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Low-dose vitamin A therapy on T lymphocyte function in neonatal pneumonia

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Abstract. – OBJECTIVE: We aimed at studying the effect of adjuvant therapy with low-dose vitamin A on the function of T lymphocytes in neonatal pneumonia.

PATIENTS AND METHODS: We recruited 60 cases of neonatal pneumonia which were randomly divided in two equal groups. The control group was treated with conventional anti-inflammatory therapy and aerosol inhalation. The experimental group received oral vitamin A soft capsules for 7 days.

RESULTS: Pre-treatment levels vitamin A level and vitamin A deficiency disorders (VADD) percentage revealed no differences between the two groups. The treatment course for the experimental group was shorter than the control group. Serum IgM, IgG, and glutathione peroxidase (GSH-Px) levels were increased, whereas the levels of malondialdehyde were decreased in the experimental group after treatment. The control group showed no changes in these factors. After treatment, both groups showed increased percentages of CD4+ and CD8+ T cells, but the experimental group showed a larger increase.

CONCLUSIONS: Neonatal pneumonia is often accompanied by a low level of vitamin A, and adjuvant therapy can shorten its disease course, improve IgM and IgG levels, and improve anti-oxidative and cellar immune function.

Key Words
Vitamin A, Neonatal pneumonia, Malondialdehyde, Glutathione peroxidase, T lymphocyte.

Introduction

The morbidity rate of neonatal pneumonia is 3.5-25% due to the easy complications with septicemia, respiratory distress syndrome, and others. Neonatal pneumonia is one of the main reasons leading to perinatal death[1]. The rate of neonatal vitamin A deficiency (<0.70 μ.70 l) is 20-50%, whereas the subclinical vitamin A deficiency (0.70-1.05 μ.70-l) is 60-90%. The rate of neonatal vitamin A deficiency in hospitalized neonates with pneumonia is even higher[2]. At present, vitamin A deficiency disorders (VADD) have become one of the top three disorders of trace elements in the world[3]. Retinol is mainly delivered to the fetus through the placenta at advanced stages of development. Therefore, some of the reasons leading to VADD include insufficient intake by pregnant women, insufficient storage of newborns, especially premature infants, feeding intolerance, insufficient intake of fat-soluble vitamins, and normal growth metabolic exhaustion[4]. Vitamin A is involved in immune function, growth of the lung trachea epithelium, and is closely related to the occurrence of neonatal pneumonia and its prognosis[5]. We aimed at analyzing the effect of adjuvant therapy with low-dose vitamin A on the function of T lymphocytes in neonatal pneumonia.

Patients and Methods

Patients

We recruited 60 cases of neonatal pneumonia confirmed in our hospital from January 2015 to June 2016. Patients selected met the diagnosis criteria of neonatal pneumonia: 1. Cough, dyspnea, and dry and wet rales of lung. 2. Symptoms of pneumonia indicated by lung X-ray and positive sputum culture. Exclusion criteria: 1. Perinatal asphyxia, hypoxic-ischemic encephalopathy, congenital disease, hereditary metabolic disease, and immunodeficiency. 2. Diseases accompanied with pregnancy, like viral hepatitis B, hypertension, diabetes, etc. 3. Intolerance to vitamin A, failure to complete treatment course, or incomplete clinical data. This study was approved by the Ethics Committee of our hospital.

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We obtained signed informed consent form the guardians of the newborns. Random number table was used to equally divide the patients into control and the experimental groups. The control group included 19 males and 11 females, with age averaging 15.6 ± 4.7 days and birth weight averaging 2.2 ± 0.5 kg. The control group contained 10 premature infants, 20 full-term deliveries, 8 cesarean section, and 22 eutocia. The average pneumonia course was 2.3 ± 0.5 days, with 10 cases of mild pneumonia, 15 moderate cases, and 5 severe cases. The experimental group included 13 males and 17 females, with age averaging 13.8 ± 5.3 days and birth weight averaging 2.3 ± 0.6 kg. There were 12 premature infants, 18 full-term deliveries, 10 cesarean sections, and 20 cases of eutocia. The average pneumonia course was 2.2 ± 0.4 days, with 8 cases of mild pneumonia, 18 moderate cases, and 4 severe cases. Basic demographic data for the two groups were comparable.

Research Methods

High-Performance Liquid Chromatography (HPLC) was used to detect the plasma Vitamin A level. 5 ml of fasting peripheral venous blood were collected in heparin anticoagulation tubes. Upper serum was collected after 20 min centrifugation at 2000 x g and kept at -70°C. Reagents were purchased from Jiangsu Biyuntian Science and Technology and were used strictly according to the manufacturer's instructions (Jiangsu, China). The control group was treated with oxygen inhalation, antibiotics, aerosol inhalation, fluids infusion, homeostasis, and other conventional therapies. The experimental group received oral vitamin A soft capsules (Sinopharm Xingsha Pharmaceuticals, Xiamen, China). Code Number Approved by SFDA: H33020231, 25000 U/d) for 7 consecutive days. Adverse reactions, such as nausea, vomiting, diarrhea, and allergy should be noted to provide timely symptomatic treatment.

Experimental Indexes

Total treatment course, serum IgM and IgG levels, malondialdehyde and glutathione peroxidase (GSH-Px) levels, and percentages of CD4+T cell and CD8+T cells were analyzed. Immune turbidimetry was used to detect IgM and IgG levels. Agents were obtained from Beijing Zhongshan Golden Bridge Biology. Malondialdehyde and GSH-Px levels were detected by spectrophotometry. Reagents were obtained from Sigma-Aldrich (St. Louis, MO, USA). The ultraviolet spectrophotometer was from Bio-Rad (Hercules, CA, USA). Percentages of CD4+T cell and CD8+T cells were detected by flow cytometry. FACS Caliber flow cytometry was from BD Company (Franklin Lakes, NJ, USA).

Statistical Analysis

SPSS 20.0 (IBM SPSS Statistics for Windows, Armonk, NY, USA) was used for statistical analysis. All measurement data were expressed by mean ± standard deviation. The comparison among groups was done with independent sample *t*-test. The intra-group comparison was performed by paired *t*-test. Enumeration data were expressed by case number or %. The comparison among groups was performed with χ^2-test. $p<0.05$ was considered statistically significant.

Results

Plasma Vitamin A Level

To first determine the vitamin A deficiency, we measured vitamin A plasma levels in both groups. The control group had 0.59-1.22 μ.59-l, with an average of 0.78 ± 0.33 .33 agl. The control group had 9 cases of VADD (30.0%). The experimental group had a range of vitamin A of 0.56-1.33 μ.56-l, with an average of0.82 ± 0.45 .45 agl per patient. The experimental group had 12 cases (40.0%) of VADD. Comparison of vitamin A level and VADD percentage between the two groups revealed no significant differences (t = 0.232, p = 0.865; n^2 = 0.659, p=0.417). The experimental group showed no adverse reactions and with good tolerance during vitamin A treatment.

Treatment Course and Levels of Serum IgM and IgG

The approved protocol indicated that treatment of the experimental group would last for up to 7 days. However, the treatment course of the experimental group was shortened to only 3.5 days compared to almost full treatment of the control group (Table I). Serum IgM and IgG levels increased significantly after treatment in the experimental group but showed no change in the control group (Table I).

Malondialdehyde and GSH-Px Levels

The control group showed no changes in the levels of malondialdehyde and GSH-Px before and after treatment (Table II). In the experimental group, the level of malondialdehyde was lower, and GSH-Px was increased after treatment.

Table I. Total treatment course and levels of serum IgM and IgG (g/L).

Group	Treatment course (d)	Before treatment		After treatment	
		IgM	IgG	IgM	IgG
Control	6.7 ± 1.2	0.62 ± 0.23	4.5 ± 1.2	0.63 ± 0.24	4.7 ± 1.3
Experimental	3.5 ± 0.6	0.60 ± 0.24	4.6 ± 1.3	0.78 ± 0.26	5.6 ± 1.5
t	4.625	0.096	0.082	4.321	4.659
p	0.021	0.869	0.936	0.025	0.019

Table II. Malondiadehyde and GSH-Px levels (nmol/ml).

Group	Before treatment		After treatment	
	Malondialdehyde	GSH-Px (U/ml)	Malondialdehyde	GSH-Px
Control	15.6 ± 2.3	356.8 ± 42.6	15.4 ± 2.4	362.5 ± 45.5
Experimental	15.9 ± 2.5	348.7 ± 38.7	12.3 ± 2.1	412.3 ± 56.2
t	0.162	0.324	4.424	5.235
p	0.855	0.735	0.023	0.012

CD4+T and CD8+T Cell Count

To determine immune system strength, we determined the levels of CD4+ and CD8+ T cells in serum. After treatment, both groups showed increased levels of CD4+ and CD8+ T cells (Table III). However, the levels of CD4+ and CD8+ T cells were significantly higher in the experimental group (Table III).

Discussion

Vitamin A plays a key role in normal metabolism maintenance, cell division and differentiation, vision, and immunity[6]. VADD can cause dysfunction of the airway epithelial cells, hyperkeratosis, and abscission of epithelial cells, reduce immune function and increase lung infection[7]. VADD induces the secretion of IL-2 and TNF-a by monocytes, inhibit the activation and proliferation of T lymphocytes, reduce cellular immunity[8], disrupt the synthesis and release of IgG, IgM (peripheral blood B lymphocytes), and other immunoglobulins, and leads to low humoral immunity function[9]. Vitamin A can promote the functional maturation and gene expression of alveolar type II cells, and promote the generation and maturation of pulmonary surfactant[10]. Retinol, the activated form of vitamin A, has antioxidant activity, prevents cell cycle arrest, and can promote pulmonary vascular growth and alveolar repair, among other functions[11]. The levels of vitamin A in newborns are independent of gender and type of delivery, but are associated with birth weight and delivery time[12]. Therefore, pregnant women and newborns should take vitamin A supplements as needed[13].

Here, we showed that vitamin A levels were low in both groups and the percentages of VADD were relatively high. The vitamin A treatment improved the levels of serum IgM and IgG levels, lowered the levels of malondialdehyde, and increased the levels of GSH-Px. After treatment, both groups showed increased levels of CD4+T cell and CD8+T cell, but the experimental group showed a more obvious increase. It should be noted that neonatal pneumonia is often accompanied with low level of vitamin A and adjuvant therapy can shorten its

Table III. Percentages of CD4+T cell and CD8+T cell (%).

Group	Before treatment		After treatment	
	CD4+	CD8+	CD4+	CD8+
Control	34.5 ± 3.6	32.3 ± 3.5	36.8 ± 4.2	35.2 ± 4.6
Experimental	33.6 ± 3.7	32.5 ± 3.3	38.2 ± 4.5	37.6 ± 4.3
t	0.252	0.196	5.324	5.659
p	0.734	0.825	0.011	0.007

disease course, improve body IgM and IgG levels, improve anti-oxidative activity, and cellar immune function. In addition, research indicates that neonatal pneumonia is related to low level of vitamin A and other trace element deficiencies, including vitamin E, zinc, and selenium[14].

Conclusions

Oral administration of complex trace elements has better efficacy than a pure supplement of vitamin A[15]. Vitamin E can maintain membrane integrity, and vitamin E insufficiency causes oxidation of fatty acids on the membrane[16]. Selenium is a critical trace element because it regulates the production of lymphocyte subsets and inhibits IL-2 levels[17]. Exogenous supplement of immunoglobulin has also some effects. Immunoglobulin can specifically bind viruses and antigens on the cellular surface; it has relatively strong effect for excreting bacteria, and can enhance body phagocytosis[18].

Conflict of Interest

The Authors declare that they have no conflict of interests.

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