Effects of vitamin D on apoptosis of T-lymphocyte subsets in neonatal sepsis

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Received September 12, 2017; Accepted May 10, 2018

DOI: 10.3892/etm.2018.6215

Abstract. Effect of vitamin D on apoptosis of peripheral blood T-lymphocyte subsets in treatment of neonatal sepsis was investigated. A total of 150 neonatal patients with sepsis were randomly divided into vitamin D treatment group (observation group) and treatment control group, while 100 healthy newborns were selected as healthy control group. T-lymphocyte subsets were detected by flow cytometer, the levels of tumor necrosis factor-α, interleukin-1 and calcitonin were determined by double-antibody immunoluminometric assay, and the effect of vitamin D on the above indicators in the treatment of sepsis was observed. Serum 25(OH)D (22.52±5.56 mg/l) in the treatment group was obviously increased compared with that in the treatment group (14.85±6.14 mg/l) ($P<0.05$), but the levels in the two groups were remarkably lower than that in the normal control group (26.38±6.56 mg/l), and the differences were statistically significant ($P<0.05$). Cluster of differentiation 4 ($CD4^+$) T-lymphocyte subset in sepsis patients was obviously reduced compared with that in the healthy control group ($P<0.01$); the difference in comparison of $CD8^+$ T-lymphocyte subset between sepsis patients and healthy people was not statistically significant ($P>0.05$). After treatment for 72 h, $CD4^+$ T-lymphocytes were increased, and the ratio of $CD4^+$ to $CD8^+$ was close to 1, suggesting that the effect was superior to that in the treatment control group. The inflammatory factor levels in children with sepsis were evidently higher than those in the healthy control group ($P<0.01$), and high-level states of inflammatory factors were significantly improved after treatment with vitamin D for 72 h, indicating that the effect was superior to that in the treatment group. The results indicated that the prognosis of sepsis patients treated with vitamin D is improved, and the mechanism may be achieved by regulating T-lymphocyte subsets and inflammatory factors.

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Key words: vitamin D, neonatal sepsis, T-lymphocyte subsets

Introduction

Neonatal sepsis is one of the most common critical diseases in pediatrics, with a higher incidence, which can be caused by a variety of pathogenic bacteria infections and often accompanied by systemic inflammatory response syndrome (SIRS). In the neonatal period, especially in premature infants, when infection occurs, blood circulation is invaded by bacteria that reproduces and produces toxins, resulting in systemic reactions (1,2). Because of insidious onset, there are no specific clinical symptoms in child patients during early stage; given that the rapid progress of the disease will lead to secondary organ damage, there is a need for early treatment, which is a huge difficulty and challenge in clinical practice; the lack of early treatment easily leads to death of children (3,4). Vitamin D is an essential nutrient; vitamin D deficiency can easily cause rickets and chondropathy. In recent years, studies by Chiesa *et al* (5), El-Mazary *et al* (6) and Cetinkaya *et al* (7) have shown that vitamin D plays an important role in immunoregulation. In this study, 150 neonates with sepsis and 100 healthy newborns were selected, and the levels of T-lymphocyte subsets, tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1) and calcitonin (CT) were detected, so as to investigate the influence of vitamin D on apoptosis of T-lymphocyte subsets, the effect on inflammatory factors and its possible mechanism.

Patients and methods

Objects of study. One hundred and fifty neonatal patients with sepsis admitted to Ruian People's Hospital (Wenzhou, China) from October 2015 to May 2017 were selected and randomly divided into observation group (n=75) and treatment control group (n=75), while 100 healthy newborns during the same period were enrolled as healthy control group. All the patients in the observation group and the treatment control group had positive blood culture results, and the imaging findings were in accordance with the positive standard diagnosis. Inclusion criteria: Observation group and treatment control group, patients aged <28 days; patients with sepsis. Exclusion criteria: Patients with immune dysfunction; patients with immune system disease; children with incomplete data; children had administration with vitamin D; patients with liver or kidney dysfunction; patients who gave up treatment. Normal control group: Neonatal without sepsis.

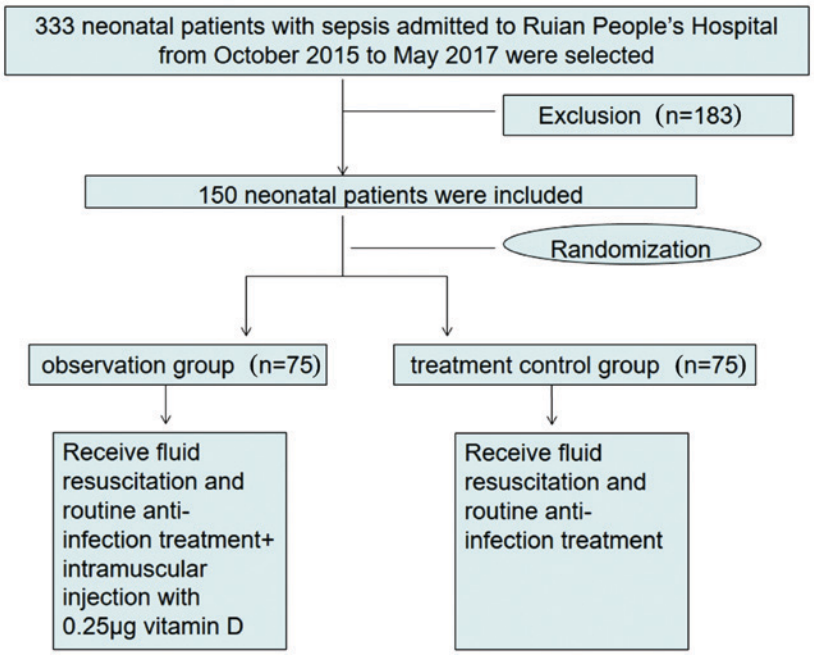

Figure 1. Flowchart of the design.

The parents of the patients or their family members signed the informed consent, and this study was approved by the Ethics Committee of Ruian People's Hospital (Wenzhou, China).

Treatment methods. We initially collected 333 patients with neonatal sepsis from October 2015 to May 2017 in Ruian People's Hospital, while 183 patients were excluded according to exclusion criteria. Therefore, only 150 patients with neonatal sepsis were included. They were randomly divided into observation (n=75) and treatment control group (n=75). Both treatment control and observation group received fluid resuscitation and routine anti-infection treatment. Based on it, the observation group was treated by intramuscular injection with 0.25 μg/per child vitamin D (8,9); when the condition was improved and remained stable, the dosage of medication could be reduced until withdrawal (Fig. 1).

Detection methods and evaluation. Observation indicators: Cluster of differentiation 8 ($CD8^+$) T-cells, $CD4^+$ T-cells, TNF-α, IL-1 and CT before treatment and at 72 h after treatment. T-lymphocyte subsets, $CD8^+$ T-cells and $CD4^+$ T-cells were detected by FACSCalibur flow cytometer (BD Biosciences, San Jose, CA, USA). TNF-α, IL-1 and CT were determined by enzyme-linked immunosorbent assay (ELISA), and the kits were provided by Jingmei Biotech Co., Ltd. (Shanghai, China). Serum 25(OH)D was detected by Roche Diagnostics GmbH (Mannheim, Germany) Cobas e411 automatic electrochemiluminescence immunoassay analyzer. Baseline levels were detected in the healthy control group.

Statistical analysis. Data were processed SPSS 22.0 (IBM Corp., Armonk, NY, USA). Data conforming to normal distribution are expressed as mean ± standard deviation and compared by t-test, and nonparametric rank sum test was used for those meeting abnormal distribution. The Chi-square test was adopted for the comparison of rate. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Clinical data of the three groups. There were 75 patients in the observation group, including 39 males and 36 females, aged 1-10 days, with an average of 4.71±1.59 days; there were 75 cases in the treatment control group, including 37 males and 38 females, aged 1-10 days, with an average of 4.45±1.42 days; there were 100 patients in the healthy control group, including 50 males and 50 females, aged 1-10 days, with an average of 4.67±1.62 days. The sex, age, mode of birth and other general data of the three groups were comparable, the differences were not statistically significant ($P>0.05$) (Table I).

Test results of vitamin D in each group. There were statistically significant differences in comparisons of 25(OH)D among three groups. Serum 25(OH)D level in the observation group was higher than that in the treatment control group ($P<0.05$), but the levels in the two groups were remarkably lower than that in the healthy control group, and the differences were statistically significant ($P<0.05$) (Table II).

Comparison of T-lymphocyte subsets, inflammatory factor and CT in three groups before and after treatment. The difference in comparison of $CD4^+$ T-cells between sepsis patients and healthy control group had statistical significance before treatment ($P<0.01$), and it showed a statistically significant difference after treatment with vitamin D for 72 h between the observation group and the treatment control group ($P<0.05$). The difference in comparison of $CD8^+$ T-cells between sepsis patients and healthy people had no statistical significance before treatment ($P>0.05$), but it showed a statistically significant difference after treatment for 72 h between the observation group and the treatment

Table I. Comparison of clinical features in the three groups.

Factor	Observation group	Treatment control group	Healthy control group	Statistic	P-value
Case	75	75	100		
Age (days)	4.71±1.59	4.45±1.42	4.67±1.62	t=0.79	0.53
Sex (n)				χ^2=0.189	0.979
Male	39	37	50		
Female	36	38	50		
Apgar score					
1 min	8.3±0.8	8.4±0.8	8.2±0.7	t=0.86	0.48
5 min	9.4±0.6	9.5±0.5	9.6±0.4	t=0.88	0.47
Mode of birth (n)				χ^2=0.202	0.912
Vaginal delivery	40	30	40		
Uterine-incision delivery	35	45	60	t=7.34	0.0051
Birth season				χ^2=0.208	0.909
Spring	19	17	24		
Summer	17	19	26		
Autumn	18	17	23		
Winter	21	22	27		

Table II. Comparison of 25(OH)D levels among three groups.

Groups	Case	25(OH)D (mg/l)
Observation	76	22.52±5.56
Treatment control	74	14.85±6.14
Healthy control	100	26.38±6.56
F-value		56.55
P-value		<0.05

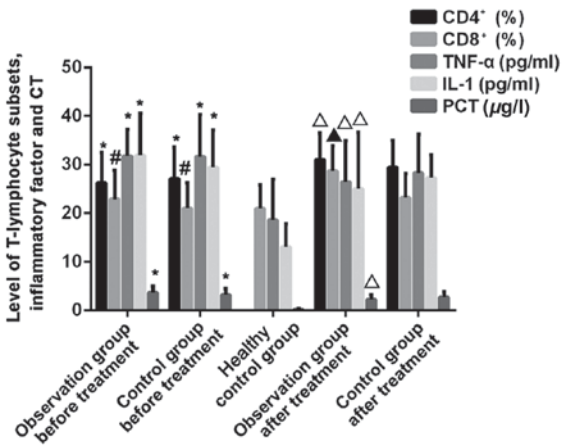

Figure 2. Comparison of T-lymphocyte subsets, inflammatory factor and CT in three groups before and after treatment. *P<0.01, compared with the healthy control group. △P<0.05, compared with the control group after treatment. ▲P<0.01, compared with the control group after treatment. #P>0.05, compared with the healthy control group. CD4+, cluster of differentiation 4; TNF-α, tumor necrosis factor-α; IL-1, interleukin-1; CT, calcitonin.

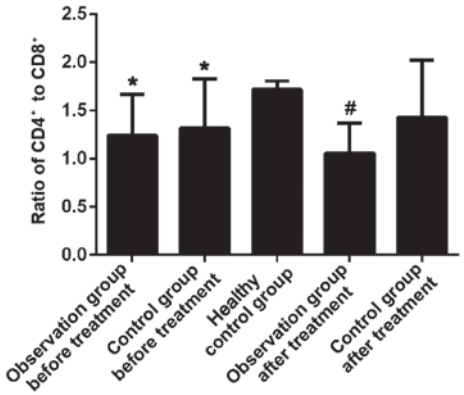

Figure 3. Comparison of the ratio of CD4+ to CD8+ in three groups before and after treatment. *P>0.05, compared with the healthy control group. #P<0.05, compared with the control group after treatment. CD4+, cluster of differentiation 4.

control group (P<0.01). After treatment for 72 h, CD4+ T-cells were increased, and the ratio of CD4+ to CD8+ was close to 1. The differences in comparisons of inflammatory factor levels between children with sepsis and healthy control group were statistically significant before treatment (P<0.01). The differences in comparisons of TNF-α, IL-1 and CT levels between the observation group and the treatment control group were statistically significant after treatment with vitamin D for 72 h (P<0.05) (Figs. 2 and 3).

Logistic regression analysis of vitamin D, CD4+ T-lymphocytes and CD8+ T-lymphocytes. There was a positive correlation between vitamin D and CD4+ T-lymphocytes and CD8+ T-lymphocytes (r=0.712, P=0.001; r=0.685, P=0.003) (Fig. 4).

Cox regression analysis. We analyzed the possibility of vitamin D, CD4+ T-lymphocytes and CD8+ T-lymphocytes as the risk factors for sepsis. The median vitamin D level before

Table III. Single factor COX regression analysis of the occurrence of sepsis.

Single factor	HR	95% CI	P-value
Vitamin D (low vs high)	1.412	1.124-1.903	0.025
CD4 +T lymphocyte (low vs high)	1.315	1.033-2.013	0.031
CD8 +T lymphocyte (low vs high)	0.512	0.235-1.059	0.024
Gender (male vs female)	0.854	0.244–2.252	0.745
Labor (natural labor vs C-section)	0.832	0.376–1.864	0.672
Premature birth (yes vs no)	1.114	0.813-1.324	0.181

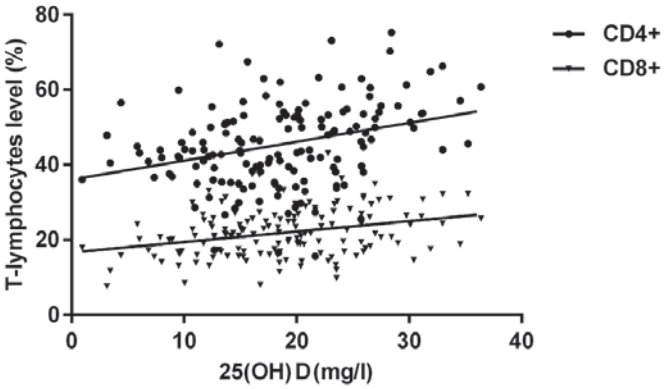

Figure 4. Logistic regression analysis of vitamin D, CD4+ T-lymphocytes and CD8+ T-lymphocytes.

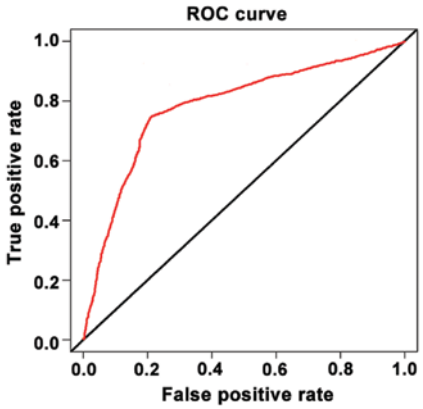

Figure 5. The ROC curve of vitamin D predicting the prognosis of children with sepsis.

treatment was 15.82 mg/l and the median percentage of $CD4^+$ T-lymphocytes was 26.58%, and the median percentage of $CD8^+$ T-lymphocytes was 21.75%. Patients were divided into high and low levels according to the median level of $CD8^+$ T-lymphocytes. The results showed that they may be risk factors for sepsis (Table III).

The outcomes of patients in different groups. The mortality rate of sepsis children with vitamin D level <15.82 mg/l is 35.2%. The mortality rate of sepsis children with vitamin D level >15.82 mg/l is 11.3%. The difference is statistically significant (P<0.05). The AUC of the ROC curve analysis of vitamin D levels predicted the prognosis of children with sepsis was 0.743, which had a good predictive value (P<0.05) (Fig. 5).

Discussion

Neonatal sepsis is a common clinical syndrome in newborns, with high morbidity and mortality. As the disease is relatively insidious, the condition is usually in a state of danger when it is found, involving complex immune dysfunction, abnormal coagulation and other aspects; systemic immune response easily occurs under the induction of infection in neonates with deficiencies in immune function (10). Admittedly, the disease can be controlled to a certain extent by adopting antibiotics, but at the same time, the bacteria died from antibiotics can release large amounts of endotoxin, resulting in the rapid increase in endotoxin levels in patients, thus activating immune response; on the contrary, it will aggravate the disease (11). The studies by Yang *et al* (12) and Cizmeci *et al* (13) have revealed that vitamin D has an important regulatory role in the immune system, which can inhibit cell proliferation and maturation, and regulate inflammatory cytokines. Xiao *et al* (14) and Nzegwu *et al* (15) investigated the relationship between vitamin D and sepsis. By detecting apoptosis of T-lymphocyte subsets and inflammatory factors, this study aimed to investigate the effect of vitamin D on apoptosis of T-lymphocyte subsets in neonatal sepsis.

Compared to other studies, the objects of this study were selected in strict accordance with inclusion and exclusion criteria, ensuring the reliability of the study. At present, there is little research on the immunological regulation mechanism of vitamin D on neonatal sepsis; however, this study investigated the possible immune regulation mechanism of vitamin D on neonatal sepsis, displaying better innovation.

As known, T-lymphocytes mainly include $CD4^+$ T-cells, $CD8^+$ T-cells, which play important roles in the organism (16,17). Moreover, the size of $CD4^+$ T-cells is closely related to immunosuppression (18). In this study, the difference in comparison of $CD4^+$ T-cells was statistically significant after treatment with vitamin D for 72 h betweent the observation group and the treatment control group. After treatment with vitamin D for 72 h, the amounts of $CD4^+$ T-cells and $CD8^+$ T-cells were increased, and the increase of $CD4^+$ T-cells was more remarkable. We analyzed the correlation between vitamin D and $CD4^+$ T-lymphocytes and $CD8^+$ T-lymphatic level. The results of logistics regression analysis showed that the correlation coefficient was 0.712, which showed that vitamin D may be able to regulate the levels of $CD4^+$ T-lymphocyte and $CD8^+$ T-lymphocytes. Chen *et al* (19) found that vitamin D can regulate the proliferation of $CD8^+$

T cells in patients with gastroenteritis and promote the development of gastrointestinal inflammation. Cantorna *et al* (20) also found that vitamin D can regulate T-lymphocyte levels in mouse. Therefore, we sepeculate that vitamin D is associated with the prognosis of neonatal sepsis. Thus, the role of vitamin D in neonatal sepsis may be achieved by reducing apoptosis in T cells via inhibiting excessive immune response (21). Under the action of inflammatory factors, nuclear factor (NF-κB) is activated, which induces inflammatory cell infiltration, thus leading to immune dysfunction (22,23). We will further test this hypothesis through *in vitro* experiment in our future studies. This study indicated that the differences in comparison of TNF-α, IL-1 and CT levels between the observation group and the treatment control group were statistically significant before treatment, and after treatment with vitamin D for 72 h, the levels of these indicators in the observation group were lower than those in the treatment control group. The high levels of inflammatory factors were obviously ameliorated, which reduced damage to the body to a certain extent. We also analyzed the prognosis of children with sepsis. We divided the children into low level group (≥15.82 mg/l) and high level group (<15.82 mg/l) according to the level of vitamin D. There is significant difference in mortality rate between two groups of children. The AUC was 0.743, indicating that vitamin D may have good prognostic value in children with sepsis.

In this study, given that there may be some bias because of the limited sample size, and the inappropriate dose of vitamin D may cause hypercalcemia (24), the dosage, time and mode of medication and other specific issues still need to be further investigated by experts worldwide. Vitamin D may be a risk factor for sepsis, but more research and larger samples are needed to verify whether it is an independent factor. More studies are needed to investigate the applications value of vitamin D in the diagnosis of sepsis. It is hoped that individualized treatment of neonatal sepsis can be carried out from the perspective of combined treatment, so as to reduce the morbidity and mortality of neonatal sepsis in China.

In conclusion, vitamin D can enhance the immune function of neonates with sepsis by regulating the levels of inflammatory factors and T-lymphocyte subsets.

Acknowledgements

Not applicable.

Funding

This study was supported by Wenzhou Science and Technology Bureau Project (Y20150128), Wenzhou Health Bureau Project (2015B37) and Ruian Science &Technology Bureau Project (MS2017006).

Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

GZ and WJ conceived and designed the study. GZ, MP and ZL were responsible for the collection and analysis of the data. WX and WJ interpreted the data and drafted the manuscript. GZ revised the manuscript critically for important intellectual content. All authors read and approved the final study.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Ruian People's Hospital (Wenzhou, China). Signed informed consents were obtained from the parents of the child patients.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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