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Original Article

Dynamic monitoring and a clinical correlation analysis of the serum vitamin A, D, and E levels in children with recurrent respiratory tract infections

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Abstract: Objective: To investigate the correlation of the serum vitamin A, D, and E (VA, VD, and VE) levels with the occurrence and development of recurrent respiratory tract infections (RRTIs). Methods: A total of 129 children with respiratory tract infections (RTIs) treated in our hospital from January 2018 to February 2020 (the RTIs group) and 50 healthy children undergoing physical examinations (the control group) in our hospital were recruited as the study cohort. The serum VA, VD, and VE levels were measured upon admission (the active phase) and at two weeks after discharge (the stable phase). The serum VA, VD, and VE levels in the children with RRTIs were compared with the levels in the control group, and the correlation between these three vitamins and the occurrence and development of RRTIs was analyzed. Results: The RRTIs group and the RTIs group witnessed markedly lower serum VA, VD, VE, and humoral immunity index levels, including IgG, IgA, and IgM, compared to the control group, with an apparent lower outcome in the RRTIs group than in the RTIs group. The serum levels of the above indexes in the RRTIs children were reduced in the active phase compared with the stable phase. A Pearson correlation analysis showed a positive correlation between VA and IgA. A multivariate logistic regression analysis revealed that a low BMI (Body mass index), prematurity, VA deficiency, VD deficiency, and VE deficiency were the risk factors for RRTIs in children, and outdoor activity was the protective factor. Conclusion: The VA, VD, and VE levels are closely related to RRTIs in children. It is important to determine and supplement the VA, VD, and VE levels to prevent RTIs in children.

Keywords: Recurrent respiratory tract infections, vitamin A, vitamin D, vitamin E

Introduction

Recurrent respiratory tract infections (RRTIs) are commonly seen in children, infections that take a toll on their health [1, 2]. Respiratory tract infections more than six times a year are defined as RRTIs [3]. In developed countries, RRTIs children aged less than 1 year account for 25%, and children aged 1-4 years old account for 18% [4, 5]. The occurrence and development of RRTIs are associated with immune function, genetic factors, and nutritional status [6]. An essential factor in the occurrence of RRTIs is the exposure of the imperfect immune system to environmental sources of infection in the nursery, and other factors may include allergies, indoor or outdoor pollution, and exposure to second-hand smoke [7, 8]. Children with a vitamin D (VD) deficiency have been found to be more susceptible to respiratory system infections [9]. The oral administration of VD plays a critical role in the prevention of recurrent pneumonia in children aged < 5 years [10]. A low level of vitamin A (VA) is considered a contributory factor for pneumonia in children, and the level of VA is associated with the severity of the pneumonia and the development of RRTIs afterward [11]. Zhang et al. [12] revealed that low serum concentrations of VA, VD, and VE contribute to RRTIs in children in northern China. Other studies found that an increase of VA in children with recurrent infections can enhance their immune levels, yielding a promising clinical efficacy. Therefore, the serum VA, VD, and VE levels in children with RRTIs and RTIs and healthy children were dynamically measured using high-performance liquid chromatography in this study, aiming to specify the correlation of the serum VA, VD, and VE levels with the occurrence and development of RRTIs.

Table 1. Comparison of the general data among the children from the three groups

Group	M/F (n)	Age (years, $\bar{x}\pm s$)	Weight (kg, $\bar{x}\pm s$)
RRTIs group	39/30	4.86 ± 2.83	18.87 ± 7.42
General RTIs group	34/26	5.23 ± 3.24	21.04 ± 8.31
Control group	24/26	4.75 ± 3.20	19.28 ± 8.79
χ^2/F *value*	1.070	0.385	0.926
P value	0.585	0.680	0.399

Materials and methods

Subjects

A total of 129 children with RTIs who were treated in our hospital from January 2018 to February 2020 and 50 healthy children who underwent physical examinations during the same period in our hospital were recruited as the study cohort. Among the 129 children with RTIs, there were 69 RRTIs children (the RRTIs group), 60 children with RTIs symptoms (the RTIs group), and 50 healthy children (the control group). No statistically significant differences in age, the male to female ratio, or weight were noted among all the children. All the children's families fully understood the study and signed the informed consent forms. This study was approved by our hospital ethics committee with the approval number of 2017-11-25. https://clinicaltrials.gov/, ClinicalTrials.gov Identifier: NCT02852011.

Inclusion and exclusion criteria

Inclusion criteria: (1) The RRTIs group: children in the active phase of the disease, 1-14 years old, and who met the enrollment criteria [13]. (2) The RTIs group: children who met the diagnostic criteria for RTIs [14] but not RRTIs, and who were in the active phase of the disease. (3) The control group: healthy children in the outpatient clinic without a previous history of RRTIs. Exclusion criteria: children with underlying diseases or other diseases, children who had recently been administered gamma globulin, blood products, hormones, or VA, VD, and VE.

Testing indicators

Fasting venous blood (2 ml) was collected on the morning of the second day after admission and centrifuged to obtain the supernatant. The VA, VD, and VE levels were measured using high-performance liquid chromatography. We defined VA less than 0.3 mg/L, VD less than 20 ug/L, and VE less than 5 mg/L as the criteria for vitamin deficiency. The deficiency rates were calculated. The humoral immunity indexes were determined using the immunoturbidimetric method by taking 5 mL of peripheral venous blood and measuring the IgG, IgA, and IgM levels using a Siemens BNII analyzer. The VA, VD, and VE levels were tested two weeks after discharge during the outpatient review of the children with RTIs.

Statistical analysis

All the data analyses were done using SPSS 22.0 software (SPSS Inc., Armonk, NY, USA). The measurement data were expressed as the means ± standard deviations ($\bar{x}\pm S$), and the inter-group comparisons were conducted using t-test, and the multi-group comparisons were conducted using one-way analyses of variance. The count data were analyzed using χ^2 tests. $P < 0.05$ was regarded as a statistically significant difference.

Results

Comparison of the general data

No statistically significant differences in the male to female ratio, the ages, or the weights were found among the patients in the RRTIs group, the RTIs group, and the control group (**Table 1**).

Comparison of the serum VA, VD, and VE levels

As shown in **Table 2**, the serum VA, VD, and VE levels were significantly lower in the RRTIs group and the RTIs group than the serum VA, VD, and VE levels in the control group. Moreover, the serum VA, VD, and VE levels were significantly decreased in the RRTIs group in comparison with the RTIs group. As shown in **Table 3**, the vitamin deficiency rates were significantly higher in the RRTIs group than the RTIs group ($P < 0.05$).

Comparison of the serum IgG, IgA, and IgM levels

As shown in **Table 4**, we observed significantly lower serum IgG, IgA, and IgM levels in the

Table 2. Comparison of serum VA, VD, and VE levels among the three groups

Group	VA (mg/L)	VD (ng/mL)	VE (mg/L)
RRTIs group	0.21 ± 0.08	13.22 ± 9.27	6.16 ± 1.65
General RTIs group	0.26 ± 0.11	17.25 ± 8.69	6.86 ± 1.36
Control group	0.36 ± 0.08	35.27 ± 8.21	7.59 ± 1.92
F value	39.413	18.512	10.963
P value	0.001	0.002	0.012

Table 3. Comparison of the VA, VD, and VE vitamin deficiency rates in the active and stable phases

	VA	VD	VE
RRTIs group	65.22 (45/69)	47.83 (33/69)	31.88 (22/69)
General RTIs group	20/60 (33.33)	15 (20.00)	8 (13.33)
χ^2 value	13.05	7.157	6.188
P value	0.001	0.023	0.013

Table 4. The serum IgG, IgA, and IgM levels in the RTIs children in the active phase between the two disease groups and the control group

Group	IgG (g/L)	IgA (g/L)	IgM (g/L)
RRTIs group	7.68 ± 1.1[4*,#]	1.05 ± 0.24[*,#]	0.94 ± 0.16[*,#]
General RTIs group	8.49 ± 1.48[*]	1.24 ± 0.23[*]	1.19 ± 0.21[*]
Control group	10.57 ± 1.62	1.55 ± 0.37	1.42 ± 0.29
F value	63.21	46.50	70.42
P value	0.014	0.002	0.001

[*]Compared with the control group, $P < 0.05$. [#]Compared with the general RTI group, $P < 0.05$.

Table 5. The serum VA, VD, and VE levels in the RRTIs children in the active and stable phases

Phase	VA (mg/L)	VD (ng/mL)	VE (mg/L)
Active phase	0.21 ± 0.08	13.22 ± 9.27	6.16 ± 1.65
Stable phase	0.29 ± 0.06	28.38 ± 8.90	7.47 ± 1.80
t value	-6.231	-6.726	-4.430
P value	0.012	0.001	0.003

Table 6. The correlation between the serum VA levels and the humoral immunity indexes in the RRTIs group

	r value (Pearson coefficient)	*P* value
IgG	0.012	0.284
IgA	0.347	0.003
IgM	0.004	0.589

RRTIs group than in the RTIs and control groups ($P < 0.05$). Moreover, the decrease in the RRTIs group was more significant in comparison with the RTIs group ($P < 0.05$).

Comparison of the serum VA, VD, and VE levels in the RRTIs children in the active and stable phases

As shown in **Table 5**, the serum VA, VD, and VE levels in the children with RRTIs were lower in the active phase than in the stable phase ($P < 0.05$).

The correlation of the serum VA, VD, and VE levels with the humoral immunity indexes in the children with RRTIs

As shown in **Tables 6**-**8**, there was a positive correlation among the serum VA and IgA levels in the patients with RRTIs ($r = 0.347$, $P = 0.003$).

Multivariate logistic regression analysis of the RRTIs in children

As shown in **Table 9**, our multivariate logistic regression analysis showed that low BMI, prematurity, VA deficiency, VD deficiency, and VE deficiency are the risk factors for RRTIs in children, and outdoor activity is the protective factor.

Discussion

Studies show that RTIs remain one of the leading causes of high morbidity and mortality in children [15]. Respiratory tract infections are frequently reported in children, susceptible adults, and the elderly. The risks of recurrence and complications are associated with both the virus and the immune function [16]. A previous study pointed out that the serum IgG, IgA, and IgM levels are significantly low in RRTIs children. Studies have shown that the serum VA, VD, and VE levels are closely related to the body's immune status, but decreased immunity may result in RRTIs in children [17]. In this paper, we dynamically monitored the serum VA, VD, and VE levels in RRTIs children, and we explored their correlation with the occurrence and development of the disease.

In this study, the serum VA, VD, and VE levels and the humoral immunity indexes, including IgG, IgA, and IgM showed a decline in the RRTIs

Table 7. The correlation between the serum VD levels and the humoral immunity indexes in the RRTIs group

	r value (Pearson coefficient)	*P* value
IgG	0.021	0.635
IgA	0.119	0.097
IgM	0.002	0.842

Table 8. The correlation between the serum VE levels and the humoral immunity indexes in the RRTIs group

	r value (Pearson coefficient)	*P* value
IgG	0.016	0.415
IgA	0.124	0.114
IgM	0.013	0.214

group compared with the control and RTIs groups. The vitamin deficiency rates showed a surge in the RRTIs group compared to the RTIs group. Our Pearson correlation analysis showed a positive correlation between VA and IgA. The multivariate logistic regression analysis showed that low BMI, prematurity, VA deficiency, VD deficiency, and VE deficiency were the risk factors for RRTIs in children, while outdoor activity was the protective factor. Micronutrient VA is defined as a class of compounds that imposes a multifunctional effect on human health. These molecules may serve as regulators in biological functions such as development, vision, and intestinal barriers [18]. The favorable effects of VA may depend on pathogen-driven immune responses [19] and genetic background [20]. It has been confirmed that VA deficiency impedes the normal regeneration of the mucosal barrier disrupted by infection and weakens the function of neutrophils, macrophages, and natural killer (NK) cells, thereby impairing innate immunity. Additionally, VA is indispensable to the adaptive immune system [21]. In this study, the serum VA level declined in the children with RRTIs, and this decline was assumed to be associated with decreased immune system function, thus increasing the risk for RTIs in the children.

Our *in vitro* data showed that, in addition to regulating the innate immune cells, VD can also improve the body's immune function. *In vivo* studies with VD supplementation in animals and humans have shown that VD exerts promising effects on immune function, particularly in the setting of autoimmunity [22]. A prior study also proposed that VD exerts an immunomodulation effect on diverse immune cells such as monocytes, macrophages, dendritic cells (DCs), T-lymphocytes, and B-lymphocytes, hence modulating both the innate and adaptive immune responses [23]. VD plays a critical role in the body's immune system. Therefore, reduced VD levels in the body will inevitably cause a decrease in immune function, thereby enhancing the risk for RRTIs in children.

Since the development, function, and regulation of DCs, macrophages, NK cells, T cells, and B cells were understated, the influence of VE on specific immunocytes has received a surge of interest [24]. Vitamin E, a potent lipid-soluble antioxidant found in higher concentrations in immunocytes than in any other cell in the blood, is thought to be one of the most effective nutrients for regulating immunological function [25]. In our study, the VE levels were significantly reduced and closely related to respiratory diseases, which clearly predisposed the children to an increased incidence of respiratory infections. Additionally, Zhang et al. [26] found that reductions in vitamin A, D3, and E levels are closely related to RRTIs in children. The timely supplementation of vitamins during routine treatment can considerably improve the condition of the children and promote recovery. However, the current status of children's vitamin-related nutrition in China is not optimistic, and a lack of vitamin A, D3, and E may give rise to RRTIs [27]. Symptomatic treatment is the mainstay for RRTI, yet it produces an undesirable outcome in improving the symptoms of the disease. Reaching the normal range of vitamin A, D3, and E levels through vitamin supplementation helps to improve immune function, thereby mitigating the inflammation and alleviating the clinical symptoms. Children who underwent the conventional treatment combined with vitamin supplementation did not experience any adverse reactions, which may be related to the enhancement of body immunity and the improvement of nutritional status after vitamin supplementation. However, it should be noted that despite the fact that vitamins are trace organic substances required by the human body, excessive supplementation can also cause damage to the body. The monitoring results should be referenced to

Table 9. A multivariate logistic regression analysis of the risk factors for RRTIs in children

	B	SE	P	OR	95% CI
Low BMI	-0.415	0.592	< 0.001	5.011	3.321~6.063
Prematurity	-0.462	0.603	< 0.001	2.453	1.762~3.561
VA deficiency	-0.162	0.521	< 0.001	6.982	5.392~8.134
VD deficiency	-0.803	0.382	< 0.001	4.024	3.213~5.574
VE deficiency	-0.762	0.582	< 0.001	2.018	1.351~3.235
Outdoor activities	0.892	0.433	< 0.001	0.672	0.493~0.704

develop medical treatment. The limitation of this study is that no further studies have been conducted on the drug targets. The VA, VD, and VE levels are closely related to RRTIs in children, but as to whether they can be used as a drug treatment target for RRTIs, future research and drug trials will be conducted to confirm the feasibility.

Furthermore, we also found that the VA, VD, and VE levels are significantly lower in the stable phase than in the active phase, providing a basis for the clinical determination of RRTIs.

Disclosure of conflict of interest

None.

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