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Original Article

The vitamin D status and serum eosinophilic cationic protein levels in infants with cow's milk protein allergy

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Abstract: Eosinophil cationic protein (ECP) is a cytotoxic protein released from eosinophils. The level of ECP increases in some allergic diseases. Recently, vitamin D deficiency has been suggested to be a risk factor for childhood allergic disease. The first aim of the study is to measure the serum vitamin D levels and ECP in infants with cow's milk protein allergy (CMPA) and compare them with controls. The second aim of this study is to investigate whether vitamin D levels are correlated with ECP or not. Sixty-two infants with CMPA were compared to 58 healthy, similar to distribution of age and sex normal infants as controls. The serum ECP levels were detected by an immunoassay system. Serum 25(OH)D levels were measured by using an enzyme-linked immunoassay kit. Vitamin D deficiency was defined as a serum 25(OH)D level of < 10 ng/mL and sufficient 30 ng/mL. The median serum ECP level in the CMPA group was significantly higher than in the control group (51.45 and 17.55 ng/mL, respectively, P = 0.001). There were no significant differences between groups with regards to median 25(OH)D levels (29.31 ± 1.67 and 27.32 ± 1.41 ng/mL, respectively, P = 0.646). The serum 25(OH)D levels were under 30 ng/mL in 38 of infants with CMPA (61.2%) and in 32 of controls (55.1%). Correlation analysis between the serum 25(OH)D level and ECP of infants with CMPA have revealed no significant relation (P = 0.888). Our results do not support the hypothesis that vitamin D deficiency may be a risk factor for CMPA.

Keywords: Cow's milk protein allergy, eosinophil cationic protein, vitamin D, infant, eosinophils

Introduction

Cow's milk protein allergy (CMPA) is an inflammatory process that is the most common food allergy seen in infants [1-3]. Studies reported that its prevalence ranges from 0.5% to 3% at age 1 year [4-6]. CMPA has three different immune mechanisms: IgE-mediated (immediate type) reactions, non-IgE-mediated (cellular, type 4) reactions and mixed-type reactions. Due to the wide range of clinical signs and symptoms, detailed history, careful physical examination, diagnostic elimination diet and use of biochemical tests (such as skin prick test, specific IgE), though not definitive, may help diagnose CMPA [7].

Eosinophilic cationic protein (ECP) is one of the four major basic proteins in specific granules in the cytoplasm of eosinophils, and elevated levels in body fluids such as saliva, serum and faeces in the course of inflammatory process and allergic diseases. Thus, ECP reflecting eosinophil activity is considered as a non-invasive indicator for diagnosing and monitoring of CMPA, allergic asthma, eczema and atopic dermatitis [8, 9].

Vitamin D plays a crucial role in the regulation of calcium and phosphorus metabolism, which is required for the healthy development of the skeletal system. There are two major forms of vitamin D, which is also known as a hormone in fat-soluble steroid structure: vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol) [10]. Vitamin D mediates its biological activity through the vitamin D receptor (VDR) found in many tissues and organs [11]. Vitamin D has also an effect on cytokine release in cells that are involved in the immune system and causes a decrease in the proliferation of lymphocytes by decreasing IL-2 activity [12]. For the role of vitamin D in food allergy, it has been hypothesised vitamin D deficiency decreases the intes-

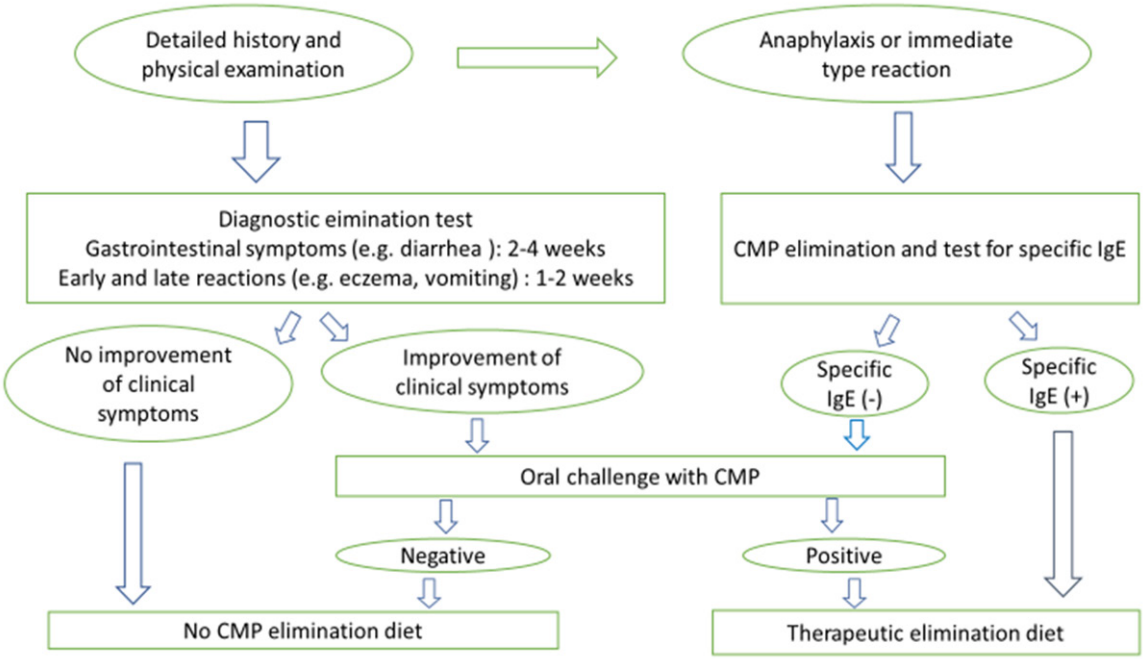

Figure 1. Algorithm for the diagnosis of CMPA. CMPA: Cow's milk protein allergy, CMP: Cow's milk protein.

tinal barrier integrity which allows low doses of food protein to infiltrate the immune system, leading to increased IgE and abnormal Th2 immune responses [13].

The limited number of studies has been conducted measuring vitamin D levels in allergic children. Most of these studies have focused on atopic dermatitis and food allergy [14-16]. In this study we aimed to investigate the serum ECP (sECP) and vitamin D levels in infants with and without CMPA and to determine for potential correlations between the sECP and vitamin D levels. To our knowledge, this is the first study in infant with CMPA to simultaneously evaluate both the sECP and their possible correlations to vitamin D level.

Materials and methods

This study was carried out at the Department of Pediatric Gastroenterology of Karabuk University Medical Faculty in Karabuk, Turkey, from January 2019 to December 2019. Sixty-two infants who were newly diagnosed with CMPA aged 2-11 months and 62 healthy infants (similar to distribution of age and sex) were enrolled in the study. 4 healthy infants were withdrawn from the study due to the request of their parents. A total of 120 infants were included in this study. The CMPA diagnosis was conducted according to the European Society for Pediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) Guideline: Diagnosis and Management of CMPA. If CMPA is suspected by medical history and physical examination, elimination of milk and dairy products is initiated. In the majority of cases with suspected CMPA, the definitive diagnosis requires to be confirmed or excluded by an oral food challenge (OFC) test, skin prick tests (SPT) and specific IgE measurements (**Figure 1**) (ESPGHAN) [17].

The sensitivity of CMPA in all cases was investigated with cow's milk-specific IgE (for parameter a value of ≥ 0.35 kU/L was accepted as positive; Dr Fooke-Achterrath Laboratorien GmbH Habicktweg 16, D41468 Neuss, Germany) and given a therapeutic CMP elimination diet. Then, the infants in the study group were distributed in two groups as infants with a positive (n = 9) and negative (n = 53) test for cow's milk specific IgE. SPT was conducted with cow's milk SPT solution (Allergopharma D-21462, Reinberk, Germany) in CMP IgE-negative infants with suspected CMPA. In order to confirm the diagnosis of CMPA, OFC testing was carried out. One drop of the cow's milk-based infant formula was dropped onto the perioral region and lips of the infants to observe for allergic reactions. The dose gradually was increased to 100 ml and administered at regular intervals

of 15-30 min. The OFC was classified as 'positive' or 'negative'. Infants with a diagnosis of multiple food allergies were excluded from the study.

Blood samples were collected for measuring ECP with vitamin D levels and stored frozen at -80°C. The ECP levels were tested by a chemiluminescence method using an Immulite 2000 XPi analyzer Immunoassay System (Germany). Serum vitamin D levels were measured by using a enzyme-linked immunoassay kit (Simens ADVIA Centaur XP). Vitamin D deficiency was defined as a serum 25(OH)D level of < 10 ng/mL, insufficiency as a 25(OH)D level between 11 and 29 ng/mL and sufficient ≥ 30 ng/mL.

The data were analyzed with SPSS version 21.0 software for Windows. Results are expressed as mean (SD) or median (range). The Kolmogorov-Smirnov test was used to determine the normality of data distribution. Values of age, ECP, absolute eosinophil count, cow's milk-specific IgE, alkaline phosphatase, duration of vitamin D supplement had abnormal data distribution, by Kolmogorov-Smirnov test, therefore, median values (interquartile range) between groups were determined and compared using Mann-Whitney U test. D vitamin, white blood cell count, calcium, phosphorus values were compared with independent t-test because of normal data distribution between groups. Correlation analyses were evaluated with Spearman's correlation test. A *P* value of less than 0.05 was considered to be statistically significant. The study was approved by the Ethics Committee for Non-invasive Clinical Research at Karabuk University. Subjects' parents provided written informed consent.

Results

The median age of 62 infants with CMPA (39 males, 63%) and 58 controls (32 males, 55%) were 4 (2-11) months and 4.5 (2-12) months, respectively. There were no statistically significance differences between the two groups with respect to age, gender, calcium, phosphorus, alkaline phosphatase, white blood cell counts, initiation time of vitamin D supplement and type of feeding (P > 0.05). The median sECP level (51.45 ng/mL) and eosinophil count (475/mm^3) in the CMPA group was significantly higher than that in the control group (17.55 ng/mL and 300/mm^3), (P < 0.001 and P < 0.001, respectively). The infants in the CMPA group were distributed in two subgroups as infants with a positive (n = 9, 14%) and negative (n = 53, 86%) test for cow's milk specific IgE. Skin prick test with milk antigen was also positive in 20.9% of infants with CMPA. Thirty-seven infants (59.7%) in CMPA group had eczema. This was followed by rectal bleeding (n = 20, 32.2%), vomiting (n = 8, 12.9%), urticaria (n = 4, 6.4%), wheezing (n = 4, 6.4%) and anaphylaxis (n = 1, 1.6%) (**Table 1**).

As for levels of the vitamin D, there were no statistically significant differences between the two groups with respect to 25(OH)D levels (29.31 ± 1.67 and 27.32 ± 1.41 ng/mL, respectively, P = 0.646). The infants were categorised into for 3 groups according to serum 25(OH)D level: ≤ 10 ng/mL, 11-29 ng/mL and ≥ 30 ng/mL. The serum 25(OH)D level was under 30 ng/mL in 38 of infants with CMPA (61.2%) and in 32 of controls (55.1%). None of infants had serum 25(OH)D level: ≤ 10 ng/mL (**Table 1**).

Correlation analysis between the serum 25(OH)D level and sECP of children with CMPA have revealed no significant relation (P = 0.888) (**Table 2**). In terms of the median sECP levels; no significant two-subgroups (infants with CMPA had 25(OH)D levels 11-29 ng/mL and ≥ 30 ng/mL) differences were observed (50.23 and 52.67 ng/mL, respectively, P = 0.256) (**Figure 2**).

Discussion

In the present study, sECP and blood eosinophil count of infants with CMPA were statistically higher than the control group. However, there was no significant difference between the two groups in terms of serum vitamin D levels. Moreover, no correlation was found between sECP and vitamin D level in infants with CMPA.

Eosinophils, which are formed in the bone marrow, have vitamin D receptor and large cytoplasmic granules containing proteins such as ECP, eosinophil protein X, eosinophil-derived neurotoxin and major basic protein. ECP encoded by the RNASE3 gene is a cytotoxic protein that enter the surrounding tissues when activated eosinophils degranulate [18]. Therefore, the numbers of circulating eosinophils and ECP level may increase in response to Th2 driven parasitic and allergic diseases such as cow's milk protein allergy, asthma and inflammatory diseases. Thus, measurement of ECP is used

Table 1. Comparison of socio-demographic and laboratory characteristics of the CMPA and control groups

	CMPA group (n = 62)	Control group (n = 58)	*P* value
Median age, months	4 (2-11)	4.5 (2-12)	> 0.899
Males, n (%)	39 (63)	32 (55)	> 0.05
Median sECP, ng/mL	51.45 (20.7-220)	17.55 (1.67-38)	0.001[a]
Median white blood cell count, $\times 10^3/\mu L$	10.4 (4.55-21.3)	9.77 (6.14-14.64)	0.470[a]
Median eosinophil count, $/mm^3$	475 (110-1720)	300 (60-990)	0.001[a]
Skin prick test (milk antigen)			
Positive n (%)	13 (20.9)	-	
Negative n (%)	49 (79.1)	-	
Specific IgE test			
Positive n (%)	9 (14)	-	
Negative n (%)	53 (86)	-	
Median Cow's milk- specific IgE (kU/L)	0.98 (0.41-3.43)	-	
25(OH)D levels ng/mL	29.31 ± 1.67	27.32 ± 1.41	0.646[b]
≤ 10 n (%)	-	-	
11-29 n (%)	38 (61.2)	32 (55.1)	
≥ 30 n (%)	24 (39.8)	26 (44.9)	
Serum calcium (mg/dL)	10.37 ± 0.41	10.01 ± 0.89	> 0.05[b]
Serum phosphorus mg/dL	5.37 ± 0.51	5.23 ± 0.90	> 0.05[b]
Serum alkaline phosphatase U/L	230 (102-432)	219 (118-388)	> 0.05[a]
Duration of Vitamin D supplement (months)	3 (1-10)	3.6 (1-11)	> 0.05[a]
Type of feeding n (%)			> 0.05
Breastfeeding	31 (50)	35 (60.3)	
Formula and breastfeeding	19 (31.7)	16 (27.6)	
Formula and complementary Foods	12 (19.3)	7 (12.8)	
Breastfeeding time (month)	3.9 (1-11)	4.4 (1-12)	
Clinical presentation n (%)			
Eczema	37 (59.7)	-	
Rectal bleeding	20 (32.2)	-	
Vomiting	8 (12.9)	-	
Urticaria	4 (6.4)	-	
Wheezing	4 (6.4)	-	
Anaphylaxis	1 (1.6)	-	

[a]Mann-Whitney U test, [b]Independent sample t-test, CMPA: Cow's milk protein allergy, sECP: serum Eosinophilic Cationic Protein.

Table 2. Correlation of vitamin D with sECP and eosinophil count

Parameters	r Value[a]	*P* value
sECP	0.018	0.888
Eosinophil, mm^3	0.140	0.279

[a]Spearman's rank correlation, sECP: serum Eosinophilic Cationic Protein.

widely as a marker in acute exacerbation and disease activity in allergic and inflammatory diseases [16, 19].

Although there are many researches evaluating the ECP level in allergic and parasitic diseases, a limited number of studies have been conducted evaluating ECP levels in infants with CMPA [20, 21]. Suomalainen et al. conducted a study in children with CMPA, they reported sECP levels and Circulating blood eosinophil counts were significantly higher after an oral cow's milk challenge [22]. In a similar study by Hidvégi E et al. found considerably higher sECP in children with CMPA compared to control group (12.4 μg/L and 4.3 μg/L, respective-

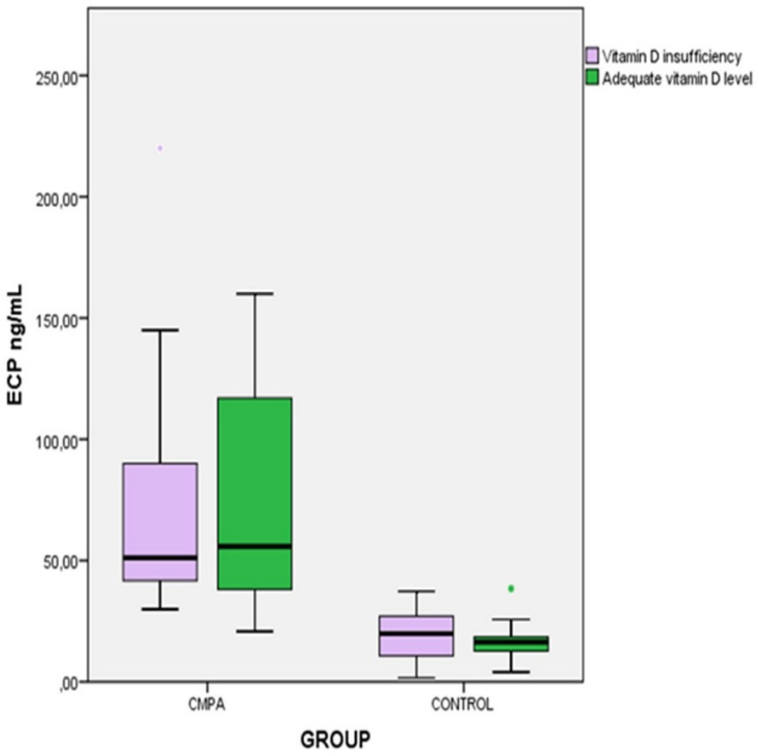

Figure 2. Levels of ECP for subgroups according to serum 25(OH)D level. ECP: Eosinophilic Cationic Protein, CMPA: Cow's milk protein allergy.

ly) [23]. Recently, Li, Jingwen et al. noted that in 6 months old infants with CMPA, blood eosinophil counts were higher than that of the controls (0.89 ± 0.45/mm^3 and 0.26 ± 0.12/mm^3, respectively, $P < 0.01$) [16]. Consistent with previous studies, we found a significantly the higher sECP level (51.45 ng/mL) and blood eosinophil counts (475/mm^3) in infants with CMPA compared to controls (17.55 ng/mL, 300/mm^3) ($P < 0.001$ and $P < 0.001$, respectively). All the above studies including ours suggest hypothesis that an increment of the numbers of circulating eosinophils and ECP levels can be seen in infants with CMPA.

It is well known that the impairment of Th1 and Th2 balance, which affects the model of the immune response, causes allergic diseases such as CMPA, asthma and atopic dermatitis [24]. Vitamin D, especially the 1.25(OH)D2 form, plays an important role in the regulation of the immune system by inhibiting both Th1- and Th2-type responses through the suppression of IL-12 production and IL-4-induced expression of IL-13. In addition, vitamin D creates an immunomodulatory effect on allergen-induced inflammatory pathways by acting on the vitamin D receptors expressed in eosinophils, B cells, T cells, dendritic cells and macrophages [25-28].

Recently, conflicting results have been reported that vitamin D deficiency may adversely affect the immune system and cause allergic diseases. Perezabad et al. conducted a study in 15 infants with CMPA they reported that vitamin D levels of infants with CMPA were significantly lower than that of controls (35.3 ± 3.5 ng/mL, 47.9 ± 3.7 ng/mL, respectively) [29]. Consistently, Silvia Cristiane M et al. noted that in infants with CMPA not taken vitamin D supplement, vitamin D levels were lower compared to the healthy controls (respectively) [30]. On the other hand, Li, Jingwen et al. reported that there was no statistical difference between infants with CMPA and their controls in terms of mean serum vitamin D level (68.3 ± 38.9 and 59.4 ± 38.1 nmol/L, respectively) and also found that 71% of infants with CMPA and 66% of controls had deficient and/or insufficient serum vitamin D levels [16]. Likewise, in a study evaluating the vitamin D status in 56 infants with CMPA, Ercan et al. reported there was no significant difference in mean vitamin D levels between infants with CMPA and the controls (33.85 ± 16.18 ng/mL vs., 30.70 ± 14.90 ng/mL, respectively). In addition, they found 41.1% of infants with CMPA and 60% of the controls had blood vitamin D levels below 30 ng/mL [31]. Consistent with the studies of Li, Jingwen and Ercan et al., we found that there was no significant difference in vitamin D levels between infants with CMPA and the controls (29.31 ± 1.67 ng/mL, 27.32 ± 1.41 ng/mL, respectively, $P = 0.646$). However, our findings are not compatible with those of Perezabad and Silva et al. These differences may be due to the fact that the study of Perezabad et al. was conducted on a small number of subjects ($n = 15$) and the study group Silvia Cristiane M et al. consisted of children who did not receive vitamin D supplements.

Different serum 25(OH)D reference ranges are used for the definition of vitamin D deficiency. In a study by Silvia et al., they defined 25(OH)D levels below 20 ng/mL as deficient. On the other hand, vitamin D deficiency for children was defined as a serum 25(OH)D level of < 12 ng/mL by Munns CF et al. [32]. However, ESPGHAN recommended to practice using 25(OH)D < 10 ng/mL as severe deficiency [33]. In this study, Vitamin D deficiency was defined as a serum 25(OH)D level of < 10 ng/mL, insufficiency as a 25(OH)D level between 11 and 29 ng/mL and sufficient ≥ 30 ng/mL. According to these reference ranges, 61.2% of infants with CMPA and 55.1% of the controls had lower than normal serum D vitamin level (< 30 ng/mL). In addition, Vitamin D deficiency (< 10 ng/mL) was not detected in none of the infants in the present study. We think that the lack of statistically significant difference between the groups may likely be due to fact that daily 400 IU vitamin D supplementation applied by the Turkish Ministry of Health to all infants until the age of 1, regardless of seasonal changes, type of nutrition and the sunlight exposure [34].

To date, no study has evaluated the relationship between serum vitamin D level and sECP in infants with CMPA. In the present study, no correlation was found between serum vitamin D level and sECP (P = 0.888). In addition, in terms of the median sECP levels; there was no statistically significant difference between the subgroups with 25(OH)D levels of 11-29 ng/mL and ≥ 30 ng/mL (P = 0.256). Considering that ECP levels can easily increase in allergic and inflammatory diseases, these results do not support the view that vitamin D deficiency may adversely affect the immune system and cause allergic diseases.

There were some limitations in this study. As there have been relatively few studies evaluating ECP and vitamin D levels in infants with CMPA, we compared our findings to only a small number of studies. A second limitation was that we did not measure the serum vitamin D levels of the mothers and the umbilical cords of the infants and also not determine the duration of daily sunlight exposure of infants and their mothers.

In conclusion, this study revealed infants with CMPA had significantly higher the sECP level, but vitamin D levels did not differ between infants with and without CMPA. Thus, the findings of the present study do not support the view that vitamin D deficiency may play a role in the etiology and physiopathology of CMPA. In addition, our results indicated that infants taking standard doses of Vitamin D supplements should be carefully monitored for vitamin D insufficiency.

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Disclosure of conflict of interest

None.

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References

[1] Sampson HA. Legumes, eggs and milk. Allergy 1998; 53: 38-43.

[2] Johansson SG, Bieber T, Dahl R, Friedmann PS, Lanier BQ, Lockey RF, Motala C, Martell JA, Platts-Mills TA, Ring J and Thien F. Revised nomenclature for allergy for global use: report of the Nomenclature Review Committee of the World Allergy Organization, October 2003. J Allergy Clin Immunol 2004; 113: 832-836.

[3] Fiocchi A, Brozek J, Schünemann H, Bahna SL, von Berg A, Beyer K, Bozzola M, Bradsher J, Compalati E, Ebisawa M, Guzmán MA, Li H, Heine RG, Keith P, Lack G, Landi M, Martelli A, Rancé F, Sampson H, Stein A, Terracciano L and Vieths S; World Allergy Organization (WAO) Special Committee on Food Allergy. World Allergy Organization (WAO) diagnosis and rationale for Action against Cow's Milk Allergy (DRACMA) guidelines. Pediatr Allergy Immunol 2010; 21 Suppl: 1-125.

[4] Savage J and Johns CB. Food allergy: epidemiology and natural history. Immunol Allergy Clin North Am 2015; 35: 45-59.

[5] Lifschitz C and Szajewska H. Cow's milk allergy: evidence-based diagnosis and management for the practitioner. Eur J Pediatr 2015; 174: 141-150.

[6] Dunlop JH and Keet CA. Epidemiology of food allergy. Immunol Allergy Clin North Am 2018; 38: 13-25.

[7] Sicherer SH and Sampson HA. Food allergy. J Allergy Clin Immunol 2006; 117: 470-475.
[8] Koh GC, Shek LP, Goh DY, Van Bever H and Koh DS. Eosinophil cationic protein: is it useful in asthma? A systematic review. Respir Med 2007; 101: 696-705.
[9] Venge P, Byström J, Carlson M, Hakansson L and Karawacjzyk M. Eosinophil cationic protein (ECP): molecular and biological properties and the use of ECP as a marker of eosinophil activation in disease. Clin Exp Allergy 1999; 29: 1172-1186.
[10] Holick MF. Vitamin D status: measurement, interpretation, and clinical application. Ann Epidemiol 2009; 19: 73-78.
[11] World Health Organization. Vitamin D Supplementation and Respiratory Infections in Children. https://www.who.int/elena/titles/vitamind_pneumonia_children/en/.
[12] Tsoukas CD, Provvedini DM and Manolagas SC. 1,25-dihydroxyvitamin D3: a novel immunoregulatory hormone. Science 1984; 224: 1438-1440.
[13] Cairncross C, Grant C, Stonehouse W, Conlon C, McDonald B, Houghton L, Eyles D, Camargo CA, Coad J and von Hurst P. The relationship between vitamin d status and allergic diseases in New Zealand preschool children. Nutrients 2016; 8: 326.
[14] Matsui T, Tanaka K, Yamashita H, Saneyasu KI, Tanaka H, Takasato Y, Sugiura S, Inagaki N and Ito K. Food allergy is linked to season of birth, sun exposure, and vitamin D deficiency. Allergol Int 2019; 68: 172-177.
[15] Samochocki Z, Bogaczewicz J, Jeziorkowska R, Sysa-Jędrzejowska A, Glińska O, Karczmarewicz E, McCauliffe DP and Woźniacka A. Vitamin D effects in atopic dermatitis. J Am Acad Dermatol 2013; 69: 238-244.
[16] Li J, Mei X, Cai X, Zhuo Y, Zhang L, Guo H, Yang H and Yang G. Association of blood eosinophilia and vitamin D insufficiency in young infants with cow milk allergy. Asia Pac J Clin Nutr 2019; 28: 550-557.
[17] Koletzko S, Niggemann B, Arató A, Dias JA, Heuschkel R, Husby S, Mearin ML, Papadopoulou A, Ruemmele FM, Staiano A, Schäppi MG and Vandenplas Y. Diagnostic approach and management of cow's-milk protein allergy in infants and children: ESPGHAN GI Committee practical guidelines. J Pediatr Gastroenterol Nutr 2012; 55: 221-229.
[18] Kang I, An XH, Oh YK, Lee SH, Jung HM, Chae SC and Lee JH. Identification of polymorphisms in the RNase3 gene and the association with allergic rhinitis. Eur Arch Otorhinolaryngol 2010; 267: 391-395.
[19] Bystrom J, Amin K and Bishop-Bailey D. Analysing the eosinophil cationic protein-a clue to the function of the eosinophil granulocyte. Respir Res 2011; 12: 10.
[20] Sugai T, Sakiyama Y and Matumoto S. Eosinophil cationic protein in peripheral blood of pediatric patients with allergic diseases. Clin Exp Allergy 1992; 22: 275-281.
[21] Jang WR, Choi JW, Nahm CH, Moon YS, Kim JJ, Kim JH and Lim DH. Significance of serum eosinophil cationic protein and high-sensitivity C-reactive protein levels in patients with allergic and non-allergic inflammatory diseases. Lab Med Online 2012; 2: 20-27.
[22] Suomalainen H, Soppi E and Isolauri E. Evidence for eosinophil activation in cow's milk allergy. Pediatr Allergy Immunol 1994; 5: 27-31.
[23] Hidvégi E, Cserháti E, Kereki E and Arató A. Higher serum eosinophil cationic protein levels in children with cow's milk allergy. J Pediatr Gastroenterol Nutr 2001; 32: 475-479.
[24] Zeyrek CD, Zeyrek F, Sevinc E and Demir E. Prevalence of asthma and allergic diseases in Sanliurfa, Turkey, and the relation to environmental and socioeconomic factors: is the hygiene hypothesis enough? J Investig Allergol Clin Immunol 2006; 16: 290-295.
[25] Mirzakhani H, Al-Garawi A, Weiss ST and Litonjua AA. Vitamin D and the development of allergic disease: how important is it? Clin Exp Allergy 2015; 45: 114-25.
[26] Hiraguchi Y, Tanida H, Sugimoto M, Hosoki K, Nagao M, Tokuda R and Fujisawa T. 1,25-Dihydroxyvitamin D3 upregulates functional C-x-C chemokine receptor type 4 expression in human eosinophils. Int Arch Allergy Immunol 2012; 158: 51-57.
[27] Ethier C, Yu Y, Cameron L, Lacy P and Davoine F. Calcitriol reduces eosinophil necrosis which leads to the diminished release of cytotoxic granules. Int Arch Allergy Immunol 2016; 171: 119-129.
[28] Li YC, Chen Y and Du J. Critical roles of intestinal epithelial vitamin D receptor signaling in controlling gut mucosal inflammation. J Steroid Biochem Mol Biol 2015; 148: 179-183.
[29] Perezabad L, López-Abente J, Alonso-Lebrero E, Seoane E, Pion M and Correa-Rocha R. The establishment of cow's milk protein allergy in infants is related with a deficit of regulatory T cells (Treg) and vitamin D. Pediatr Res 2017; 81: 722-730.
[30] Silva CM, Silva SA, Antunes MM, Silva GA, Sarinho ES and Brandt KG. Do infants with cow's milk protein allergy have inadequate levels of vitamin D? J Pediatr (Rio J) 2017; 93: 632-638.
[31] Ercan N, Bostancib İB, Ozmen S and Tekindal MA. Is there an association between vitamin D levels and cow's milk protein allergy at infancy? Arch Argent Pediatr 2019; 117: 306-313.

[32] Yesiltepe Mutlu G and Hatun S. Use of vitamin D in children and adults: frequently asked questions. J Clin Res Pediatr Endocrinol 2018; 10: 301-306.

[33] Turck D, Braegger CP, Colombo C, Declercq D, Morton A, Pancheva R, Robberecht E, Stern M, Strandvik B, Wolfe S, Schneider SM and Wilschanski M. ESPEN-ESPGHAN-ECFS guidelines on nutrition care for infants, children, and adults with cystic fibrosis. Clin Nutr 2016; 35: 557-577.

[34] The Turkish Ministry of Health - General Directorate of Mother and Child Health and Family Planning. The Project on Preventing Vitamin D Deficiency in Infants and Protecting Bone Health, 2005. http://www.istanbulsaglik.gov.tr/w/sb/halksag/belge/mevzuat/bebek_dvit_yet_kemik_kor_projesi.pdf.