Effect of Rotavirus Infection on Serum Micronutrients and Atopy in Children

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Abstract

Objective Rotavirus is a highly infectious and prevalent ribonucleic acid (RNA) virus that causes fatal gastroenteritis in children. Despite vitamin D deficiency is associated with susceptibility to infections, the relationship between ferritin and vitamin B12 levels is not known. This study aimed to investigate and compare the effect of rotavirus on micronutrient levels, atopy, and the frequency of allergic diseases in children with rotavirus.

Methods There were rotavirus gastroenteritis (RVG) ($N = 92$) and non-rotavirus (control) groups ($N = 95$). Serum micronutrient levels (B12, ferritin, and 25-hydroxyvitamin D [25-OH-D3]) were checked during the first control after gastroenteritis healed. Patients were also examined for allergic diseases on an average of 17 (14–32) months following rotavirus infection. Serum immunoglobulin E (IgE), eosinophil count, and percentage were analyzed. Skin tests and respiratory function tests were also performed on patients with allergic disease and asthma symptoms.

Results Mean ferritin, B12, and 25-OH-D3 levels were lower in the RVG group compared with the control group. Allergic diseases in the RVG group were more frequent than in the control group. The prevalence of the allergic disease in the RVG group was 16.3%, as opposed to 5.2% in the control group ($p = 0.014$). The IgE level was significantly higher in the RVG group.

Conclusion Children with rotavirus infection should be followed closely in terms of allergic diseases and micronutrient deficiency. Furthermore, rotavirus infection should be prevented in the society and early treatment should be made available via tests detecting micronutrient deficiency.

Keywords
- ferritin
- gastroenteritis
- sensitivity
- rotavirus

Introduction

Rotavirus is a highly infectious and prevalent double-stranded ribonucleic acid (RNA) virus that causes gastroenteritis in children and approximately 30% of hospitalizations related to diarrhea.[1] Exposure to rotavirus in children aged <5 years causes initial symptoms of fever and vomiting within a few days, followed by non-bloody diarrhea.[2] In comparison to

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these severe symptoms observed in children, healthy adults are generally resistant to rotavirus, having much milder symptoms or none.[3] Although rotavirus vaccination has been available since 2006, gastroenteritis caused by rotavirus still results in the death of approximately 200,000 children annually, most in developing countries.[4]

The action mechanism of rotavirus gastroenteritis (RVG) is primarily through the interaction between rotavirus and columnar epithelial tissue. The resulting diarrhea leads to malabsorption, and subsequently dehydration, electrolyte, and pH imbalance.[5] Cellular atrophy and ischemia of the villus disrupt filtration of micronutrients through villi and stimulate the enteric nervous system.[6,7]

The relationship between infectious diseases including rotavirus infection and 25-hydroxyvitamin D3 (25-OH-D3) has been reported in numerous studies. A meta-analysis has indicated that 25-OH-D3 might exhibit antibiotic activity and its deficiency is associated with increased infection rates.[8,9] In addition, a low 25-OH-D3 level has been linked to an increase in the incidence of rotavirus infection.[10] However, to date, no study has yet investigated the relationship between rotavirus infection, vitamin B12, and ferritin levels. Moreover, it has been proposed that RNA virus infection before the age of five is strongly associated with future development and sensitivity to atopic diseases and the development of eosinophilia.[11–13] The present study aims to determine the effects of RVG on B12, ferritin, and 25-OH-D3 levels, as well as the immunoglobulin E (IgE) level, peripheral eosinophilia count as a marker of atopy.

Materials and Methods

The study protocol was approved by the Taksim Training and Research Hospital Ethics Committee for Clinical Research on June 26, 2019 (registration no. 85). Moreover, all participants provided informed consent in the format required by the relevant authorities and/or boards.

This study was performed with a retrospective analysis of patient records in Gaziosmanpaşa Training and Research Hospital, Istanbul. The sociodemographic, clinical, and laboratory data of children who had rotavirus and were hospitalized (RVG) were recorded. Patients hospitalized due to RVG from the age of 6 months to 6 years between 2011 and 2018 were included in the study. An independent pediatrician has reviewed the information in patient files retrospectively.

The control group (non-rotavirus) with a resembling age and gender profile was created from a sample of children who did not have chronic diseases. The children in the control group displayed normal growth. Patients with chronic diseases such as protein-energy malnutrition, type I diabetes mellitus, nephrotic syndrome, celiac disease, and short bowel syndrome were excluded from the study. Furthermore, patients who were born prematurely, not breastfed during the first 6 months following birth, or those cases with high (>4,000 g) or low (<2,500 g) birth weight were also excluded from the study. Furthermore, patients who had received the rotavirus vaccine were excluded from the study.

Ferritin, B12, and 25-OH-D3 levels of the patients who were hospitalized due to RVG were measured between 2 weeks and 1 month after recovery from gastroenteritis. Moreover, patients were also examined for allergic diseases on an average of 17 (14–32) months following rotavirus infection. Serum IgE, eosinophil count, and percentage were analyzed. Patients with atopy symptoms (such as atopic dermatitis, eczema, urticaria, asthma, food allergy, and allergic rhinitis) were examined with prick and patch tests. Thus, non-IgE-mediated allergies were investigated. Pulmonary function tests were also performed in cases with asthma symptoms. Since some allergy types (such as milk allergy) disappear with age or allergy findings become more visible through time, only final data from the latest examination were included in the study. Serum ferritin, B12, 25-OH-D3 levels were compared in the two groups with and without the presence of patients with allergic diseases. Likewise, while comparing serum IgE level, eosinophil count, and percentage, a comparison was made with and without the presence of patients with allergic diseases. Thus, it was aimed to investigate the atopy potential of patients who had not yet been diagnosed with allergic diseases. The reference ranges for the micronutrients were as follows: ferritin: 20 to 200 ng/mL; B12: 126.5 to 505 pg/mL; and 25-OH-D3: 20 to 100 ng/mL. An eosinophil count >400 µL and a percentage of >4% were considered abnormal and indicative of allergic disease. Blood analysis results were compared between the RVG and the control groups.

Statistical analysis was performed using International Business Machines (IBM) Statistical Package for the Social Sciences (SPSS) Statistics for Windows v.20.0 (IBM Corp., Armonk, New York, United States). The Shapiro-Wilk test with histogram analysis was used to test the normality of the dataset. The Mann-Whitney U test was used with nonparametric datasets to identify differences between the RVG and control groups. Pearson's Chi-square test was used to compare the prevalence of allergic diseases in the RVG and control groups. The level of statistical significance was set at $p < 0.05$.

Results

The RVG group included 92 patients (49.2%) that were hospitalized and followed-up due to RVG. The control group consisted of 95 volunteers (50.8%) who applied to the outclinic for a routine check-up. The study population comprised 103 males (55.1%) and 84 females (44.9%) with a mean age of 50 ± 17 (19–91) months. There was no difference between the groups in terms of age and gender (►**Table 1**).

When comparing laboratory values between groups, the findings show that the mean ferritin, B12, and 25-OH-D3 levels in the RVG group were significantly lower than the control group (►**Fig. 1**). Similarly, the ferritin, B12, and 25-OH-D3 levels were significantly lower in the RVG patients without an allergic disease than the control participants without an allergic disease (►**Fig. 1**).

The number of participants with ferritin and 25-OH-D3 deficiency was significantly higher in the RVG group than in

Table 1 Clinical and demographic qualifications

	RVG-positive Mean ± SD ($n = 92$)	Control Mean ± SD ($n = 95$)	*p*-Value
Number (*n*)	92 (49.2%)	95 (50.8%)	
Age (month)	47.4 ± 17.4	51.8 ± 17.2	0.093
Gender			
Girl	41 (44.6%)	43 (45.3%)	0.924
Boy	51 (55.4%)	52 (54.7%)	
Allergic diseases[a]			
Milk allergy[c]	2	–	
Egg allergy	1	1	
Mite allergy	6	3	
Pollen allergy	2	1	
Atopic dermatitis	–	–	<0.001[b]
Asthma	4	-	
Allergic rhinitis	–	–	

Abbreviations: min–max, minimum–maximum; RVG, rotavirus gastroenteritis; SD, standard deviation.
[a]Diseased patient number.
[b]Mite allergy and asthma disease prevalence.
[c]Patch test was applied in the detection of milk and egg allergy whereas prick test was used in locating mite and pollen allergy.

the control group after excluding those with an allergic disease.

Patients were examined for allergic disease and atopy, approximately 17 (14–32) months after rotavirus infection.

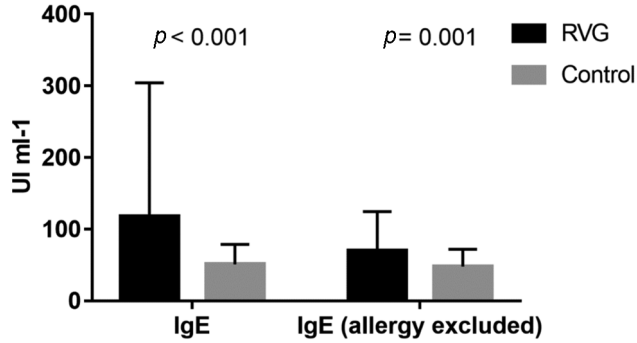

Fig. 2 Comparative analysis of mean laboratory findings in the RVG group and control group, both including and excluding patients with allergic disease for IgE concentrations.

Among the 92 patients in the RVG group, 15 (16.3%) had RVG and allergic disease. Among the 95 control group participants, 5 (5.2%) did have an allergic disease. Furthermore, the IgE level was significantly higher in the RVG group (►**Fig. 2**). In addition, the IgE level was significantly higher in the RVG patients without an allergic disease than in the control group subjects who did not have an allergic disease (►**Fig. 2**). The frequency of asthma and mite allergy in the RVG group was significantly higher compared with the control group (►**Table 1**). Moreover, the eosinophil count and percentage were higher, but not significantly, in the RVG group than in the control group after excluding the participants with allergic disease (►**Table 2**; ►**Fig. 3**). Finally, the prevalence of the allergic disease in the RVG group was 16.3% as opposed to 5.2% in the control group ($p = 0.014$).

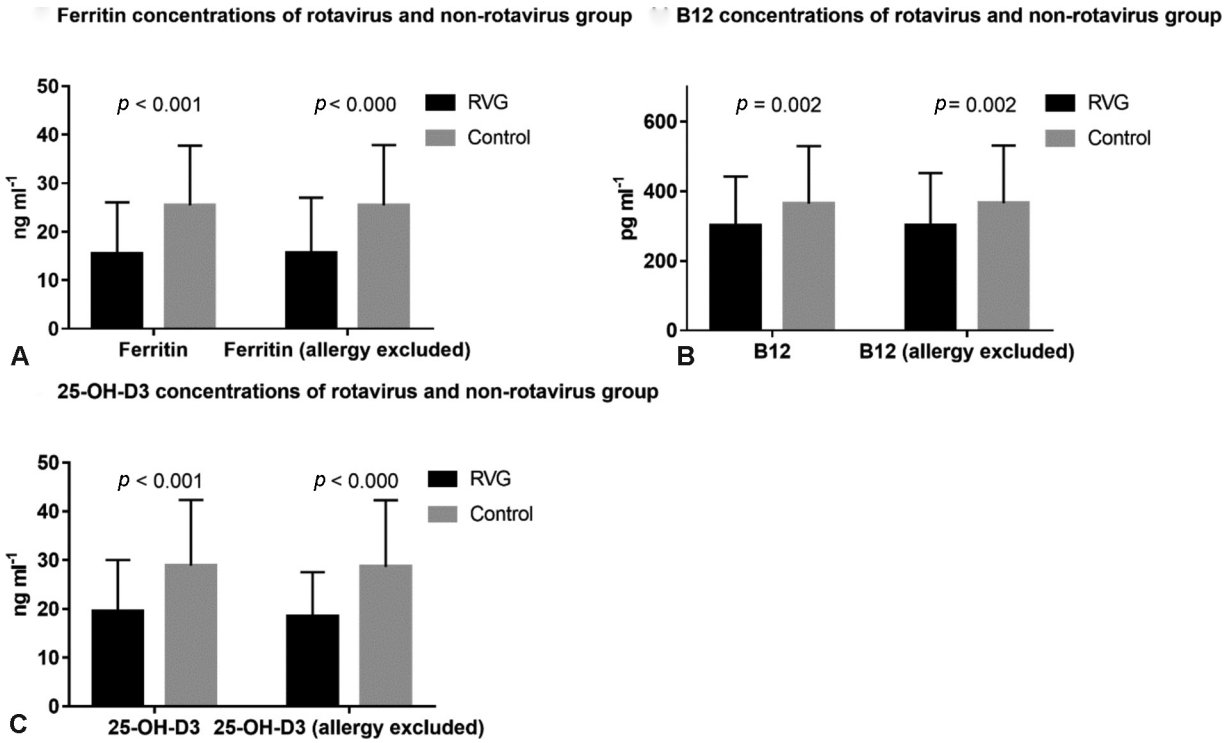

Fig. 1 Comparative analysis of mean laboratory findings in the RVG group and control group, both including and excluding patients with allergic disease for micronutrients. (**A**) Ferritin concentrations; (**B**) B12 concentrations; (**C**) 25-OH- D3 concentrations.

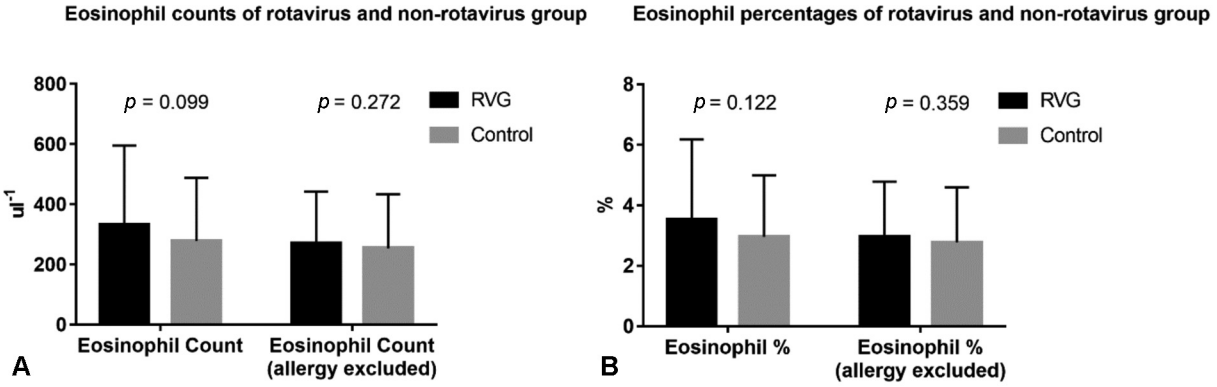

Fig. 3 Comparative analysis of mean laboratory findings in the RVG group and control group, both including and excluding patients with allergic disease for eosinophil variables. (**A**) Eosinophil counts; (**B**) eosinophil percentages.

Table 2 Laboratory examination results of the groups

Blood test qualifications, (normal values)	RVG Mean ± SD ($n = 92$)	Control Mean ± SD ($n = 95$)	*p*-Value	RVG (allergy excluded) Mean ± SD ($n = 77$)	Control (allergy excluded) Mean ± SD ($n = 90$)	*p*-Value
Hb (11–18 g/L)	13 ± 1	13 ± 0.9	0.716	13 ± 1	13 ± 0.9	0.933
Eosinophil count (<400/μL)	332.7 ± 262.1	277.9 ± 209.9	0.099	271.1 ± 170.4	253.4 ± 179.3	0.272
Eosinophil % (<4%)	3.52 ± 2.6	2.9 ± 2.03	0.122	2.96 ± 1.8	2.8 ± 1.8	0.359
Ferritin (20–200 ng/mL)	15.5 ± 10.6	25.4 ± 12.3	<0.001	15.6 ± 11.4	25.4 ± 12.4	<0.001
B12 vitamin (126.5–505 pg/mL)	301.9 ± 141.2	364.05 ± 165.2	0.002	301.9 ± 150.6	365.5 ± 165.5	0.002
25-OH- D3 (20–100 ng/mL)	19.5 ± 10.5	28.8 ± 13.5	<0.001	18.5 ± 9.03	28.6 ± 13.6	<0.001
IgE (0–52 kIU/L)	117.7 ± 186.4	50.9 ± 27.8	<0.001	69.64 ± 54.8	47.7 ± 24.1	0.001

Abbreviations: *n*, number of patients; RVG, rotavirus gastroenteritis; SD, standard deviation.

Discussion

Rotavirus infection is a major cause of mortality and morbidity in children in less developed and developing countries. Viral infections are a serious public health problem that has the potential to cause pandemics. Coronavirus disease 2019 (COVID-19) pandemic, which began at the end of 2019, has affected the health system in many countries worldwide. Other accompanying outbreaks (such as Marburg virus, Rift Valley Fever Virus) in some underdeveloped regions such as in Africa have substantially deteriorated the situation and increased the burden on the health system in these regions. Consequently, diagnosis and treatment of these viruses have proven more difficult during the COVID-19 outbreak.[14,15] Morbidity and mortality have risen more rapidly due to these viruses other than COVID-19. While there have been approximately 88,000 reported deaths due to COVID-19 infection particularly in Africa, 200,000 deaths per year occur due to viral hepatitis in the continent.[16] Most viral infections that result in pandemics have no specific treatment. It is essential that the health system is well coordinated during the process of diagnosis, follow-up, and treatment. In addition to early diagnosis, treatment, isolation, and infection control, it is also necessary to provide public hygiene. The low vaccination rates in developing countries also complicate the infection control. Rotavirus is a major viral infection that can be prevented by vaccination. A study conducted in India indicated that rotavirus-related gastroenteritis and deaths decreased with rotavirus vaccination.[17] In Turkey, rotavirus vaccine is not a part of the national vaccination program. The widespread use of the vaccine can reduce mortality rates and prevent morbidity due to rotavirus and other viruses.

The 25-OH-D3 concentration has been well studied in patients with various diseases. It is known that 25-OH-D3 deficiency is associated with infectious diseases and other conditions such as polycystic ovary syndrome, cardiovascular disease, and type II diabetes mellitus.[18–20] In addition, 25-OH-D3 is known to be an immune-modulator.[21] Furthermore, 25-OH-D3 is used as a preventative intervention against infectious diseases.[22] The literature is limited with respect to serum vitamin D levels in children with rotavirus diarrhea. Thornton et al concluded that school-age children (mean age: 8.9 years) with low 25(OH)D3 levels had diarrhea more frequently.[23] The vitamin D level was found to be significantly lower in children who had rotavirus in comparison to healthy children in our study. This may be the result of

a deficiency of the immunomodulating effect of vitamin D and its protective effect against infections. Despite the numerous studies focused on the relationship between 25-OH-D3 deficiency and infectious diseases, there remains a lack of knowledge about the relationship between infectious diseases and micronutrients such as ferritin and B12. Similar to 25-OH-D3 deficiency in children with RVG, the overall decrease in ferritin and B12 levels may be due to rotavirus-based cellular atrophy and ischemia of the villus, which disrupts the pH of the environment necessary for proper villus absorption. In our study, ferritin and B12 levels were significantly lower in children with RVG compared with healthy children. A similar study on this subject has not been identified in the literature.

Eosinophilia is associated with atopic diseases such as atopic dermatitis.[24] RNA virus infections such as rotavirus infection predispose patients to atopic diseases and exacerbation of such diseases. As such, it was expected that the eosinophil count and percentage in the present study's RVG group would be higher than in the control group due to rotavirus infection conferring sensitivity to allergic reactions; however, the difference between the two groups was not significant. This situation can be explained by the presence of non-IgE allergies. Patients with atopy symptoms were therefore also given prick and patch testing. The patients presented early period atopy findings, as well. In the long-term follow-up, the severity of clinical findings and its reflection on laboratory parameters could be observed. In this study, the mean eosinophil count and percentage, and the mean IgE level, decreased in both groups after patients with an allergic disease, were excluded. Overall, however, the IgE level in the RVG group was significantly higher than in the control group. In our study, the frequency of asthma and mite allergy was significantly higher in children who had a rotavirus infection, and in general, the frequency of all allergic diseases was statistically higher in children who had rotavirus infection. Genetic factors, gender, and past infections play a role in the etiology of allergic diseases. In our study, the frequency of allergic diseases and asthma frequency in children who had rotavirus was significantly higher than the control group.

Due to the small number of participants with allergic disease in the present study (15 in the RVG group and five in the control group), it was not possible to compare the effects of allergic diseases on the study parameters effectively. The literature on the relationship between rotavirus and atopy in children is limited. One of the most important limitations of this study is its retrospective design. Prospective and large-scale studies are necessary to strengthen the accuracy of the results obtained.

Conclusion

25-OH-D3, ferritin, and B12 levels were lower in subjects who had rotavirus infection. Moreover, the frequency of allergic diseases and asthma was more common in those who had rotavirus infection. Rotavirus infection and micronutrient deficiency are significant components of a vicious circle. To prevent rotavirus, vaccination as well as treatment for early malnutrition and micronutrient deficiencies is essential.

Authors' Contributions
M.K.B., C.D., and F.O. contributed to the conception and design of this study. M.K.B. and C.D. collected the data. A.S. and F.O. contributed to analysis and interpretation of data. M.K.B., A.S., and F.O. performed the statistical analysis and drafted the manuscript and figures. M.K.B., A.S., C.D., and F.O. critically reviewed the manuscript and supervised the whole study process. All authors read and approved the final manuscript. All authors agree to be accountable for all aspects of work ensuring integrity and accuracy.

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Conflict of Interest
None declared.

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