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*Corresponding Author: Fadia Thamir Ahmed
Email: fadia.ahmed@copharm.uobaghdad.edu.iq

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Abstract

Rotaviruses are a leading cause of severe gastroenteritis in newborns and young children. The fat-soluble vitamin D plays an important role in immune regulation and in gut health.

Assessment of the Anti-Inflammatory Effect of Vitamin D in Rotavirus Infection among Iraqi Children

Mazin Wafi Abdullrazzaq¹ , Fadia Thamir Ahmed² , Hussein Abdul Kareem Noah³

¹ Clinical pharmacist/ Al-Kadhimiya Pediatric Hospital, Baghdad, Iraq

² Clinical Pharmacy Department, College of Pharmacy, University of Baghdad, Baghdad, Iraq.

³ Al-Kadhimiya Pediatric Hospital, Baghdad, Iraq.

The current study aimed to evaluate the anti-inflammatory effect of vitamin D in pediatric patients with rotavirus gastroenteritis.

A prospective, non-randomized clinical study was conducted at Mohammed Baqir Al-Hakim Hospital on 60 children under 5 years of age with vitamin D deficiency and confirmed rotavirus infection. Participants were divided into two equal groups: Group I (control) received conventional rotavirus gastroenteritis treatment. In contrast, Group II received the same treatment plus a single oral dose of vitamin D of 100000 IU for ages less than 1 year and 300000 IU for ages more than 1 year. Serum vitamin D levels were measured at baseline and after 7 days. Laboratory parameters were measured on days 1, 4, and 7.

According to baseline characteristics, there were no significant differences in demographic and baseline laboratory parameters between groups I and II. Significant time-related changes in C-reactive protein (CRP) and lymphocyte-to-monocyte ratio (LMR) were observed in both groups ($P < 0.05$). In Group I (control), CRP decreased from 32.8 ± 13.15 to 27.80 ± 10.39 , and LMR showed a minimal change from 3.07 (2.49–3.51) to 3.11 (2.56–3.61). In Group II (vitamin D), more pronounced improvements were noted, with CRP declining from 33 (24–36) to 12 (6–12) and LMR increasing from 2.34 (2.10–3.69) to 4.75 (4.30–5.12) ($P < 0.001$).

The findings indicated that vitamin D acts as an immunomodulatory and anti-inflammatory agent, particularly by lowering pro-inflammatory markers in individuals with baseline deficiency or high inflammation.

Keywords: Anti-inflammatory; Gastroenteritis; Pediatrics; Rotavirus; Vitamin D.



Introduction

Rotavirus gastroenteritis (RG) is a leading cause of severe gastroenteritis in newborns and young children (1,2). It has been demonstrated that rotaviral gastroenteritis accounts for about 40% of all outpatient visits to pediatric primary care practices for acute gastroenteritis in babies and young children (3).

Particularly in underdeveloped nations, diarrhea and its consequences continue to be a leading cause of morbidity and mortality in children. It ranks as the second most frequent cause of death for kids younger than five (4).

Compared with other viral infections that cause gastroenteritis, rotavirus causes more severe illness; children are more likely to present with fever, dehydration, and metabolic acidosis, and they are more likely to be admitted or readmitted to the hospital (5). In the absence of dehydration, physical examination findings for rotavirus infection are often normal (6).

As rotavirus gastroenteritis severity increased, children with acute gastroenteritis may have a low lymphocyte-to-monocyte ratio (LMR). In the differential diagnosis of infantile diarrhea, serum C-reactive protein (CRP) levels were highly significant (7).

There is no specific treatment for a rotavirus infection. Antibiotics and antivirals cannot be used to treat a rotavirus infection. The ailment often clears up in three to seven days, but staying hydrated is crucial (8).

Vitamin D (calciferol) is a fat-soluble vitamin that can be bought as a dietary supplement, added to other foods, and found naturally in some foods. Furthermore, the skin produces it naturally when ultraviolet (UV) rays from the sun initiate vitamin D synthesis (9).

The assessment of vitamin D deficiency entails determining serum levels of the 25-hydroxy form; levels between 20 and 30 ng/mL suggest insufficiency, while values below 20 ng/mL indicate deficiency (10).

Vitamin D is most likely safe when taken at the recommended dosages (11). Despite being uncommon, a vitamin D overdose can have serious consequences that impact both long-term renal function and the short-term vital prognosis. In developing nations, vitamin D poisoning is still a concern (12).

Serum 25(OH)D concentrations >30 ng/mL were observed as early as 1 day after 600,000 IU of D3 and 540,000 IU of D3; the largest increases in serum 25(OH)D consistently happened between days 1 and 30. Peak levels were detected at 3 and 7 days after dose (13).

Vitamin D supplementation appears to exert its anti-inflammatory effects through several interconnected mechanisms. One key pathway is the reduction of CRP, an acute-phase reactant that rises during systemic inflammation; studies consistently show that restoring or maintaining adequate vitamin D status is associated with lower CRP concentrations (14,15).

Vitamin D augments lymphocyte regulatory function by facilitating the development and activity of regulatory T cells, promoting regulated lymphocyte proliferation, enhancing the secretion of the anti-inflammatory cytokine IL-10, and diminishing lymphocyte apoptosis by inhibiting inflammatory and oxidative pathways. Collectively, these mechanisms sustain or enhance circulating lymphocyte numbers, thereby augmenting the lymphocyte-to-monocyte ratio (16).



This study aims to evaluate the anti-inflammatory effect of vitamin D in pediatric patients with rotavirus gastroenteritis.

Material and Methods:

Study design and setting:

A prospective, non-randomized clinical research study was conducted at Mohammed Baqir al-Hakim Hospital from December 2024 to May 2025.

Study population:

A total of 60 patients diagnosed with RG according to the inclusion criteria were enrolled in the study. Participants were selected from those attending the Mohammed Baqir al-Hakim Hospital. The current study included patients who were conveniently selected and had confirmed rotavirus infection. The study population was divided into 30 RG patients in group I and 30 RG patients in group II as follows:

Group I (control): received standard treatment, including rehydration and antipyretic therapy.

Group II (Treatment) group: received a single oral dose of vitamin D 100000 IU for age < 1 year and a dose of 300000IU for age > 1 year in addition to standard treatment. The study lasted 6 months, with follow-ups at 7-10 days (10,17).

Inclusion Criteria

The following were the study's inclusion criteria:

1. Children with confirmed rotavirus infection by rotavirus stool examination by the

rotavirus rapid test from CerTest BIOTEC company in Spain.

2. Serum vitamin D levels indicate deficiency (below 20 ng/mL).

Exclusion Criteria

The following were the study's exclusion criteria:

1. Children with other major infections, severe dehydration, or underlying health conditions.
2. Children with current vitamin D supplementation.

Outcome Measures:

1. Measuring lymphocyte to monocyte ratio (LMR) and C-reactive protein (CRP) at admission, after 4 days, and after 7 days in both groups. LMR was measured by calculating the percentage of blood cells from complete blood count (CBC) tests performed in the hospital using an automated hematology analyzer.

CRP was measured in the hospital using an agglutination test with a kit produced by Linear Chemicals S.L. (Barcelona, Spain) (16).

2. Measuring serum vitamin D at admission and after 7 days. Serum vitamin D was measured by electrochemiluminescence (ECL) using the Roche serum vitamin D Kit in Germany. Electrochemiluminescence (ECL) is a sophisticated immunoassay for serum 25-hydroxyvitamin D. Its excellent sensitivity, specificity, and dynamic range are enabled by electrochemical activation and light signal detection. When electrically stimulated, antibodies labeled with ruthenium bind vitamin D in the sample and emit light. Light intensity correlates with vitamin D levels, allowing



rapid, precise quantification. Due to their reliability, compatibility with automated systems, and low analytical variability, clinical laboratories use ECL tests (18-20).

Statistical Analysis:

Data was entered and analyzed using IBM SPSS Statistics (Version 24). Descriptive statistics were used to summarize participants' baseline characteristics. Continuous variables (e.g., lab data) were presented as mean ± standard deviation (SD). A normality test was performed on the data using the Shapiro-Wilk test. The paired t-test (for normally distributed variables) or the Wilcoxon signed-rank test (for non-normally distributed variables) was used to compare laboratory data on rotavirus infections within the same group. In contrast, the Mann-Whitney test (for non-normally distributed variables) was used to compare laboratory data of rotavirus infections between group I (control) and group II (treatment).

Spearman's correlation analysis was used to examine associations between changes in vitamin D levels and non-normally distributed laboratory parameters.

Results:

The study recruited 60 pediatric patients: 30 in group I (control) and 30 in group II (treatment). Group I includes 21 males (70%) and nine females (30%), and group II includes 22 males (73.3%) and 8 females (26.6%). The average age of the patients in group I was 4 (3.75–6) months. The average age of the patients of group II was 4 (3–6.25) months. There was no significant difference between the two groups regarding sex and age, as shown in Table 1.

Baseline laboratory parameters between group I (control) and group II (treatment) before intervention showed no statistically significant differences ($P > 0.05$), as shown in Table 2.

Table 1: Demographic characteristics of study participants.

Parameter		Group I	Group II	P value
Age (in months) Median (IQR)		4 (3.75–6)	4 (3–6.25)	0.946*
Gender n (%)	Male	21 (70%)	22 (73.3%)	0.082#
	Female	9 (30%)	8 (26.6%)	

*Statistical analysis was done using the Mann-Whitney test

Statistical analysis was done using the Chi-square test

Table 2: Baseline lab data of the patients.

Parameter	Group I	Group II	P value
CRP1 Median (IQR)	36 (24–42)	33 (24–36)	0.856*
LMR1 Median (IQR)	3.07 (2.49–3.51)	2.34 (2.10–3.69)	0.1*
VIT D1 Median (IQR)	15 (13.75–18)	16 (14–17)	0.709*

*Statistical analysis was done using the Mann-Whitney test



Table 3: Association between Vitamin D Level Change and the laboratory Change between day 1 and day 4

Parameter	Group I (Control)			Parameter	Group II (Treatment)		
	Day 1	Day4	P value		Day 1	Day4	P value
CRP (Mean±SD)	32.8±13.15	27.80±10.39	<0.001*	CRP Median (IQR)	33 (24–36)	12 (6–12)	<0.001#
LMR Median (IQR)	3.07 (2.49–3.51)	3.11 (2.56–3.61)	0.002#	LMR Median (IQR)	2.34 (2.10–3.69)	4.75 (4.30–5.12)	<0.001#

*Statistical analysis was done using the Paired T-test test

#Statistical analysis was done using the Wilcoxon signed rank test

In Table 3, the results showed an association between Vitamin D level change and the laboratory change, with statistical comparisons between group I (control) and group II (treatment) across lab parameters (CRP and LMR). Here is a structured interpretation:

These findings revealed that all parameters have statistically significant changes in both control and treatment groups ($P < 0.05$). In Group II, all parameters demonstrated highly significant changes, including CRP and LMR (all $p < 0.001$).

Table 4 shows statistical comparisons of various laboratory parameters between the control and treatment groups on days 1 and 7. Here is a summary interpretation: Results of CRP and LMR

findings showed statistically significant differences ($P < 0.001$) in both the control and treatment groups, suggesting changes over time in both groups. Vitamin D levels did not change significantly in the control group ($P = 0.161$). Significant increase in the treatment group ($P < 0.001$).

The correlation analysis revealed that greater increases in vitamin D levels were significantly associated with improvements in laboratory parameters. Specifically, moderate negative correlations were found between CRP levels. Positive correlations were seen with LMR, suggesting favorable hematological changes as shown in Table 5.

Table 4: The Change of Laboratory Data between the Two Groups between day 1 and day 7

Parameter	Group I (Control)			Parameter	Group II (Treatment)		
	Day 1	Day7	P value		Day 1	Day7	P value
CRP (Mean±SD)	32.8±13.15	10.4±7.37	<0.001*	CRP Median (IQR)	33 (24–36)	0 (0–6)	<0.001#
LMR Median (IQR)	3.07 (2.49–3.51)	3.26 (2.66–3.68)	<0.001#	LMR Median (IQR)	2.34 (2.10–3.69)	6.85 (6.15–7.53)	<0.001#
Vit D Median (IQR)	15(13.75–18)	15 (12.75–17)	0.161#	Vit D Median (IQR)	15.5±2.16	39 (33.75–45.25)	<0.001#

*Statistical analysis was done using the Paired T-test test

#Statistical analysis was done using the Wilcoxon signed rank test



Table 5: The correlation between laboratory variables and vitamin D level change

Variable	Correlation Coefficient	P-value
CRP	-0.453	<0.001*
LMR	0.741	<0.001*

*Statistical analysis was done using the Spearman correlation test

Discussion

This study found that Vitamin D significantly inhibits plasma CRP ($P < 0.001$), indicating that Vitamin D supplementation affects the body's inflammatory response. In this study, a single dose of vitamin D was found to significantly reduce plasma CRP levels in participants with rotavirus gastroenteritis, faster than in participants who did not receive Vitamin D. These findings agree with several studies worldwide (21-23). These findings agree with a study on the effect of vitamin D supplementation on circulating high-sensitivity C-reactive protein levels conducted in China, which found that vitamin D supplementation has a notably positive impact on reducing circulating high-sensitivity CRP (hs-CRP) levels ($p = 0.004$) (21). The current findings agree with another bidirectional Mendelian randomization study on the effect of Vitamin D deficiency and C-reactive protein conducted in the UK. That study found that raising 25-hydroxyvitamin D₃ levels in individuals with poor vitamin D status significantly reduced CRP levels ($P < 0.001$) (14).

Also, the current findings agree with a study on Vitamin D and C-Reactive Protein conducted in the Netherlands. The study concluded that there was a significant inverse relationship between blood vitamin D levels and CRP ($p = 0.027$) (22).

The current results align with a 2024 study in China exploring the impact of vitamin D

supplementation and C-reactive protein and systolic and diastolic blood pressure in postmenopausal women, which found that vitamin D supplementation significantly correlates with decreased CRP levels ($p < 0.001$) (23).

The current study's findings agree with a study from China, which found a significant inverse relationship between Vitamin D and C-Reactive Protein in newborns ($p < 0.001$). The study showed that neonates with low 25(OH)D levels or those born in winter or spring had cord blood CRP levels inversely linked to vitamin D levels (24).

This action of vitamin D on CRP occurs by increasing anti-inflammatory T regulatory cell activity to reduce excessive immune responses and by downregulating pro-inflammatory cytokines such as IL-6 and TNF- α that stimulate the liver's production of CRP (25).

The current findings do not agree with a 2014 study conducted in Iran that examined the impact of vitamin D supplementation on C-reactive protein levels in patients with non-alcoholic fatty liver disease. The results showed that, as compared to the placebo group, vitamin D supplementation did not affect CRP or other variables in the intervention group ($p = 0.2$) (26).

This may result from the dosage, duration, and initial vitamin D status of the subjects, which likely affected the response; a brief supplement.



Durations or near-normal baseline levels frequently do not yield significant biochemical alterations (27).

The current study found that LMR was significantly higher in group II receiving Vitamin D than in group I ($P < 0.001$). This agrees with a study conducted in Turkey on the relationship between vitamin D insufficiency and hemogram-derived inflammatory biomarkers in children, in which Vitamin D levels were significantly positively correlated with LMR (correlation coefficient = 0.218) ($P < 0.001$) (28).

Vitamin D augments lymphocyte regulatory function by facilitating the development and activity of regulatory T cells, promoting regulated lymphocyte proliferation, enhancing the secretion of the anti-inflammatory cytokine IL-10, and diminishing lymphocyte apoptosis by inhibiting inflammatory and oxidative pathways. Collectively, these mechanisms sustain or enhance circulating lymphocyte numbers, thereby augmenting the lymphocyte-to-monocyte ratio (15).

Limitations

Limitations of the present study include the relatively small sample size, constrained by the limited study duration, and the inability to reassess serum vitamin D levels on Day 4 to evaluate the short-term biochemical response.

Conclusion

The findings indicated that vitamin D acts as an immunomodulatory and anti-inflammatory agent, particularly by lowering pro-inflammatory markers in individuals with baseline deficiency or high inflammation.

Ethical Approval:

The proposal for the current study was submitted to the "College of Pharmacy, University of Baghdad," and approval was obtained from the "Scientific and Ethical Committee" under approval number (REC06202507R) on 5-1-2025. In addition, approval of the Ministry of Health was obtained according to approval number (120624) on 31-12-2024. At the same time, verbal consent was obtained from patients. This study was registered in the Clinical Library of Medicine in (clinicaltrials.gov) according to approval number (NCT07167797) on 2025-09-19, and informed consent was taken from the parents of all participants.

Author contributions

All authors contributed to the collection of data, the preparation of the draft, and the review and approval of the final version of the manuscript.

Conflict of Interest: None to declare

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