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The correlation between levels of vitamin D (25(HD)D) and the occurrence of necrotizing enterocolitis (NEC) in preterm infants

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ABSTRACT

Introduction: One of the causes of gastrointestinal emergencies in newborns is necrotizing enterocolitis (NEC). A fetus is fully dependent on transfer from the mother as it lacks endogenous 25(OH)D production. Transplacental vitamin D transmission mainly occurs due to the elevated risk of vitamin D insufficiency in preterm newborns during the third trimester of pregnancy. This research is important to raise awareness about the importance of early initial screening of vitamin D levels in preterm infants in Indonesia.

Methods: This study used an observational research design which is a cohort study, taking place during March–May 2020 in the neonatology inpatient ward of dr. Saiful Anwar General Hospital Malang. The enzyme-linked immunosorbent assay (ELISA) technique was used to measure the amount of vitamin D in the blood. The Kolmogorov-Smirnov test and the Levene test were used to determine whether the data were normal and homogeneous, respectively.

Result: It was found that vitamin D levels in preterm infants with NEC were lower (10.18 ± 4.07 ng/mL) than in preterm infants without NEC (16.95 ± 4.45 ng/mL). The bivariate analysis result showed that vitamin D deficiency increased the risk of NEC by 10.7 times with p-value = 0.033 (95% CI 1.2-24.9). Furthermore, multivariate analysis found that vitamin D deficiency increased the occurrence of NEC by 19.4 times with a p-value = 0.043 (95%CI 0.7-21.7) and respiratory distress could increase the incidence of NEC by 9.4 times with a p-value =0.02 (95%CI 1.2-70).

Conclusion: Vitamin D (25(OH)D) levels were lower in preterm infants with necrotizing enterocolitis compared to preterm infants without necrotizing enterocolitis. This study showed a significant correlation between vitamin D(25(OH)D) levels and the case of necrotizing enterocolitis in preterm infants.

Keywords: 25(OH)D vitamin D levels, cohort, necrotizing enterocolitis preterm infants.
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INTRODUCTION

One of the causes of gastrointestinal emergencies in newborns is necrotizing enterocolitis (NEC), a multifactorial condition of rapid intestinal ischemia necrosis. 1 per 1000 live births (5%–10%) is the incidence of NEC, and preterm newborns account for more than 90% of all cases. In 2009, there were 31 cases of NEC out of approximately 737 preterm births in the Perinatology Division of the Department of Pediatrics (IKA) at Cipto Mangunkusumo Hospital (RSCM). The mortality rate from NEC ranges from 15%-30%, is higher in infants of younger gestational age, and is one of the leading causes of death in the neonatal intensive care unit (NICU).[1]

The mortality rate can be as high as 40% in neonates who develop NEC with perforation and require surgical intervention. Neonates with NEC are also more likely to experience nosocomial infections, inadequate nutritional intake, slow growth, higher incidence of bronchopulmonary dysplasia and retinopathy of prematurity, and require long intensive care. The cause of NEC is not known up to now, but several things are suspected to be the cause, namely a hyperreactive immune system response, ischemia, infection, introduction of enteral food, abnormal microflora colonization, or response to microflora translocation in the gastrointestinal tract. Preterm infants' immature intestinal epithelial cells and gastrointestinal tract immune response mechanisms encourage the permeabilization of the intestinal barrier and the colonization of aberrant microbiota.[1]

Necrotizing enterocolitis (NEC) is a necrotic inflammatory bowel disease affecting premature infants.[2] Intestinal barrier failure, bacterial invasion, immunological activation, uncontrolled inflammation with reactive oxygen (ROS) and nitrogen generation, vasoconstriction followed by ischemia in perfusion, intestinal necrosis, sepsis, and shock are all thought to contribute to NEC.[3]

Recent studies have shown that vitamin D is a key modulator of immune function and inflammation with broad regulatory effects on the adaptive and innate immune systems cells. The vitamin D receptor (VDR), which is expressed in several human organs and tissues in addition to bone cells, including the kidneys, colonic mucosa, and immune

cells, is the mechanism by which vitamin D exerts its biological effects. It has been demonstrated that all immune system cells express VDR. VDR controls cell division, proliferation, and the triggering of apoptosis in the gut. Inflammatory bowel disease (IBD) has been the subject of numerous studies looking at the effect of vitamin D in immune-mediated disorders. 60% of people with IBD have been found to have vitamin D insufficiency. This is because vitamin D deficiency's effects on the digestive system result in lowered colonic bacterial clearance, decreased tight junction (TJ) expression in the intestinal epithelium, and elevated Th1-mediated inflammation in the gut.[3]

Some evidence supports that Vitamin D3 has a role in maintaining intestinal barrier integrity and immune homeostasis. Vitamin D3 regulates many types of cells by controlling hundreds of genes in tissues and cells. Previous studies have highlighted the potential effect of Vitamin D3 as an inducer in the differentiation of normal intestinal epithelial cells and through maintaining the morphological differentiation typical of epithelial phenotypes. There is evidence that Vitamin D3 can improve the intestinal brush border affected by the inflammatory process. It can increase colonic mucosal cell proliferation and show no erosion and minimal ulceration suggesting a healing process.[4]

The fetus is highly dependent on the mother because it cannot make endogenous 25(OH)D. Preterm newborns are at high risk for vitamin D deficiency because transplacental vitamin D transmission primarily occurs in the third trimester of pregnancy. Previous research has demonstrated a link between maternal vitamin D deficiency and preterm birth risk as well as delayed fetal growth and lower fetal weight.[5–7]

Only a few studies on the prevalence of vitamin D insufficiency in preterm newborns are currently available, despite the significance of vitamin D's involvement in the digestive system. Furthermore, there has never been a study on the relationship between vitamin D levels in preterm infants and the occurrence of necrotizing enterocolitis in Indonesia. Thus, this research is important to raise awareness about the importance of early initial screening of vitamin D levels in preterm infants in Indonesia.

METHODS

This study used an observational research design which is a cohort study. The allocation of research subjects into research groups was done by consecutive sampling on the subjects who met the inclusion and exclusion criteria until the sample size requirements for each group were met during March–May 2020 in the neonatology inpatient ward of dr. Saiful Anwar General Hospital Malang. The inclusion criteria for this study were: (a) all preterm infants born and treated in the inpatient ward of dr. Saiful Anwar General Hospital Malang (b) preterm infants with a gestational age of 28-37 weeks (c) infants with exclusive or predominantly breastfeeding nutrition (d) parents or guardians willing to include their children in the study. The exclusion criteria were as follows: (a) patients suffering from congenital anomalies (congenital anomalies of kidney and urinary tract, congenital heart disease) (b) parents/guardians stated that they were not willing to participate in the study. Dropout criteria: (a) the patient died during the first 24 hours of observation (b) the parents/guardians stated that they were not willing to participate in the study. The procedure for establishing the diagnosis of necrotizing enterocolitis (NEC) was based on Bell's modification criteria and confirmed by a neonatologist. Using the enzyme-linked immunosorbent assay (ELISA) technique, serum vitamin D levels were assessed in the first 24 hours of life for all neonatal participants using blood samples drawn from the umbilical cord vein. Because the data were regularly distributed, the Kolmogorov-Smirnov test was used to determine the normality of the data, and the Levene test was used to determine the homogeneity of the data. Then, univariate and multivariate logistic regression analysis was performed. Furthermore, the Spearman test was carried out because the data were not normally distributed.

RESULTS

Vitamin D (25(OH)D) Levels in Premature Babies

This research was conducted in the neonatology ward of dr. Saiful Anwar General Hospital Malang for preterm newborns with a gestational age of 28 - 37 weeks who met the inclusion and exclusion criteria. The main objective of this study was to prove the relationship between serum 25(OH)D levels and the occurrence of necrotizing enterocolitis in preterm infants. By using a cohort research design, 51 research subjects were obtained.

Table 1 showed that there were more preterm infants with NEC compared to preterm infants without NEC in this study. The overall mean gestational age of preterm infants with and without NEC was 33.61 ± 2.08 weeks with the mean gestational age of preterm infants without NEC being 34 ± 3.23 weeks and the mean gestational age of preterm infants with NEC was 32 ± 8.01. The average birth weight was 1953.90 ± 409 grams with birth weight in preterm infants without NEC of 2025.43 ± 356 grams and birth weight in preterm infants with NEC of 1870.20 ± 314 grams. The average level of vitamin D (25(OH)D) overall was 13.38 5.44 ng/mL. Several maternal risk factors for the occurrence of necrotizing enterocolitis include severe preeclampsia, eclampsia, antepartum bleeding, premature rupture of membranes, history of vaginal discharge, intrapartum fever, no lung maturation induction, maternal age of more than 35 years old, and passive smoking. Risk factors for infants include preterm infants with GA <32 weeks, very low birth weight, and the presence of respiratory distress. The outcomes of preterm infants with NEC obtained were 23 patients recovered and 4 patients died.

Based on Table 2, vitamin D levels in preterm infants with NEC were lower (10.18 ± 4.07 ng/mL) than in preterm infants without NEC (16.95 ± 4.45 ng/mL). Among the preterm infants with NEC, there were 26 infants with vitamin D deficiency and 1 infant with vitamin D insufficiency. In the group of preterm infants without NEC, there were 17 infants with vitamin D deficiency and 7 infants

Table 1. Research Subjects Characteristics

Variables	No NEC (n = 24)	NEC (n = 27)	Total (n = 51)
Sex			
Male (n)	11	13	24/51
Female (n)	13	14	27/51
Gestational age (mean ± SD) [weeks]	34 ± 3.23	32 ± 8.01	33.61 ± 2.08
BW (mean ± SD) [grams]	2025.43 ± 356	1870.20 ± 314	1953.90 ± 409
Childbirth delivery method			
SC	16	19	35/51
Vaginal delivery	8	8	16/51
Maternal Risk Factors			
Severe preeclampsia (n)	13	13	26
Eclampsia (n)	6	4	10
APB (placenta previa) (n)	3	8	11
PROM (n)	2	3	5
History of vaginal discharge (n)	3	7	10
Intrapartum fever (n)	4	8	12
No fetal lung maturity induction (n)	19	16	35
Maternal age > 35 years old (n)	3	8	11
Passive smoker (n)	10	20	30
Infant Risk Factors			
Very Preterm (GA < 32 weeks) (n)	4	15	19
VLBW (n)	1	7	8
Respiratory distress (n)	3	17	20
Serum Vitamin D level (mean ± SD) [ng/mL]	16.95 ± 4.45	10.18 ± 4.07	13.38 ± 5.44
The outcome of infants with NEC			
Survived			23/27
Died			4/27

*NEC: necrotizing enterocolitis; SD: standard deviation; BW: birth weight; SC: sectio cesarean; APB: antepartum bleeding; PROM: premature rupture of membrane; GA: gestational age; VLBW: very low birth weight.

Table 2. Characteristics of Vitamin D (25(OH)D) Levels

Groups	NEC (n=27)	No NEC (n=24)
Vitamin D levels (ng/mL)	10.18 ± 4.07	16.95 ± 4.45
Vitamin D Classification		
Deficiency (<20 ng/mL)	26/27	17/24
Insufficiency (20-30 ng/mL)	1/27	7/24

with vitamin D insufficiency. According to the classification from the Endocrine Society, the normal vitamin D level is > 30 ng/ml, vitamin D insufficiency is 20-30 ng/mL, and vitamin D deficiency is < 20 ng/mL.

Before further analysis, the data were tested for normality and homogeneity as prerequisites for the parametric test. The data normality test result showed a p-value of 0.062 (p>0.05) which means that the data is normally distributed. The data homogeneity test was carried out using Levene's test, which resulted in a p-value of 0.072 (p>0.05), meaning the data is homogeneously distributed.

In this study, an evaluation of several factors that influence the occurrence of NEC was carried out, including maternal factors and infant factors. Maternal factors from this study were severe preeclampsia, eclampsia, antepartum bleeding, premature rupture of membranes, history of vaginal discharge, intrapartum fever, cesarean delivery, not given lung maturation induction, maternal age > 35 years old, and passive smoker. While the infant factors were vitamin D status, early preterm (gestational age <32 weeks), very low birth weight, and respiratory distress.

Table 3 showed that one of the significantly related factors to the incidence of NEC was vitamin D deficiency with p-value = 0.033 and OR = 10.7 (95% CI 1.2-24.9). This indicates that vitamin D deficiency increases the risk of developing NEC by 10.7 times. In addition, intrapartum fever can increase the incidence of NEC by 3.5 times with p-value = 0.09 (95% CI 0.8-14.9). Not given fetal lung maturation induction was a factor that increases the incidence of NEC by 4 times with a p-value = 0.039 (95% CI 1-14.9). Passive smoking can increase the incidence of NEC by 2.8 times with a p-value = 0.049 (95% CI 0.9-8.9). Very preterm birth (gestational age <32 weeks) has increased the incidence of NEC by 6.2 times with a p-value = 0.006 (95% CI 1.7-23.2) and respiratory distress can increase the incidence of NEC by 20 times with a p-value = 0.000 (95% CI 4.5-88.2).

Furthermore, to find out which factors are influential when several risk factors were to be analyzed simultaneously, a multivariate analysis test was carried out. Risk factor variables with a significance

Table 3. Bivariate Analysis Test Results of Risk Factors for the Occurrence of NEC

Variables	P-value	OR	95% CI	
			Lower Limit	Upper Limit
Vitamin D Deficiency	0.033*	10.706	1.207	24.959
Severe eclampsia	0.668	1.273	0.423	3.831
Eclampsia	0.365	1.917	0.469	7.831
APB	0.148	0.339	0.078	1.468
PROM	0.740	0.727	0.111	4.768
History of vaginal discharge	0.237	2.450	0.555	10.813
Intrapartum fever	0.090*	3.500	0.821	14.927
SC delivery	0.563	0.700	0.209	2.345
No lung maturation induction	0.039*	4.000	1.074	14.896
Maternal age > 35 years old	0.148	0.339	0.078	1.468
Passive smoker (n %)	0.049*	2.807	0.887	8.882
Very Preterm (GA < 32 weeks)	0.006*	6.250	1.678	23.275
VLBW	0.061	8.050	0.911	71.163
Respiratory distress	0.000*	20.000	4.532	88.271

Note: *significant at p<0.05

Table 4. Multivariate Analysis Results of Risk Factors for the Occurrence of NEC

Variables	P-value	OR	95%CI	
			Lower Limit	Upper Limit
Vitamin D Deficiency	0.043*	19.406	0.714	21.743
APB	0.457	2.364	0.245	22.765
History of vaginal discharge	0.695	1.654	0.134	20.469
Intrapartum fever	0.272	3.519	0.373	33.177
No lung maturation induction	0.215	3.645	0.472	28.128
Maternal age > 35 years old	0.221	6.677	0.318	140.128
Passive smoker (n %)	0.420	2.263	0.310	16.504
Very Preterm (GA < 32 weeks)	0.535	1.923	0.244	15.189
VLBW	0.533	11.042	0.006	20.984
Respiratory distress	0.028*	9.443	1.273	70.043

* Note: *significant at p<0.05

p-value < 0.250 were included in the multivariate analysis test.

Respiratory distress and vitamin D deficiency were strongly linked with the incidence of NEC, according to Table 4. Respiratory discomfort can raise the incidence of NEC by 9.4 times with a p-value of 0.02 (95% CI 1.2-70) and vitamin D insufficiency can increase it by 19.4 times with a p-value of 0.043 (95% CI 0.7-21.7).

Correlation of Vitamin D (25(OH)D) levels with the onset of NEC

The mean onset of NEC in this study was 5.7 days. To determine the relationship between 25(OH)D levels and the incidence of NEC, Spearman's correlation test was conducted. It was found that there was a significant correlation between vitamin D levels and the onset of NEC (p=0.029). In Figure 1, it is seen that vitamin D levels

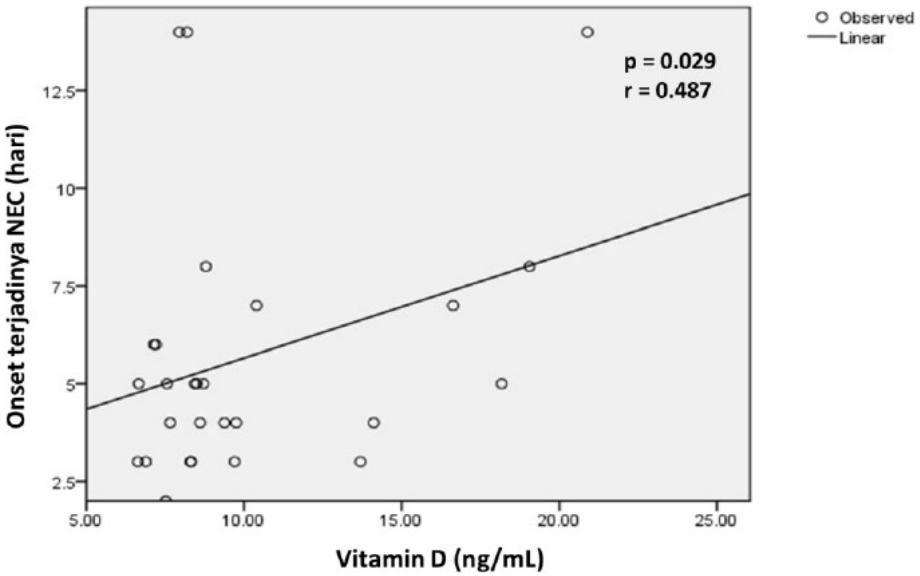

Figure 1. Results of the Spearman Correlation Test between Serum 25(OH)D Levels on the Onset of NEC.

are positively correlated with the onset of NEC. The higher the vitamin D level, the longer the onset of NEC and vice versa. The results of the correlation test also revealed the strength of the correlation of r = 0.487 which was interpreted as a moderate positive correlation. The graph showed that patients with higher vitamin D levels had an increasing trend from the bottom left to the top right which shows a positive correlation

DISCUSSION

Vitamin D (25(OH)D) Levels in Premature Babies

In this study, we found 51 patients who met the inclusion and exclusion criteria. Research subjects were determined based on gestational age according to the Ballard Score, which was those with a gestational age of 28-37 weeks who were born via vaginal or abdominal delivery. Then each subject was measured for 25(OH)D levels and grouped based on vitamin 25(OH)D levels into normal, vitamin D deficiency, and vitamin D insufficiency. Based on the birth weight of the research subjects, the average birth weight distribution was 1953.90 ± 4.09 grams. As many as 43 (84.3%) preterm infants had vitamin D deficiency which made up the majority of the subject.

A previous study conducted by Selviana in 2020, compared vitamin D levels in full-term infants with preterm infants in the hospital. Saiful Anwar Malang, found 23% of infants with vitamin D deficiency, 68% with vitamin D insufficiency, and 9% with normal vitamin D levels among full-term infants. The mean serum 25(OH)D level in full-term infants, was 22.94 ng/mL. Meanwhile, in preterm infants, the mean serum 25(OH)D level was 13.42 ng/mL. Based on the results of the Mann-Whitney test, the value of sig = 0.000 (sig < 0.05) indicates that there was a significant difference in serum 25(OH)D levels between preterm and full-term infants. Levels of 25(OH)D in preterm infants were lower than in full-term infants.[8]

Based on the levels of vitamin D 25(OH)D in the study, there were 43 preterm infants with vitamin D deficiency (< 20ng/mL), 8 preterm infants with vitamin D insufficiency (20-30 ng/mL), and no preterm infants with normal vitamin D status (>30 ng/mL). Several studies have shown that premature infants born <32 weeks (and especially those <28 weeks) are at greater risk of developing vitamin D deficiency.[9] In a study in India by Dadwal and Narayan in 2020, it was found that in a study of 100 newborns, 53 infants had vitamin D deficiency, 24 infants had vitamin D insufficiency and 23 infants had sufficient vitamin D levels. Of the 53 newborns with vitamin D deficiency, 32 (60%) were premature and 21 (40%) were term. Of the 24 newborns with vitamin D deficiency, 14 (58%) were premature and 10 (42%) were term. Of the 23 newborns who had adequate vitamin D levels, 4 (17%) were premature and 19 (83%) were term. The difference in vitamin D levels between premature and full-term newborns was statistically significant (p=0.002).[10]

This is consistent with the study of Burris et al., with 471 study subjects who compared 25(OH)D levels in the umbilical cord of premature and full-term infants. When compared to full-term newborns, infants under 32 weeks of age had a significantly higher risk of vitamin D insufficiency (25(OH)D 20 ng/mL) (OR 2.2, 95% CI 1.1, 4.3).[11]

Previous studies conducted by Selviana in 2020, compared vitamin D levels in adequate infants with infants less month at the hospital. Saiful Anwar Malang is obtained by a baby enough for vitamin D deficiency of as much as 23%, insufficiency of 68%, and normal levels of 9%. The baby is in enough months to get an average serum level of 25 (OH) D of 22.94 ng/ml while in infants less than 13.42 ng/ml. Based on the Mann-Whitney test results obtained sig = 0,000 (sig <0.05) show that there are differences in serum levels of 25 (OH) D in infants less than a month and enough months. 25 (OH) D levels in infants born less than lower months than infants enough months.[8]

NEC Incidence in Preterm Babies

In the study, after 14 days of observation, the number of preterm infants with necrotizing enterocolitis was 27 patients and the number of preterm infants without necrotizing enterocolitis was 24 patients. Based on the birth weight of the research subjects, the average birth weight and distribution were 1953.90 ± 409 grams with birth weight in preterm infants without NEC of 2025.43 ± 356 grams and birth weight in preterm infants with NEC of 1870.20 ± 314 grams. The mean gestational age was 33.61 ± 2.08 weeks with the mean gestational age in preterm infants without NEC of 34 ± 3.23 weeks and the mean gestational age in preterm infants with NEC of 32 ± 8.01.

A previous study on 6113 NICU patients found that 172 (2.8%) out of those patients developed NEC. The incidence of NEC decreases with increasing gestational age. The NEC incidence in infants between 28 and 36/7 weeks was 7%. The NEC incidence was higher in the moderately preterm (28–24 weeks) group (n=99) than in the other two gestational age groups (n=73). The NEC incidence in full-term infants was 0.4% (30 infants).[12]

A similar study conducted in Denmark in 2019 found that the incidence of NEC increased significantly with a decrease in gestational age, which was 0.82 per 10,000 birth for full-term infants compared to 1063.2 per 10,000 birth for infants born before 28 weeks (p<0, 0001). Incidence also increased with weight loss (p<0.0001). From the first to the last period, the incidence increased from 6.27 to 7.89 per 10,000 births (p = 0.006). This increase in incidence over time was due to an increase among most premature infants.[13]

In 2012, Yee, et al., conducted a study using a population-based cohort study of 16,669 infants at 33 weeks gestation admitted to 25 NICUs participating in CNN between January 1, 2003, and December 31, 2008. That study revealed variations in the incidence of NEC among the participating NICUs during the study period. Researchers categorized early-onset NEC as NEC occurring before the infant was 14 days old and late-onset NEC occurring beyond that cut-off. Multivariate logistic regression analysis was performed to identify risk factors for early-onset NEC. The time of diagnosis of NEC (in days) was documented in 841 of 858 (98%) infants. Of the 841 infants, 336 (40%) infants had early-onset NEC, whereas 505 infants had late-onset NEC. The mean (SD) age at diagnosis in the early-onset NEC group was 7.6 (3.1) days compared with 32 (17.2) days in the late-

onset NEC group. The incidence of surgical NEC was significantly higher in the early-onset NEC group (40%) compared with the late-onset NEC group (28%; P<0.001). The peak onset in this group was 32 weeks post-menstrual age.[14]

The exact etiology of necrotizing enterocolitis is still not fully understood, it is considered to be multifactorial, with several contributing causes being extensively studied over the last 40 years. Prematurity is the best-known predisposing factor for necrotizing enterocolitis and is rare in full-term infants. Vice versa, most infants with necrotizing enterocolitis are born prematurely, and the risk of developing necrotizing enterocolitis is inversely related to gestational age and birth weight. In an infant with prematurity, there is the immaturity of intestinal motility and digestion, regulation of intestinal blood circulation, intestinal barrier function, and immune defense.[15]

Necrotizing enterocolitis is an inflammatory bowel disorder primarily seen in premature infants, characterized by a variety of damage to the intestinal tract, from mucosal lesions to full-thickness necrosis and perforation. In the presence of some of the risk factors described earlier, postnatal disturbance of the developing gut is the etiology of NEC. Early epithelial lesions caused by these variables result in an inflammatory bowel response and the release of inflammatory mediators. The development of NEC is significantly influenced by nitric oxide. Following intestinal barrier collapse, bacterial translocation results in an increase in endotoxins and other bacterial products in the lamina propria. In turn, this results in enterocyte death or necrosis, poor enterocyte proliferation, and impaired epithelial repair by enterocyte migration. The mismatch between tissue damage and repair further fuels the inflammatory process that leads to NEC.[16]

It is known that the intestinal epithelium of preterm newborns expresses TLR4 more frequently. TLRs are crucial in the activation of adaptive immunity because they can identify particular microbial component patterns. TLR4 signaling pathway mutations have been reported in human NEC, and TLR4 expression is elevated in both mice and humans with NEC. Lipopolysaccharide causes Gram-negative bacteria to express TLR4. When gut luminal germs activate TLR4, the intestinal barrier is damaged and unable to heal, which permits luminal bacterial translocation, vasoconstriction, intestinal ischemia, and NEC. Additionally, dysbiosis is a disturbance of the gut microbiota's development and equilibrium, which has been linked to the emergence of NEC. Due to a lack of helpful commensal microbes and a lack of bacterial diversity, this pathological process results in an overgrowth of pathogenic bacteria that triggers an inflammatory response.[16]

Relationship of Vitamin D (25(OH)D) Levels with NEC Incidence

In this study, the mean onset of NEC was 5.7 days. The Spearman's correlation test was then carried out and found that there was a significant correlation between vitamin D levels and the onset of NEC (p = 0.029). Vitamin D levels are positively correlated with the day of onset of NEC. The higher the vitamin D level, the longer the onset of NEC and vice versa. The results of the correlation test also gave the strength of the correlation of r = 0.487 meaning there was a moderate positive correlation.

Thus, the hypothesis in this study was accepted. This hypothesis was in line with the previous hypothesis in a 2017 study by Cetinkaya et al., that stated each 1 ng/mL increase in serum 25(OH) D levels in mothers and preterm infants was associated with a 0.751 and 0.582-fold reduction in the risk of NEC in neonates, respectively. Therefore, lower serum 25(OH)D levels in mothers and preterm infants may be associated with the occurrence of NEC in preterm infants and low serum 25(OH)D levels may become one of the NEC risk factors in preterm infants.[6]

In a related study conducted in 2018 by Yang et al., preterm infants and their mothers in the group with necrotizing enterocolitis had significantly lower serum 25(OH)D levels than those in the control group (P <0.001). Mothers and preterm infants in both groups had a positive correlation between their serum 25(OH)D levels (P<0.001). Between the groups with necrotizing enterocolitis and those without necrotizing enterocolitis, there were significant differences in the distribution of vitamin D levels (normal vitamin D levels, low vitamin D levels, vitamin D deficiency, and severe vitamin D deficiency) (P< 0.001). Necrotizing enterocolitis was associated with gestational age, birth weight, mothers' and babies' 25(OH)D levels, duration of mechanical ventilation, duration of inhaled oxygen, and length of hospital stay (P0.05), according to univariate logistic regression analysis.[6]

This is consistent with a previous study involving 145 enrolled patients, among which 26 patients developed NEC. NEC typically manifested 11.1 6.5 (3–29) days after delivery. Of the NEC patients, stage IIA and stage IIB NEC were present in 18 (69.2%) and 7 (26.9%) of the neonates. Only one patient's (3.8%) stage III NEC was diagnosed. In the NEC group compared to the group without NEC, maternal and neonatal 25(OH)D levels were considerably lower (P=0.001 and 0.004, respectively). In the NEC group, none of the moms or babies had 25(OH) D levels that were sufficient (>32 ng ml-1). While a much higher number of patients were found to have 25(OH)D levels in the insufficiency range (11 and 32 ng ml-1) in the group without NEC, the percentage of infants with 25(OH)D levels 10 ng ml-1 was significantly higher in the NEC group.[3]

In both NEC and IBD, a damaged intestinal tight junction plays a key role in the beginning and progression of intestinal inflammation. According to several studies, active vitamin D has been shown to upregulate the tight junction proteins, defending the intestinal barrier. According to the work by Chen et al., intestinal barrier dysfunction is caused by LPS at clinically significant concentrations by lowering tight junction protein and VDR levels. This finding raises the possibility that active vitamin D can reverse VDR expression and lessen LPS's effects on downregulation and redistribution. This research offers medical proof that vitamin D can help treat intestinal conditions including NEC and IBD that are linked to LPS.[3]

Recent studies have also shown that vitamin D is a key modulator of immune function and inflammation with broad regulatory effects on cells of the adaptive and innate immune systems. The vitamin D receptor (VDR), which is expressed in several human organs and tissues in addition to bone cells, including the kidneys, colonic mucosa, and immune cells, is the mechanism by which vitamin D exerts its biological effects. It has been demonstrated that all immune system cells express VDR. VDR controls cell division, proliferation, and the triggering of apoptosis in the gut. Numerous research has looked into how vitamin D affects immune-mediated illnesses including inflammatory bowel disease (IBD). 60% of people with IBD have been found to have vitamin D insufficiency. This is due to the effects vitamin D deprivation has on the digestive system, which includes decreased colonic bacterial clearance, decreased tight junction (TJ) expression in the intestinal epithelium, and increased Th1-mediated inflammation in the gut.3 A recent study found some associations between NEC and vitamin D insufficiency. Premature newborns frequently have vitamin D deficiency, especially if the pregnancy was under 32 weeks. Numerous studies have demonstrated that the vitamin D/vitamin D receptor pathway (VDR), one of the TLR4 regulators, protects the stomach.[17]

In 2017, Shi et al. conducted a study on 15 premature infant rats with NEC, 12 uncomplicated premature infant rats, and 20 full-term healthy infant rats. Those Wistar rats had been induced by food additives and stress asphyxia. To compare the microscopic structure, apoptotic protein expression, intestinal barrier function, inflammatory response, and TLR 4 expression, vitamin D was administered. The vitamin D/VDR pathway and NEC are significantly related, according to the study's findings. Vitamin D helps to preserve intestinal barrier integrity, prevent structural damage, and boost survival rates. This is partially accomplished by restoring VDR expression and inhibiting TLR4. As a result, enterocyte apoptosis and the release of proinflammatory cytokines are significantly reduced. Vitamin D therapy may help prevent NEC since TLR4 activation causes the pathogenic alterations that are characteristic of the condition.[18]

In other studies, mouse models were given dietary vitamin D restriction and genetic manipulation of vitamin D metabolism and signaling systems. Mice lacking the gene for the vitamin D receptor (VDR) that binds 1,25-(OH)2D exhibit increased severity of experimentally induced colitis resembling irritable bowel disease. Similar observations have also been made for mice that lack the gene for 1-hydroxylase, the enzyme that converts 25-OH-D to 1.25-(OH)2D. Thus, the inability to synthesize or recognize 1,25-(OH)2D in mice is associated with increased severity of irritable bowel disease. This is due, in part, to impaired anti-inflammatory adaptive immune function, but has also been associated with disruption of the normal gastrointestinal epithelial barrier. Interestingly, in vitamin D-restricted mice, an increase in colitis severity was associated with an increase of normal commensal bacteria in the sub-mucosal gastrointestinal epithelium, further highlighting the importance of vitamin D in maintaining barrier integrity and controlling the gut microbiota.[19]

Other Factors Associated with NEC in Preterm Infants

In this study, an evaluation of several factors that influence the occurrence of NEC was carried out, including maternal factors and infant factors. The results of the univariate logistic regression test revealed several factors that were significantly related to the incidence of NEC, including vitamin D deficiency with a p-value = 0.033; OR=10.7 (95%CI 1.2-24.9). This indicates that vitamin D deficiency increases the risk of developing NEC by 10.7 times. In addition, intrapartum fever can increase the incidence of NEC by 3.5x with p-value = 0.09 (95% CI 0.8-14.9). Not given fetal lung maturity induction was a factor that increases the incidence of NEC by 4x with a p-value = 0.039 (95% CI 1-14.9). Passive smoking can increase the incidence of NEC by 2.8x with a p-value=0.049 (95%CI 0.9-8.9). Very preterm birth (gestational age <32 weeks) has increased the incidence of NEC by 62 times with a p-value=0.006 (95%CI 1.7-23.2) and respiratory distress can increase the incidence of NEC by 20 times with a p-value = 0.000 (95%CI 4.5-88.2).

Antenatal factors play an important role in NEC. A study by Duci et al., in 2019 aimed to identify antenatal risk factors associated with the development of NEC, the role of placental changes, and the presence of prenatal signs that predispose to NEC. This study found a higher prevalence of preeclampsia ($p = 0.0024$), chorioamnionitis ($p < 0.001$), and abnormal antenatal umbilical artery flow ($p < 0.0001$) in the NEC group. Further, this chorioamnionitis can increase the incidence of NEC by 5 times (95% CI 1,260–19,716).[20]

A total of 33 relevant studies were identified. Clinical chorioamnionitis was significantly associated with the incidence of NEC (12 studies; n = 22601; OR, 1.24; 95% CI, 1.01-1.52; P = 0.04; I2 = 12%), but the association between histologic chorioamnionitis and NEC were not statistically significant (13 studies; n = 5889; OR, 1.39; 95% CI, 0.95-2.04; P = 0.09; I2 = 49%). However, histologic chorioamnionitis with fetal involvement was strongly associated with NEC (3 studies; n = 1640; OR, 3.29; 95% CI, 1.87-5.78; P# .0001; I2 = 10%). Necrotizing enterocolitis is a multifactorial disease, and additional factors contribute to its pathogenesis. Chorioamnionitis has recently been shown to be associated with spontaneous bowel perforation in premature infants. Increased gastrointestinal neutrophil counts and IL-6 levels have been shown in preterm infants exposed to chorioamnionitis, reflecting a pro-inflammatory state of the gut immediately after birth.[21]

The administration of antenatal steroids (ANS) lowers neonatal mortality. According to a meta-analysis of 10 randomized controlled trials including 4702 newborns, ANS reduced the relative risk of NEC by 50% (RR: 0.50; 95% CI: 0.32-0.78). A meta-analysis of observational studies, however, found no evidence that ANS had any advantage for NEC at very early gestational age (OR: 1.01; 95% CI: 0.84-1.22; seven studies, 8737 newborns <25 weeks gestation).[22] This is consistent with the study of Battarbee, et al., in 2020, which discovered that

one of the best strategies for improving newborn outcomes is providing prenatal corticosteroids before preterm birth. Exposure to glucocorticoids accelerates the development of several embryonic organ systems, including the respiratory, digestive, and central neurological systems. Antenatal corticosteroid administration decreased rates of several prematurity-related complications, such as respiratory distress syndrome (RDS) (relative risk [RR] 0.66, 95% confidence interval [CI] 0.59-0.73), intraventricular hemorrhage (RR 0.54, 95% CI 0, 43-0.69), necrotizing enterocolitis (RR 0.46, 95% CI 0.29-0.74), and neonatal death (RR 0.69, 95% CI 0. Because of this, prenatal corticosteroids are advised for all women who run the risk of giving delivery too soon.[23]

Numerous research has looked into potential pregnancy-related risk factors for infant deaths brought on by necrotizing enterocolitis. Between 2000 and 2004, data from the US Linked Livebirth and Infant Death records identified infant death in preterm newborns related to NEC. Three categories were created based on the average daily cigarette use indicated by pregnant smoking mothers: non-smokers, light smokers (10 cigarettes/day), and heavy smokers (10 cigarettes/day). The relationship between prenatal smoking and infant mortality related to NEC was investigated using logistic regression analysis. women of light and heavy smokers had a greater risk of NEC-related infant mortality compared to women who did not smoke [heavy smokers: adjusted odds ratio (aOR) = 1.30, 95% confidence interval (CI), 1.12-1.52; light smokers: adjusted odds ratio (aOR) = 1.21, respectively. Additionally, the link was greater in Caucasians and female babies (light smokers: aOR = 1.31, 95% CI, 1.02-1.69; heavy smokers: aOR = 1.62, 95% CI, 1.29-2.02) (aOR = 1.69, 95% CI, 1.34-2.13; heavy smokers: aOR = 1.44, 95% CI, 1.18-1.75). Preterm children, particularly Caucasian and female infants, have a higher risk of infant mortality owing to NEC when their mothers smoke during pregnancy.[24]

Exposure to cigarette smoke can cause changes in the immune system of pregnant women. These changes include an increase in activated leukocytes and a decrease in the percentage of regulatory T lymphocytes (Treg cells). Smoking during pregnancy also affects the balance of function between Th1 cells (T helper lymphocytes) and Th2 cells, leading to increased production of cytokines, proinflammatory chemokines, and Th1 growth factors. In addition, the percentage of macrophages and residual NK cells was higher in smokers in the first trimester.[25]

Furthermore, to find out which factors are influential when several risk factors were to be analyzed simultaneously, a multivariate analysis test is carried out. Risk factor variables with a significance p-value <0.250 were included in the multivariate analysis test. From the results of the multivariate analysis test, it was found that vitamin D deficiency and respiratory distress were significantly associated with the incidence of NEC. Vitamin D deficiency increases the incidence of NEC by 19.4 times with a p-value = 0.043 (95% CI 0.7-21.7) and respiratory distress can increase the incidence of NEC by 9.4 times with a p-value = 0.02 (95% CI 1.2-70).

In a study conducted by Reffat et al., measurement of vitamin D levels in premature neonates with RDS (9.52 ± 2.48ng/mL) compared to healthy preterm infants (31.25 ± 1.23ng/mL) showed that preterm infants with RDS had severe deficiency ($p < 0.001$).[7,26]

Low levels of vitamin D 25(OH) D were found in 57 babies in a study by Fettah et al. (Group 1, median 8.0 ng/mL [interquartile range, IQR, 5-10]; Group 2, median 21 ng/mL [IQR, 19-24.7]). In comparison to Group 2 (n = 3.5.4%), RDS levels were substantially greater in Groups 1a (n = 18, 32.7%) and 1b (n = 34, 61.8%) (p = 0.001). A greater 25(OH)D level may be protective against the development of RDS, according to multivariate analysis (odds ratio, 0.6; 95% confidence range, (0.5-0.8); p = 0.001). In conclusion, RDS risk may be enhanced in very low birth weight preterm newborns with lower cord blood 25(OH)D levels. However, there was no correlation between vitamin D levels and the prevalence of lung illness or other morbidities in premature newborns in the study by Joung et al.[27,28]

A study was conducted by Reffat et al., in 2020 to detect an important risk of NEC and help prevent NEC in neonates using a retrospective method with a sample of 36 neonates admitted to the NICU of Assiut University Children's Hospital from April 2017 to March 2018. This study found several risk factors for the incidence of NEC, including premature, cesarean section, formula administration, sepsis in neonates, and respiratory distress syndrome (RDS). In this study, we found that there was an association between RDS and NEC, where 55.6% of NEC cases were diagnosed as having RDS. This is in line with a study by Ahle et al., which revealed that 43% of infants with NEC had RDS. In addition, Muhammad et al. reported that there is an association between hyaline membrane disease and NEC, in which 14% of NEC cases were diagnosed as having hyaline membrane disease.[26]

In prematurity, respiratory distress syndrome (RDS) often occurs due to a lack of surfactant, causing impaired adequate gas exchange in the lungs. RDS and related complications are the leading cause of death in premature infants. One of these complications is necrotizing enterocolitis. Necrotizing enterocolitis is associated with interactions in the immature intestine with aggressive enteral feeding and intestinal epithelial damage due to hypoxia or altered intestinal blood flow. Some of the possible consequences of gut immaturity are decreased gut motility, abnormal microbiota, immature gut barrier, and imbalance in the inflammatory response to various agents.[29–31]

Research Limitations

In this study, there was no examination of the nutritional status and vitamin D levels of the research subjects' mothers. In several studies, it is said that the nutritional status and vitamin D levels of the mother can affect the vitamin D levels of the baby. So maternal nutritional factors and maternal vitamin D levels can be confounding factors in this study. In this study, Predicting the risk of prenatal vitamin D supplementation's impact on the infant's vitamin D status was not achievable due to a lack of information on maternal 25(OH)D levels and prenatal vitamin D supplementation. Maternal vitamin D deficiency is likely to impact child health by altering fetal and newborn vitamin D availability. Although 1,25-dihydroxy

vitamin D [1,25(OH)2D], the major bioactive form, does not readily cross the placenta, the umbilical cord concentration of its precursor, 25(OH)D, is similar to that of the mother. According to a study by Kovacs (2008), Maternal 25(OH)D concentrations do not appreciably change throughout pregnancy, and umbilical cord plasma 25(OH)D concentrations are roughly 90% of maternal values. In contrast, vitamin D deficiency in pregnant women increases the risk of vitamin D deficiency in infants because fetal and neonatal concentrations of 25(OH)D depend on maternal serum levels. Normal vitamin D shortage in mothers puts their offspring at risk for 25(OH)D deficiency, whereas vitamin D insufficient moms are almost certainly deficient in their offspring.[11,32,33]

Necrotizing enterocolitis is a gastrointestinal disease with multifactorial causes. Maternal factors are one of the predisposing factors for NEC, namely mode of delivery, lifestyle (smoking), chorioamnionitis, and pre-eclampsia. Early diagnosis remains a challenge and its pathogenesis is elusive. So, many confounding factors need to be limited. In this research, a multivariate regression test was used to reduce confounding factors for the occurrence of NEC.

The influence of other biomarkers is also one of the predispositions for the occurrence of NEC. Lipopolysaccharides and TLR4 are several predisposing factors to NEC in preterm infants.

CONCLUSION

Vitamin D (25(OH)D) levels were lower in preterm infants with necrotizing enterocolitis than in preterm infants without necrotizing enterocolitis. There is a relationship between levels of vitamin D(25(OH)D) with the occurrence of necrotizing enterocolitis in preterm infants. Further research is needed to study other biomarkers that can predispose to the occurrence of NEC, including TLR4 and LPS. Experimental research on administering vitamin D to infants is needed.

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Conflict of Interest

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Author Contribution

All of the authors participated in selecting the topic until manuscript preparation for publication.

Ethical Clearance

This study already got permission from the local Ethical Committee.

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