- confidence interval

In the final model of the hierarchical analysis, the factors that maintained a statistically significant association with NEC were: use of corticosteroids, reduced amniotic fluid, resuscitation at birth, birth weight less than or equal to 1500g, blood transfusion and use of (Table 5).

Table 5 - Final model of the hierarchical analysis of the variables of the distal level + intermediate level + proximal level associated with NEC. São Luís-MA. 2015

Variables	OR*	IC** 95%	p
Male	0,55	0,30-1,02	0,06
Use of antenatal corticosteroids	2,58	1,11-6,00	0,03
Apnea	1,67	0,91-3,05	0,09
Resuscitation at birth	1,35	1,09-1,66	0,01
Reduced Amniotic Fluid	1,80	1,21-2,67	<0,001
Birth weight ≤1500g	1,89	0,80-4,44	0,14
Transfusion	2,11	1,05-4,21	0,04
Enteral nutrition / artificial formula	2,88	0,87-9,51	0,08
Surfactant	2,41	1,17-4,96	0,02
Beginning of diet (days of life)	1,11	0,96-1,29	0,16

OR- *Oddsratio* IC- confidence interval

DISCUSSION

It was found that the majority of cases were female, unlike other studies where there was a greater number of NECs among male NBs. However, no clinical or genetic justifications for this finding were found in the literature.[1,4,6]

The hierarchical analysis revealed that the use of antenal corticosteroids almost triples the chance of occurrence of NEC, corroborating with a study[7] that showed the importance of this therapy as a strong ally in the prevention of prematurity, even being pointed out as a protective factor for NEC. This type of medication accelerates the maturation of the intestinal mucosa, which would explain the decrease in the incidence of this disease and the attenuation of the clinical presentation in neonates whose mothers received corticosteroids early in pregnancy.

The use of antennal corticosteroids can also be justified, as it is a drug used routinely on the verge of premature birth and the sample of this study was predominantly composed of premature newborns. On the other hand, it is questionable whether the development of this disease may be being influenced by the inappropriate use of antenatal corticosteroids in an attempt to accelerate lung maturation in infants with intrauterine suffering.

Another finding pointed out by the hierarchical analysis as a factor associated with NEC was the reduction of amniotic fluid, which may have been triggered by arterial hypertension and urinary tract infection, although there is no tested correlation. Studies indicate that maternal infections, especially intrauterine infections, when not treated with antibiotics have an important relationship with the pathogenesis of ECN.[8-9] In connection with this, oligodramnia has been revealed as a risk factor for in-hospital death in the unit neonatal care in Brazil, as it is associated with operative delivery, fetal distress and malformations.

Weight equal to or less than 1500g has a greater association for NEC, which can be explained by the inverse relationship between low weight and the main neonatal morbidities, that is, the lower the birth weight, the greater the likelihood of severe hyaline membrane disease, intracranial hemorrhage, bronchopulmonary dysplasia, persistence of the ductus arteriosus, which contributes to the occurrence of hypoxic-ischemic events, increasing the risk for ECN.[10-11] Linked to low birth weight are also the greatest need for resuscitation to the newborn, data still found in the study above, as associated with the pathogenesis of this disease, which may be related to intrauterine fetal distress and hypoxemia, caused by unfavorable environmental conditions at birth.

Apnea was found to have a significant association, through univariate analysis, significant association (p = 0.010), however, this significance disappeared in the hierarchical analysis. A similar finding was seen in a study with 394 NBs younger than 30 weeks, showing no association between episodes of apnea, severe desaturation or bradycardia and the development of the disease in premature infants.[12] However, it is worth noting that despite the apnea not having revealed a significant association, it was present together with other disorders of the respiratory system, as an indication for the use of ventilatory support and long duration of invasive mechanical ventilation, thus predisposing this population to infectious conditions.

Regarding the use of surfactant, its use on a large scale in order to reduce the number of deaths has been observed in recent years, which is confirmed by results that revealed that this medication had a positive impact on neonatal mortality, reducing the number of deaths, but on the other hand, its use did not change the incidence of complications related to prematurity, such as necrotizing enterocolitis.[3] Our findings showed a high chance of developing NEC among NBs who use this medication, concluding that even though there are proven benefits of surfactant therapy, its use should be carefully evaluated. However, it is observed in clinical practice, that this medication has been used late, which ends up not generating positive impacts in reducing neonatal mortality.

With regard to blood transfusions received by the NB in the present study, it was found that this type of therapeutic approach is associated with the development of NEC, increasing the chance of being affected by about two times. On the other hand, authors found in research with newborns

with the same weight, low association between the transfusion of red blood cells and severe conditions of NEC.[13] These divergences, however, do not nullify the risk of this type of therapeutic conduct, and should also be used cautiously.

As for the nutritional aspects, it was found that the use of artificial formula showed a significant association only in the univariate analysis, while the speed of progression of the diet above 20ml / Kg / day did not reveal an association in this study. However, other research reveals that consumption of human milk is associated with low risk of NEC, especially in premature infants, and their mothers should be encouraged to supply their own milk, except in cases of special situations.[14] In addition, use of artificial formula increases the risk for gastrointestinal disorders with no literary consensus on the ideal speed of progression of the diet. It is worth mentioning that because our research was carried out in public hospitals that have a human milk bank, with the title Hospital Amigo da Criança, and there is a policy to encourage breastfeeding, it may have contributed to the reduced number of newborns using artificial formula.

FINAL CONSIDERATIONS

Despite the existing limitation with the lack of information in the medical records, due to its incomplete filling, especially with regard to neonatal variables, such as prolonged hospital stay, this study allowed a more detailed understanding of the behavior of this pathology in low weight newborns and, consequently , serve as a subsidy for the elaboration of interventions both for the early detection of signs and symptoms and for the prevention of complications that affect the quality of life of this population.

Thus, it is concluded that maternal aspects related to the gestational period and neonates related to birth and hospitalization may be influencing the appearance of NEC.

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