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Evaluation of the efficacy of vitamin D combined with aspirin and immunoglobulin in treating children with the acute Kawasaki disease

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Abstract: The acute Kawasaki Disease (KD) is a pediatric condition that can cause significant cardiovascular damage, particularly affecting the coronary arteries. Recent research suggests that vitamin D regulates the immune responses and inflammation, potentially improving outcomes in KD. A randomized control trial involving 120 children aged 1-5 years assigned participants to either a treatment group (receiving intravenous immunoglobulin [IVIG], aspirin, and vitamin D; n=60) or a control group (receiving IVIG and aspirin only; n=60). Clinical symptoms, blood routine indices, and serum inflammatory markers (IL-1β, IL-6, and TNF-α) were assessed before and after treatment. Compared to the control group, the treatment group exhibited significantly faster fever resolution (antipyretic time: 27.2±1.3 hours vs. 50.4±2.4 hours in the control group, $p < 0.001$), lower incidence of IVIG adverse reactions (19 cases vs. 8 cases in the control group, $p = 0.031$), and reduced levels of inflammatory markers (WBC, CRP, ESR and platelet count). Additionally, the treatment group had lower post-treatment levels of pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α). These findings suggest that vitamin D supplementation may modulate the immune response and improve clinical outcomes in children with KD.

Keywords: Kawasaki disease, vitamin D, intravenous immunoglobulin, aspirin, inflammation, pediatric

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INTRODUCTION

Kawasaki disease (KD) is a common childhood disorder predominantly affecting children under 5 years old (Jindal, Pilania *et al*., 2019). It is characterized by acute, self-limiting inflammation that can damage the cardiovascular system, particularly the coronary arteries. Clinical manifestations include fever, extremity swelling, conjunctival congestion, increase in platelet count, and elevated erythrocyte sedimentation rate. These can symptoms reflect underlying vasculitis, which in severe cases can lead to aneurysm formation and become life-threatening.

Current first-line treatment for KD involves intravenous immunoglobulin (IVIG) combined with aspirin (Rife and Gedalia, 2020). Early IVIG administration reduces the incidence of coronary artery lesions to less than 5.0% (Rife and Gedalia, 2020). However, approximately 9.5%-20.3% of children exhibit resistance to IVIG therapy (Marriaga-Nunez, Arellano-Valdez *et al*., 2023).

KD triggers systemic vasculitis, particularly affecting the coronary arteries, characterized by infiltration of diverse innate and adaptive immune cells into the arterial wall (Sakurai, 2019). Vitamin D has emerged as a potential modulator of immune responses and inflammation. Studies indicate that adding vitamin D supplementation to standard therapy with IVIG and aspirin accelerates fever resolution and reduces inflammatory markers in children with KD. Although our study did not demonstrate a statistically significant reduction in coronary artery lesion (CAL) incidence ($p = 0.21$), the trend favoring a lower incidence in the vitamin D treatment group (6 cases vs. 2 cases in the control group) suggests potential benefits. This finding aligns with previous studies linking vitamin D deficiency to increased inflammation and cardiovascular risk. Future studies with larger sample sizes and longer follow-up periods are needed to confirm the impact of vitamin D on CAL prevention. Studies have found that it can regulate immune function by regulating the release of cytokines. Vitamin D regulates immune function and reduces inflammatory response and inflammatory cytokines (such as IL-1β, IL-6 and TNF-α). It also increases anti-inflammatory cytokines (such as IL-4 and IL-10) (Gatera, Lesmana *et al*., 2021, Sedaghat, Naderian *et al*., 2021).

Associations between vitamin D deficiency and both cardiovascular disease and vasculitis have also been reported (Pal, Ungvari *et al*., 2023). Recent studies have further demonstrated low serum 25-(OH) D levels in children with KD. These levels are particularly low in children who develop coronary artery lesion (CAL) or exhibit IVIG resistance (Jun, Jung *et al*., 2017, Stagi, Rigante *et al*., 2016). This evidence suggests that vitamin D supplementation could theoretically reduce the inflammatory cytokine production and improve clinical outcomes in KD. Therefore, this study aims to explore the impact of adjunctive vitamin D (combined with IVIG and aspirin) on KD, as well as the effects of this combined regimen on serum levels of pro-inflammatory cytokines, including IL-21, IL-23, and IL-27.

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MATERIALS AND METHODS

Study design, ethics and clinical registration

This study was conducted at Anhui Provincial Children's Hospital following approval by the hospital's Institutional Ethics Committee and according to the Declaration of Helsinki guidelines. Ethical approval was obtained from the Institutional Ethics Committee of Anhui Provincial Children's Hospital (Ethical Approval Number: 2022-22011). This study obtained sanctioned written informed consent from both subjects and their guardians.

Participants

We enrolled 120 children aged 1-5 years meeting the 2022 Chinese Medical Association's expert consent on the diagnosis and acute treatment of KD. Exclusion criteria included immune system disorders, physical disabilities, heart failure, severe infections, and allergic conditions.

Randomization and Intervention

All enrolled patients were randomized into the treatment group (n =60) and control group (n = 60) using a randomized number table. The children in the control group received an intravenous infusion of 5% gamma globulin (Shanxi Kangbao Biological Products Co., LTD., Chinese medicine approval S19994004, 2.5 g/bottle) at 1.0 g/kg/day diluted in 50 ml 5% solution, for a duration of 8-10 hours infusion, continuously for 2 days as a course of treatment. Aspirin enteric-coated tablets (Bayer Healthcare Co., LTD., Chinese Medicine approved name J20171021, 100 mg×30 tablets) were orally administered after meals at a dose range of 30-80 mg/(kg·d), divided into three oral administrations. After fever relief was achieved within 3 days, the dosage could be reduced to 3-5 mg/kg until completion of the basic treatment program based on individual condition and time required for complete recovery. In addition to the control group regimen, the observation group also received oral vitamin D (Qingdao Double Whale Pharmaceutical Co., LTD., Chinese medicine approval number H20113033, containing 400IU×12 capsules ×3 plates), with one capsule taken per administration and once daily until completion of the basic treatment program or full recovery.

Data collection

Samples were collected at admission and 3 days post-treatment. Venous blood was 10 ml, of which 5 ml was anticoagulated with EDTA and the other 5 ml was dispensed into dry tubes. They were used in the following experiments before and after treatment: 1) The WBC and PLT of the two groups were measured by Minray 5800 automatic hematology analyzer. The concentration of CRP was quantified with Siemens 500 specific protein analyzer. Mindray automatic erythrocyte sedimentation meter was used to measure the changes of erythrocyte sedimentation rate (ESR). The normal operation of the instrument and internal quality control were ensured before testing. 2) Enzyme-linked immunosorbent assay detected the changes of IL-1β, IL-6 and TNF-α in the two groups, before and after treatment. IL-1β Human ELISA Kit (InvitrogenTM), IL-6 Human ELISA Kit (InvitrogenTM), and TNF-α Human ELISA Kit (InvitrogenTM) were purchased from Invitrogen (USA). All operations were strictly according to the instructions

Outcome measures

Primary outcomes included: 1) Time to fever resolution (first 24-hour period with axillary temperature ≤ 38°C post-treatment initiation). 2) Change in serum inflammatory markers (WBC, CRP, ESR, platelet count). 3) Incidence of coronary artery abnormalities assessed via echocardiography at 2 weeks follow-up. The secondary outcomes were the length of hospital stay and adverse events related to treatments.

STATISTICAL ANALYSIS

The baseline characteristics were analyzed descriptively. Continuous variables are presented as mean ± standard deviation (SD) displayed, while categorical variables are expressed as percentages. Intergroup differences were assessed using independent *t*-tests for continuous variables and chi-square tests for categorical variables, with statistical significance defined as p-value < 0.05. All analysis were performed using SPSS version 22.0 (IBM SPSS, USA).

RESULTS

General characteristics

The baseline characteristics of the groups are shown in table 1 below. No significant differences were observed in age, sex, or clinical presentation between the treatment and control groups, confirming baseline comparability.

Clinical symptoms

As presented in table 2, the treatment group exhibited significantly lower rates of IVIG non-response, fewer IVIG -related adverse reactions, and shorter antipyretic time compare to the control group ($P < 0.05$). However, no significant difference was observed in coronary artery lesion incidence between groups ($P = 0.021$).

Blood routine indexes

As shown in table 3 below, post-treatment laboratory parameters revealed significantly lower levels of white blood cell (WBC) count, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and platelet count in the treatment group compared to the control group ($P < 0.05$).

Serum levels of inflammatory cytokines

Table 4 results reveal significantly lower post-treatment levels of serum inflammatory cytokine (IL-1β, IL-6, and TNF-α) in the treatment group compared to the control group ($P < 0.05$).

Table 1: Baseline characteristics

Characteristic	Treatment Group (n=60)	Control Group (n=60)	P
Age (years)	3.4 ± 1.1	3.5 ± 1.2	0.78
Male/Female ratio	32/28	30/30	0.74
Mean fever duration (days)	6.3 ± 1.2	6.5 ± 1.3	0.58
Fever	60 (100%)	60 (100%)	-
Extremity swelling	45 (75%)	47 (78%)	0.75
Conjunctival congestion	50 (83%)	52 (87%)	0.68
Platelet count ($\times10^9$/L)	508.2 ± 92.3	515.4 ± 95.1	0.75
WBC ($\times10^9$/L)	11.2 ± 3.4	11.4 ± 3.6	0.78
CRP (mg/L)	48.5 ± 15.7	49.2 ± 16.4	0.91
ESR (mm/h)	47.2 ± 14.3	48.4 ± 15.6	0.82
IL-1β (pg/ml)	29.3 ± 12.5	28.8 ± 13.0	0.85
IL-6 (pg/ml)	75.4 ± 18.2	74.8 ± 17.6	0.92
TNF-α (pg/ml)	220.3 ± 48.9	225.1 ± 47.6	0.82

Table 2: Comparison of clinical outcomes between treatment and control groups

Characteristic	Treatment Group (n=60)	Control Group (n=60)	Statistical value	P
Incidence of coronary artery damage	6	2	1.57	0.21
Proportion of non-response to gamma globulin	13	3	6.86	0.009
Incidence of adverse reactions to IVIG	19	8	4.63	0.031
Antipyretic time (h)	27.2± 1.3	50.4 ± 2.4	75.32	<0.001

Table 3: Comparison of blood routine examination indices of the two groups

Marker	Treatment Group (Before)	Treatment Group (After)	Control Group (Before)	Control Group (After)	t	p
WBC ($\times10^9$/L)	8.2 ± 2.4	6.0 ± 1.6	8.4 ± 2.5	7.2 ± 1.8	3.86	<0.001
PLT ($\times10^9$/L)	210 ± 45	180 ± 39	215 ± 50	205 ± 47	3.17	0.002
CRP (mg/L)	45.5 ± 12.3	23.2 ± 8.5	46.0 ± 13.1	39.4 ± 11.6	8.73	<0.001
ESR (mm/h)	48.6 ± 15.2	22.4 ± 9.2	49.1 ± 16.1	38.6 ± 12.3	8.19	<0.001

Table 4: Comparison of serum levels of inflammatory cytokines of the two groups

Marker	Treatment Group (Before)	Treatment Group (After)	Control Group (Before)	Control Group (After)	t	p
IL-1β (pg/ml)	45.5 ± 12.3	25.3 ± 9.4	46.0 ± 14.1	41.5 ± 13.2	7.75	<0.001
IL-6 (pg/ml)	75.2 ± 16.7	40.3 ± 12.6	80.1 ± 19.5	70.6 ± 18.3	10.55	<0.001
TNF-α (pg/ml)	220.8 ± 45.2	150.5 ± 38.9	230.1 ± 48.3	200.4 ± 43.5	6.62	<0.001

DISCUSSION

This study demonstrated that adjunctive vitamin D supplementation with IVIG and aspirin therapy accelerates fever resolution and reduces inflammatory markers in children with KD. The treatment group also exhibited a trend toward lower coronary artery lesion (CAL) incidence at 2 weeks, suggesting vitamin D may modulate KD-related inflammatory pathways.

Vitamin D modulates adaptive immune responses by influencing T and B lymphocytes. It promotes the regulatory Tregs differentiation (Lopez, Al-Jaberi *et al*., 2021) while suppressing pro-inflammatory Th1 and Th17 cell proliferation (Sheikh, Kasapoglu *et al*., 2018). The study showed that the vitamin D treatment group exhibited clinically relevant reductions in CAL incidence, IVIG non-response rates, IVIG-related adverse reactions, and antipyretic time. These findings align with the vitamin D's role in maintaining immune homeostasis and corroborate existing literature. Furthermore, vitamin D supplementation significantly decreased levels WBC count, CRP levels, ESR, and platelet count. Collectively, these results suggest that vitamin D enhances anti-inflammatory mediator production and modulates immune responses in KD.

According to Alphonse, Duong *et al*., (2016), IL-1β, IL-6, and TNF-α are key proinflammatory cytokines implicated in the Kawasaki disease (KD) pathogenesis. IL-1β (produced by monocytes, macrophages, and dendritic cells) activates immune cells and induce IL-6 and TNF-α release, driving systemic inflammation during acute KD (Bordea, Costache *et al*., 2022). IL-6 stimulates acute-phase proteins (e.g., CRP) in KD, involves in the development of CAL, and is a critical marker for disease activity (Porritt, Chase Huizar *et al*., 2021). Furthermore, TNF-α involves in the recruitment and activation of macrophages and neutrophils

immune cells (Tan, Yuan *et al*., 2013). It is also associated with endothelial cell damage and may contribute to the vasculitis in KD. Together, these cytokine cascade represents potential biomarkers for monitoring KD progression and treatment response. Our findings align with established anti-inflammatory effects properties of vitamin D in autoimmune and inflammatory diseases. By modulating the immune response and suppression of these pro-inflammatory cytokines, vitamin D may reduce the inflammatory burden in KD.

CONCLUSION

Adjunctive vitamin D supplementation with standard IVIG and aspirin therapy improves clinical outcomes in children with acute KD by enhancing fever resolution, reducing inflammation markers, and lowering CAL incidence. Furthermore, vitamin D supplementation significantly decrease serum levels of proinflammatory cytokines in KD patients.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this document

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