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Study on the correlation between levels of vitamin A and vitamin E and neonatal immune function and necrotizing enterocolitis

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Abstract: Vtamin A (VA) and vitamin E (VE) deficiencies are common in premature infants and may impair immune function, increasing the risk of necrotizing enterocolitis (NEC). This study examined the link between VA and VE levels and neonatal immune function and NEC in 220 premature infants from 2022 to 2024. Infants were divided into VA, VE, VA+VE supplementation groups and a control group. After one month, VA and VE levels and immune function markers (T lymphocyte subsets) were reassessed. Results showed that VA and VE levels increased significantly in supplemented groups ($P<0.05$). Immune function improved, with the best results in the VA+VE group ($P<0.05$). NEC incidence was lowest in the VA+VE group (1.82%, $P=0.031$). Correlation analysis revealed that higher VA and VE levels were associated with better immune function and lower NEC risk ($P<0.05$). The concentrations of VA and VE are closely related to neonatal immune function and the occurrence of NEC. Supplementation with VA and VE may help prevent the occurrence of NEC in newborns.

Keywords: Vitamin A; vitamin E; neonates; immune function; necrotizing enterocolitis

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INTRODUCTION

Neonatal necrotizing enterocolitis (NEC) is a severe intestinal inflammatory condition mainly affecting preterm infants, with a global incidence of 7% and a mortality rate of about 25% (Roberts *et al.*, 2024). It is characterized by acute intestinal ischemia, necrosis and symptoms such as abdominal distension, vomiting and bloody stools (Kaplina *et al.*, 2023). NEC is most common in the ileum but can affect any part of the gastrointestinal tract (Dong *et al.*, 2023). Over 90% of cases occur in preterm neonates under 36 weeks' gestation and prevalence is 5%-10% among low birth weight infants (Egozi *et al.*, 2023). The immature digestive and immune systems of these infants lead to genetic susceptibility, weak gut defenses, abnormal microbial colonization and reactive mucosa, driving NEC pathogenesis (Chen *et al.*, 2022). Excessive immune responses triggered by microbial abnormalities, ischemia, infection and external stimuli are also key factors (Doikova *et al.*, 2024).

Premature infants often have immature organs and nutrient deficiencies, particularly in vitamin A (VA) and vitamin E (VE) (Assunção *et al.*, 2022; Kumar and Anjankar, 2022). VA is crucial for epithelial tissue integrity and immune system function, but premature infants struggle with liver storage and intestinal absorption, leading to lower serum VA levels compared to full-term infants (Liu *et al.*, 2022; Rakshasbhuvankar *et al.*, 2021).. VE, an antioxidant nutrient, protects cell membranes and modulates immune responses (Ge *et al.*, 2021). Its storage neon inates correlates with birth weight and gestational duration, with premature infants having lower reserves due to shorter gestation (Kolnik and Wood, 2022). VE absorption in the small intestine is also affected by conditions like malabsorption and reduced bile salt synthesis in premature infants (Yang *et al.*, 2024).

The levels of VA and VE are significantly associated with the immune competence of newborns. Vitamin A modulates the differentiation and functionality of dendritic cells (DCs), natural killer (NK) cells and macrophages via its derivative, retinoic acid. (Bos *et al.*, 2021). Retinoic acid promotes the transformation of DCs into antigen-presenting cells, enhancing their antigen-presenting capacity, while inhibiting excessive inflammatory responses in macrophages, such as reducing the expression of tumor necrosis factor-alpha (TNF-α) and cyclooxygenase-2 (COX-2) (Devalaraja *et al.*, 2020). Additionally, VA maintains immune tolerance by regulating the T-helper 1/T-helper 2 (Th1/Th2) cell balance, suppressing the development of pro-inflammatory T-helper 17 (Th17) lymphocytes and enhancing the production of regulatory T (Treg) cells (Nagy *et al.*, 2024). VA plays a vital role in preserving the structural integrity of mucosal barriers in the gastrointestinal and respiratory systems, as it supports the differentiation of epithelial cells and the secretion of mucus, establishing the primary defense mechanism against pathogenic invaders (Bezerra *et al.*, 2022). VA in breast milk also synergizes with live bacteria and oligosaccharides in colostrum to initiate the neonatal intestinal immune system (Nimmannun *et al.*, 2023). As a fat-soluble antioxidant, VE protects immune cell

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membranes from oxidative damage and maintains the phagocytic functions of NK cells, neutrophils and macrophages (Lai *et al.*, 2021). Owing to insufficient vitamin E reserves in preterm neonates, supplementation can lower the likelihood of hemolytic anemia and chronic pulmonary disease. VE suppresses the COX-2 and nuclear factor kappa-B (NF-κB) signaling pathways, thereby decreasing the secretion of pro-inflammatory mediators like interleukin-6 (IL-6) and TNF-α (Mesalam *et al.*, 2023; Wu *et al.*, 2001); it additionally stimulates the proliferation of T lymphocytes and the formation of immune synapses, modulates the equilibrium between Th1 and Th2 cells and strengthens adaptive immune reactions (Garcia *et al.*, 2022). Furthermore, studies have found that VE indirectly regulates immune responses by influencing the composition of the gut microbiota, maintaining the expression of tight junction proteins in the intestine and reducing pathogen translocation (Calik *et al.*, 2022).

The investigation conducted by Su Q *et al.* utilized a neonatal NEC murine model and administered VA supplementation, revealing that VA might mitigate apoptosis in intestinal epithelial cells during NEC by enhancing the phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt) signaling cascade and regulating apoptotic pathways (Su *et al.*, 2025). Luo S et al. generated mice with targeted deletion of glutathione peroxidase 4 (GPX4) in Treg cells to examine the influence of Treg cell ferroptosis on intestinal injury and localized inflammation in NEC. The findings demonstrated that the development of NEC is strongly associated with a reduction in intestinal Treg cell populations, primarily attributed to ferroptosis (a type of cell death driven by lipid peroxidation). As a powerful antioxidant, VE can bolster the resilience of Treg cells against lipid peroxidation by increasing the expression of GPX4, thus preventing their ferroptosis (Luo *et al.*, 2024). This protective effect helps maintain intestinal immune homeostasis and reduce intestinal inflammatory damage associated with NEC. These studies suggest that deficiencies in VA and VE may be related to the onset and progression of neonatal NEC, but further research is needed to explore the correlation between VA and VE levels, neonatal immune function and NEC. Elucidating the association between the concentrations of VA and VE, neonatal immune function and NEC has significant clinical implications for early prevention and intervention of NEC. Therefore, the primary purpose of this study is to elucidate the association between the concentrations of VA and VE, neonatal immune function and NEC, with the aim of providing new insights for the early prevention and intervention of NEC in clinical practice.

MATERIALS AND METHODS

Study design

This study was a retrospective controlled clinical study conducted in neonatal intensive care (NICU) in our hospital. The design operation flow is shown in the fig. 1.

General information

A cohort of 220 preterm neonates admitted to our institution between January 2022 and December 2024 were enrolled as participants in this investigation. Inclusion criteria: gestational age<37 weeks; birth weight<2500g; no severe diseases such as congenital intestinal malformations, inherited metabolic disorders, or congenital heart disease; and complete clinical data. Exclusion criteria: death within 24 hours after birth; concurrent severe infectious diseases; presence of congenital intestinal diseases, congenital malformations, genetic disorders, or hematological system diseases; accompanied by severe heart, lung, liver, or kidney diseases; and mothers with severe complications during pregnancy or who took medications affecting vitamin metabolism. The 220 premature infants were divided into four groups according to the treatment method, with 55 infants in each group: the VA supplementation group, the VE supplementation group, the VA+VE supplementation group and the control group.

Sample size calculation

This research employed One-way ANOVA for group comparisons and utilized G*Power to estimate the sample size (Faul *et al.*, 2007). With an effect size set at a moderate level (Cohen's f = 0.25), a significance level (α) of 0.05 and a test power (Power) of 0.80, the calculated total sample size was 180 participants. However, in this study, we recruited 55 participants per group, amounting to 220 individuals in total. This sample size substantially surpasses the minimum required, thereby enhancing the statistical power and further bolstering the reliability and robustness of the findings.

Methods

All four groups of preterm infants received standard therapeutic and nursing interventions, including thermal support, nutritional provision and infection control measures. The VA group was administered oral VA capsules (2000 IU/d) once daily (Ye *et al.*, 2022); the VE group received oral VE capsules (5 mg/d) once daily (Bronsky *et al.*, 2018); the VA+VE group was provided with both vitamins at the aforementioned dosages concurrently; and the control group did not receive supplementary vitamin administration.

Observation index

(1) Serum concentrations of VA and VE (Morikawa *et al.*, 2023): Before the intervention and one month after the intervention, the serum levels of VA and VE were analyzed utilizing high-performance liquid chromatography (HPLC) with an Agilent 1260 HPLC apparatus and a C18 column (4.6 mm × 250 mm, 5 μm). The eluent comprised methanol: water (98:2, v/v) at a flow rate of 1.0 ml/min, with detection wavelengths set at 325 nm (for VA) and 292 nm (for VE).

(2) Immune function parameters (Schultze-Florey *et al.*, 2021): Peripheral venous blood samples (5mL) were obtained both prior to and one month following the

intervention and the levels of T lymphocyte subsets ($CD3^+$, $CD4^+$, $CD8^+$) were quantified using flow cytometric analysis.

(3) Incidence of NEC: The occurrence of NEC in the four groups of premature infants during hospitalization was observed and recorded. The diagnosis of NEC was based on clinical symptoms, signs and abdominal X-ray findings, following the modified Bell staging criteria (Cai *et al.*, 2023).

STATISTICAL ANALYSIS

Data were analyzed using SPSS 25.0. Continuous variables (mean ± SD) were compared between groups using analysis of variance (ANOVA) and within groups using paired t-tests. Categorical variables (%) were compared using the chi-square test. Pearson correlation analyzed VA/VE levels and NEC incidence. $P<0.05$ was considered significant.

RESULTS

Comparison of general characteristics among the four groups of premature infants

The baseline demographic features of the neonates across the four groups were compared and the outcomes are presented in table 1. Statistical evaluation revealed no notable variations in gender distribution, gestational age, birth weight and childbirth method among the four groups of preterm infants ($P>0.05$).

Comparison of serum VA and VE levels before and after treatment among the four groups of premature infants

Prior to the intervention, no significant variations were observed in the serum concentrations of VA and VE among the four groups ($P>0.05$). Following one month of treatment, the serum VA levels in the VA group and the VA+VE group exhibited a marked elevation compared to pre-treatment levels and were significantly greater than those in the control group ($P<0.05$). Similarly, the serum VE levels in the VE group and the VA+VE group demonstrated a substantial rise compared to baseline measurements and were significantly higher than those in the control group ($P<0.05$). Refer to table 2 and fig. 2 for details.

Comparison of immune function indicators before and after treatment among the four groups of premature infants

Prior to the intervention, no statistically significant variations were observed in the levels of T lymphocyte subsets ($CD3^+$, $CD4^+$, $CD8^+$, $CD4^+/CD8^+$) among the four groups of preterm infants ($P>0.05$). Following one month of treatment, the concentrations of $CD3^+$, $CD4^+$ and $CD4^+/CD8^+$ in the VA group, VE group and VA+VE group were significantly elevated compared to pre-treatment levels ($P<0.05$), whereas the level of $CD8^+$ was significantly reduced compared to baseline measurements ($P<0.05$). Furthermore, the enhancement in immune function parameters in the VA+VE group was more pronounced than that in the VA group and the VE group ($P<0.05$). In the control group, no statistically significant differences were noted in the levels of $CD3^+$ and $CD8^+$ before and after treatment ($P>0.05$); however, the levels of $CD4^+$ and $CD4^+/CD8^+$ in the control group were significantly lower post-treatment compared to pre-treatment ($P<0.05$). Refer to table 3 and fig. 3 for details.

Comparison of NEC incidence among the four groups of premature infants

Throughout the hospital stay, 9 instances of NEC were recorded in the control group, representing an incidence rate of 16.36%; 4 cases were observed in the VA group, with an incidence rate of 7.27%; 3 cases were identified in the VE group, yielding an incidence rate of 5.45%; and 1 case was noted in the VA+VE group, with an incidence rate of 1.82%. The variation in NEC occurrence among the four groups was statistically significant ($\chi^2=8.861$, $P=0.031$). Refer to table 4 for further details.

Correlation analysis between serum VA, VE levels and NEC incidence in newborns

Pearson correlation analysis indicated that the levels of VA, VE, $CD3^+$, $CD4^+$ and $CD4^+/CD8^+$ exhibited an inverse relationship with the occurrence of NEC ($P<0.05$); conversely, the concentration of $CD8^+$ demonstrated a positive association with the incidence of NEC ($P<0.05$). Further investigation revealed that the quantities of $CD3^+$, $CD4^+$ and $CD4^+/CD8^+$ were directly correlated with the levels of VA and VE ($P<0.05$); whereas the level of $CD8^+$ showed an inverse correlation with the concentrations of VA and VE ($P<0.05$). Refer to table 5 for further details.

DISCUSSION

NEC is a severe intestinal disease in premature infants, with a complex pathogenesis involving the interaction of multiple factors. The core pathophysiological processes include impaired intestinal mucosal barrier function, dysbiosis of gut microbiota, immune deficiency and enhanced oxidative stress response (Kinstlinger *et al.*, 2021). The intestinal mucosa of premature infants is immature and the mucosal barrier function is weak, making it susceptible to factors such as hypoxia, ischemia-reperfusion injury and infection, which can lead to increased intestinal permeability, bacterial and toxin translocation into the submucosa and subsequent inflammatory responses (George *et al.*, 2021; Willis and Ambalavanan, 2021). Additionally, the immune system of premature infants is underdeveloped, with imbalances in T lymphocyte subsets and weak cellular and humoral immune functions, making it difficult for them to effectively clear pathogens in the intestine, thereby increasing the risk of infection and inflammatory responses (Mani *et al.*, 2023).

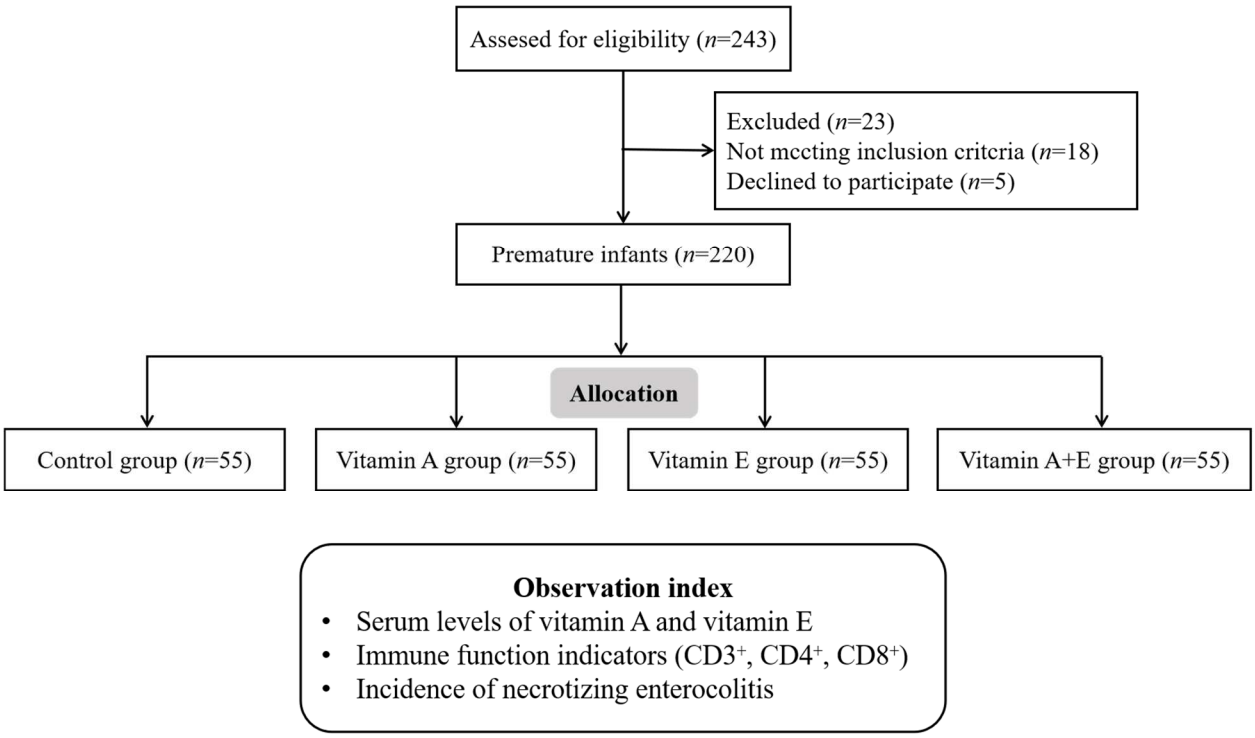

Fig. 1: The design operation flow.

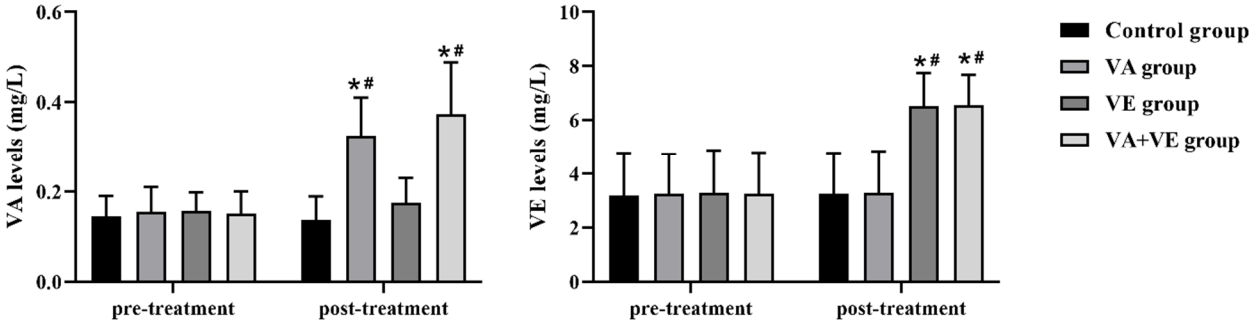

Fig. 2: Comparison of serum VA and VE levels

Table 1: Comparison of baseline characteristics

group	*n*	Gender (n,%)		Gestational age ($\overline{x}\pm s$, weeks)	Birth weight ($\overline{x}\pm s$, g)	Childbirth method (n,%)	
		Male	Female			Spontaneous labor	Cesarean section
Control group	55	22 (40.00)	33 (60.00)	32.45±2.44	1866±233	24 (43.64)	31 (56.36)
VA group	55	24 (43.64)	31 (56.36)	32.71±2.39	1879±224	27 (49.09)	28 (50.91)
VE group	55	21 (38.18)	34 (61.82)	32.65±2.18	1852±235	28 (50.91)	27 (49.09)
VA+VE group	55	26 (47.27)	29 (52.73)	32.44±2.88	1876±254	25 (45.45)	30 (54.55)
χ^2/t		1.099		0.171	0.151	1.592	
P		0.777		0.916	0.929	0.661	

Table 2: Comparison of serum VA and VE levels ($\overline{x}\pm s$, mg/L)

group	*n*	VA		*t*	*P*	VE		*t*	*P*
		pre-treatment	post-treatment			pre-treatment	post-treatment		
Control group	55	0.15±0.04	0.14±0.05	0.876	0.383	3.21±1.58	3.27±1.52	0.204	0.839
VA group	55	0.15±0.06	0.33±0.09*#	12.364	0.000	3.24±1.51	3.28±1.58	0.113	0.911
VE group	55	0.16±0.04	0.17±0.06	1.790	0.076	3.28±1.59	6.53±1.22*#	11.950	0.000
VA+VE group	55	0.15±0.05	0.37±0.11*#	13.195	0.000	3.24±1.55	6.55±1.13*#	12.792	0.000
F		0.689	109.715			0.024	103.853		
P		0.559	0.000			0.995	0.000		

Note: Compared with pre-treatment, *P<0.05; Compared with Control group, #P<0.05

Table 3.1: Comparison of T lymphocyte subsets ($\overline{x}\pm s$)

group	*n*	$CD3^+$ T (%)		*t*	*P*	$CD4^+$ T (%)		*t*	*P*
		pre-treatment	post-treatment			pre-treatment	post-treatment		
Control group	55	51.45±4.63	50.26±4.77	1.317	0.191	32.12±3.22	30.28±4.55*	2.451	0.016
VA group	55	51.88±5.21	58.46±5.33*#	6.547	0.000	32.45±3.46	36.88±5.23*#	5.252	0.000
VE group	55	52.07±4.78	57.95±5.45*#	6.031	0.000	33.17±3.65	36.67±5.41*#	3.981	0.000
VA+VE group	55	51.37±4.99	67.33±6.18*#	14.903	0.000	32.46±3.57	39.67±3.13*#	11.248	0.000
F		0.262	90.008			0.894	39.846		
P		0.853	0.000			0.445	0.000		

Table 3.2: Comparison of T lymphocyte subsets ($\overline{x}\pm s$)

group	*n*	$CD8^+$ T (%)		*t*	*P*	$CD4^+$ T/$CD8^+$ T		*t*	*P*
		pre-treatment	post-treatment			pre-treatment	post-treatment		
Control group	55	31.45±3.33	32.46±4.46	1.333	0.185	1.04±0.17	0.95±0.21*	2.263	0.026
VA group	55	30.28±3.67	26.33±2.42*#	6.678	0.000	1.08±0.16	1.41±0.23*#	8.794	0.000
VE group	55	31.26±3.17	26.08±2.37*#	9.723	0.000	1.07±0.14	1.42±0.26*#	8.795	0.000
VA+VE group	55	32.07±3.88	24.18±2.16*#	13.166	0.000	1.03±0.17	1.66±0.22*#	17.043	0.000
F		2.417	78.919			1.553	90.061		
P		0.067	0.000			0.202	0.000		

Table 4: Comparison of NEC incidence [n (%)]

group	n	NEC occurrence	No NEC
Control group	55	9 (16.36)	46 (83.64)
VA group	55	4 (7.27)	51 (92.73)
VE group	55	3 (5.45)	52 (94.55)
VA+VE group	55	1 (1.82)	54 (98.18)
χ^2		8.861	
P		0.031	

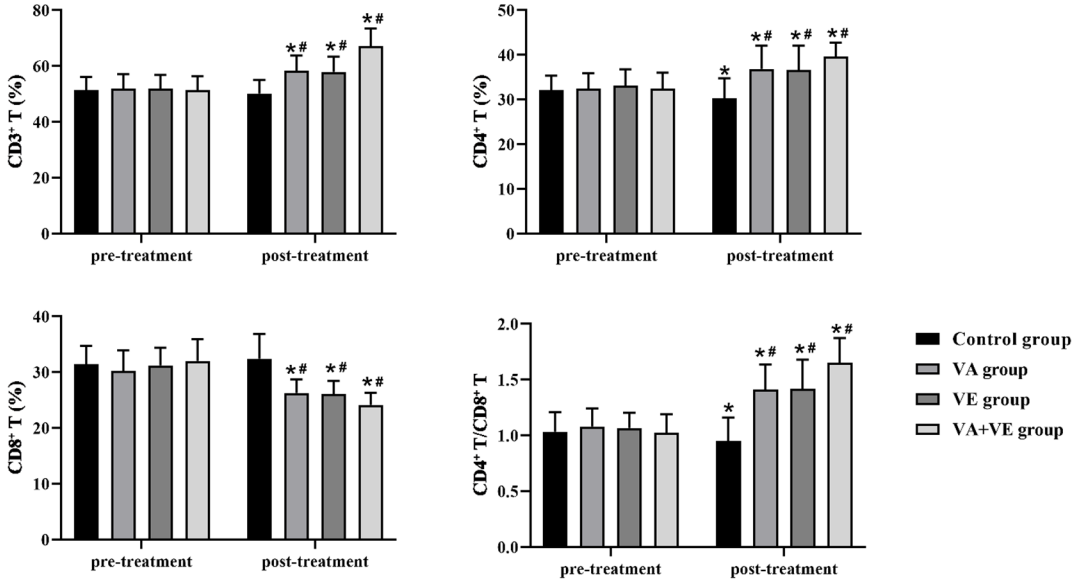

Fig. 3: Comparison of T lymphocyte subsets

Table 5.1: Correlation analysis between VA, VE, T lymphocyte subsets and neonatal NEC

Pearson	VA	VE	$CD3^+$	$CD4^+$	$CD8^+$	$CD4^+/CD8^+$
r	-0.364	-0.442	-0.453	-0.453	0.400	-0.445
P	0.000	0.000	0.000	0.000	0.000	0.000

Table 5.2: Correlation analysis between VA and T lymphocyte subsets

Pearson	$CD3^+$	$CD4^+$	$CD8^+$	$CD4^+/CD8^+$
r	0.581	0.489	-0.512	0.575
P	0.000	0.000	0.000	0.000

Table 5.3: Correlation analysis between VE and T lymphocyte subsets

Pearson	$CD3^+$	$CD4^+$	$CD8^+$	$CD4^+/CD8^+$
r	0.514	0.443	-0.464	0.513
P	0.000	0.000	0.000	0.000

Therefore, finding effective preventive and therapeutic measures is of great significance in decreasing the prevalence and fatality rates of NEC. VA and VE, as important fat-soluble vitamins, play key roles in immune regulation, antioxidant defense and intestinal mucosal protection, respectively. This study aims to provide new strategies for the prevention and treatment of NEC by observing the correlation between VA and VE levels and neonatal immune function, as well as the incidence of NEC.

The findings of this investigation revealed that the serum concentrations of VA in the VA group and the VA+VE group were markedly elevated, indicating that exogenous supplementation of VA can effectively elevate the VA content in newborns. In the complex physiological system of the human body, the absorption of VA relies on specific transport proteins in the intestine and it is predominantly carried in the bloodstream bound to retinol-binding protein (RBP) (Suri *et al.*, 2021). Due to immature liver development and limited RBP synthesis capacity in premature infants, their ability to store and utilize VA is insufficient. After VA supplementation, it is absorbed through the intestine into the bloodstream, binds to RBP and is transported to various tissues and organs, thereby increasing serum VA levels (Honarbakhsh *et al.*, 2021). Previous related studies have also indicated that serum VA levels in premature infants exhibit a similar upward trend after VA supplementation, further confirming the results of this study (Phattraprayoon *et al.*, 2022). Likewise, the serum concentrations of VE in the VE group and the VA+VE group exhibited an increase, highlighting the efficacy of the VE supplementation approach. As a lipid-soluble antioxidant, VE primarily exists in the form of α-tocopherol within the body and its absorption in the intestine requires the assistance of bile acids and fat microparticles (Noguchi and Niki, 2024). Premature infants have insufficient bile secretion and weak fat digestion and absorption capabilities, which affect the

absorption of VE. After VE supplementation, it is absorbed into chylomicrons in the intestine and then enters the bloodstream through the lymphatic system, thereby increasing serum VE levels (Kiyose, 2021). Studies have shown that after receiving VE supplements, serum VE levels in adult patients undergoing hemodialysis increased significantly, which was associated with improvements in oxidative stress and vascular and systemic inflammation (Skouroliakou *et al.*, 2010). This aligns with the hypothesis in this study that VE improves oxidative stress status in premature infants.

The levels of $CD3^+$, $CD4^+$ and $CD4^+/CD8^+$ rose, while $CD8^+$ levels declined after treatment in the VA group, VE group and VA+VE group, with the immune function indicators showing better improvement in the VA+VE group. As a marker on the surface of T lymphocytes, the increase in $CD3^+$ levels indicates an overall increase in the number of T lymphocytes in the body, which is a crucial manifestation of enhanced cellular immune function (Frank *et al.*, 2020). From the standpoint of cellular development, VA modulates the differentiation of T lymphocyte precursor cells via retinoic acid receptors and retinoid X receptors, facilitating the maturation of T lymphocytes (Fujiki *et al.*, 2022). VE, on the other hand, can indirectly affect the activation and proliferation of T lymphocytes by modulating cell membrane fluidity and signal transduction (Oyama *et al.*, 2023). $CD4^+$ helper T cells hold a pivotal role in the immune regulatory system, releasing cytokines like IL-2 and IFN-γ to stimulate antibody generation by B cells and boost the function of killer T cells (Ruterbusch *et al.*, 2020). The biologically active form of VA, retinoic acid, fosters the production of Treg cells and hinders the development of Th17 cells to uphold immune tolerance (Ashrafizadeh, 2024; Ren, 2024). VE, through its antioxidant effects, protects immune cells from oxidative damage and maintains the normal function of $CD4^+$ cells. Studies have shown that VE deficiency impairs $CD4^+$ cell function, inhibiting the proliferation capacity, cytokine secretion and immune synapse formation ability of $CD4^+$ cells (Patwardhan *et al.*, 2020). The $CD4^+/CD8^+$ ratio is often regarded as a key indicator for assessing immune balance in the body, with an increased ratio indicating an active and coordinated immune function (Galli *et al.*, 2023). Although $CD8^+$ cytotoxic T cells serve a vital function in immune defense by killing pathogen-infected cells and tumor cells, excessively high levels can disrupt immune balance and trigger excessive immune responses (Reina-Campos *et al.*, 2021). VA and VE may affect the activation and function of $CD8^+$ cells by regulating the cytokine network. The more significant improvement in immune function in the VA+VE group suggests a synergistic effect between the two. In the antioxidant defense system, VE can directly scavenge free radicals, interrupt the chain reaction of lipid peroxidation and protect the integrity of cell membranes; VA, by maintaining normal cellular metabolism, reduces the production of free radicals and indirectly alleviates oxidative stress. In immune regulation, VA regulates the differentiation of immune cells, while VE maintains the function of immune cells, with both jointly promoting the enhancement of immune function from different levels.

During hospital stay, there were notable disparities in the occurrence of NEC among the control group, VA group, VE group and VA+VE group. Moreover, the levels of VA, VE, $CD3^+$, $CD4^+$ and the $CD4^+$ to $CD8^+$ ratio exhibited a negative association with the occurrence of NEC, whereas $CD8^+$ levels demonstrated a positive correlation with it. From an immunology standpoint, VA and VE bolster the immune barrier of the intestinal mucosa by regulating immune response. The intestinal mucosal immune barrier, composed of intestinal epithelial cells, immune cells and secretory immunoglobulin A (sIgA) (Di Tommaso *et al.*, 2021). VA is an essential nutrient for sustaining the wellness of intestinal epithelial cells, fostering the development of intestinal villi and enhancing crypt depth, thus upholding the wholeness of the intestinal mucosa (Pham *et al.*, 2021). Through its derivative retinoic acid, VA modulates the specialization and activity of immune cells, stimulates the release of sIgA and bolsters localized immune protection in the intestine (Pu *et al.*, 2024). VE protects intestinal epithelial cells through its antioxidant effects, reducing oxidative stress-induced damage to the intestinal barrier and indirectly strengthening the intestinal mucosal immune barrier (Liu *et al.*, 2021). In the pathological progression of NEC, the ferroptosis of Treg cells is a pivotal element contributing to intestinal tissue injury and exaggerated inflammatory reactions. Through the suppression of Treg cell ferroptosis, VE markedly enhances their population and augments their functionality, thereby playing an essential role in mitigating intestinal tissue damage and inflammatory responses associated with NEC (Hu *et al.*, 2021). Intestinal ischemia-reperfusion injury represents a pivotal stage in the development of NEC, characterized by the production of an abundance of reactive oxygen species, including superoxide anions and hydroxyl radicals. These reactive species target the cellular membranes, protein structures and genetic material within intestinal tissues, leading to cellular impairment and demise (Subramanian *et al.*, 2020). VA and VE possess potent antioxidant capabilities, capable of scavenging free radicals and mitigating oxidative stress damage. VE can directly react with free radicals, converting them into stable products (Kagan *et al.*, 2024); VA indirectly exerts antioxidant effects by regulating the activity of intracellular antioxidant enzymes (de Lima-Reis *et al.*, 2022). Therefore, supplementation with VA and VE helps protect intestinal tissues and reduce the incidence of NEC.

This study has significant practical implications for the clinical management of premature infants. Firstly, it is recommended to routinely supplement VA and VE in the nutritional intervention for premature infants to improve their immune function and reduce the risk of necrotizing

enterocolitis (NEC). Secondly, clinicians should strengthen the monitoring of vitamin nutritional status in premature infants, particularly the concentrations of VA and VE, to facilitate timely intervention. Additionally, the results of this study also provide a scientific basis for formulating nutritional guidelines for premature infants.

Research limitations

This study has certain limitations. It focuses solely on the premature infant population and has a relatively short observation period. The situation for term infants and the long-term effects and safety of VA and VE supplementation remain unclear. The exploration of related immune regulatory mechanisms and signaling pathways is also insufficient. Future research needs to expand the sample scope to include term infants, conduct large-scale, multi-center, long-term clinical studies and delve deeper into the molecular mechanisms to further verify the efficacy and safety of VA and VE supplementation in preventing NEC in neonates.

CONCLUSION

This study shows that VA and VE levels are closely linked to neonatal immune function and NEC. VA and VE supplementation in premature infants significantly boosts their vitamin levels, enhancing immune function by increasing $CD3^+$, $CD4^+$ and $CD4^+/CD8^+$ levels while decreasing $CD8^+$ levels. Combined VA and VE supplementation has a stronger effect on immune function. Additionally, higher VA and VE concentrations are associated with a lower risk of NEC, suggesting that these vitamins can help reduce NEC incidence in neonates.

Consent to participate

We secured a signed informed consent form from every participant.

Ethical approval

This experiment was approved by Shijiazhuang No.4 Hospital Ethics Committee. Ethics Approval Number: KZ20210602.

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Author contribution

[Jing Ma]: Developed and planned the study, performed experiments and interpreted results. Edited and refined the manuscript with a focus on critical intellectual contributions.

[Zhexia Hu, Weina Liu]: Participated in collecting, assessing and interpreting the date. Made significant contributions to date interpretation and manuscript preparation.

[Xingyu Bai]: Provided substantial intellectual input during the drafting and revision of the manuscript.

Conflicts of interest

The authors declare that they have no financial conflicts of interest.

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