Kocatepe Tıp Dergisi
Kocatepe Medical Journal
24:343-349/Temmuz 2023 Sayısı

ARAŞTIRMA YAZISI / RESEARCH ARTICLE

ÇOCUKLARDA HELİKOBAKTER PYLORİ ENFEKSİYONU İLE PLAZMA 25 HİDROKSİ VİTAMİN D3 DÜZEYİ ARASINDAKİ İLİŞKİ

RELATIONSHIP BETWEEN PLASMA 25 HIDROXY VITAMIN D3 LEVEL AND HELICOBACTER PYLORI INFECTION IN CHILDREN

Gülseren EVİRGEN ŞAHİN[1], Erhun KASIRGA[1], Semin AYHAN[2]

[1]Dr. Sami Ulus Çocuk Hastanesi, Çocuk Gastroenteroloji, Hepatoloji ve Beslenme Bölümü
[2]Celal Bayar Üniversitesi Tıp Fakültesi, Patoloji Ana Bilim Dalı

ÖZET

AMAÇ: Helicobacter pylori (H.pylori) enfeksiyonunun şiddeti ve ilişkili hastalıkların varlığı konakçı, bakteriyel ve çevresel faktörlerden etkilenir. Bu çalışmada, H.pylori enfeksiyonu saptanan çocuklarda inflamatuar yanıtın düzenlenmesinde önemli rolü olan plazma 25 hidroksi vitamin D3 (vitD3) ile H.pylori enfeksiyonu arasındaki ilişkinin gösterilmesi amaçlanmıştır.

GEREÇ VE YÖNTEM: Mart 2010 - Mart 2011 tarihleri arasında Celal Bayar Üniversitesi Tıp Fakültesi gastroentroloji bölümünde dispeptik yakımalar ve medikal tedaviye dirençli demir eksikliği anemisi nedeni ile üst gastrointestinal endoskopi yapılan 3-18 yaş arası 201 hasta bu çalışmaya alındı. Biyopsi örneklerinin histopatolojik tanıları değerlendirildiğinde, H.pylori pozitif grupta 98, H.pylori negatif grupta 103 hasta vardı. Vitamin D3 düzeyi, H.pylori pozitif ve H.pylori negatif gruplarda karşılaştırıldı. Helicobacter pylori pozitif grupta ise gastrik mukozal aktivite ve inflamasyon şiddetinin derecesi ile vitD3 düzeyleri karşılaştırıldı.

BULGULAR: Helicobacter pylori pozitif hastaların 80'inde (81.6%), H.pylori negatif hastaların 76'sında (73%) vitD3 düzeyleri düşüktü. Bu fark istatistiksel olarak anlamlı değildi (p>0.05). Helicobacter pylori pozitif ve negatif grupların plazma vitD3 düzeylerinin ortalama değeri sırasıyla 15.64±8.9 ng/mL ve 16.36±1.35 ng/mL idi. Gruplar arasındaki fark istatistiksel olarak anlamlı değildi (p>0.05). Helicobacter pylori pozitif ve negatif gruptaki hastalar, plazma vitD3 düzeyine göre eksiklik, şiddetli eksiklik, yetersizlik ve yetmezlik olarak dört farklı grupta sınıflandırıldığında ise H.pylori pozitif grupta vitD3 eksikliği H.pylori negatif gruba göre daha sıktı. Bu sonuç istatistiksel olarak anlamlıydı (p<0.05). Her iki grupta kronik inflamasyonun şiddeti ve doku H.pylori aktivitesi ortalama vitD3 düzeyi ile ters orantılı olarak arttığı görüldü (p<0.05).

SONUÇ: Çalışmamızın sonuçları bölgemizde çocuklarda vitD3 düşüklüğünün yaygın olduğunu göstermektedir. Vitamin D3 eksikliği H.pylori enfeksiyonu için risk faktörüdür. Bu çalışma H.pylori enfeksiyonunda vitD3'ün antibakteriyel etkiyi artırdığını ve inflamasyonun şiddetini azalttığını göstermektedir.

ANAHTAR KELİMELER: Çocukluk Çağı, Helicobakter pylori, Vitamin D3, Risk Faktörleri, Komplikasyonlar.

ABSTRACT

OBJECTIVE: The severity of the Helicobacter pylori (H.pylori) infection and the presence H.pylori related diseases are affected by host, bacterial and environmental factors. In this study it is aimed to show relationship between H.pylori infection and plasma Vitamin D3 (vitD3) has significant role in regulation inflamatory response in children with H.pylori infection.

MATERIAL AND METHODS: Two hundred one patients aged between 3-18 years, referred to pediatric gastroentrology department of Celal Bayar University Medical Faculty between March 2010 to March 2011 and performed upper gastrointestinal endoscopy because of gastrointestinal symptoms and iron deficiency anemia refractory to medical therapy were enrolled in this study. Histopathologic diagnosis of biopsy speciments of the patients were evaluated. There were 98 patients in H.pylori positive group and 103 patients in H.pylori negative group. Plasma level of vitD3 of H.pylori positive and H.pylori negative cases were compared. Relationship between gastric mucosal activity the degree of inflammation severity and vitD3 level were evaluated in the H.pylori positive group.

RESULTS: Vitamin D3 levels were low in 80 (81.6%) H.pylori positive patients and in 76 (73%) H.pylori negative patients. This difference was not statistically significant (p>0.05). The mean value of plasma level of vitD3 H.pylori positive and negative groups were 15.64±8.9 ng/mL and 16.36±11.35 ng/mL respectively. The difference between the groups was not statistically significant (p>0.05). When patients in H.pylori positive and negative groups were classified according to plasma vitD3 level in four different groups as severe deficiency, deficiency, insuficiency and sufficiency. Vitamin D3 severe deficiency in H.pylori positive group was statistically more frequent than H.pylori negative group (p<0.05). Severity of chronic inflamation and tissue H.pylori activity increased inversely with the mean vitD3 level in both groups (p<0.05).

CONCLUSIONS: Results of this study suggests that vitD3 deficiency is common in children in our region. Vitamin D3 deficiency is risk factor for H.pylori infection. The findings of this study shows vitD3 increasing antibacterial effect and reducing the severity of inflammation in H.pylori infection.

KEYWORDS: Childhood, Helicobacter pylori, Vitamin D3, Risk Factors, Complications.

Geliş Tarihi / Received: 15.02.2022
Kabul Tarihi / Accepted:11.11.2022
Yazışma Adresi / Correspondence: Uzm. Dr. Gülseren EVİRGEN ŞAHİN
Dr. Sami Ulus Çocuk Hastanesi, Çocuk Gastroenteroloji, Hepatoloji ve Beslenme Bölümü
E-mail: evirgen2005@yahoo.com
Orcid No (Sırasıyla):0000-0001-6146-3509, 0000-0002-1098-9604, 0000-0002-8546-0705
Etik Kurul / Ethical Committee: Celal Bayar Üniversitesi Etik Kurulu (2010/46).

INTRODUCTION

Vitamin D3 has well known effects on calcium phosphor and bone metabolism however nowadays roles of vitD3 on other biological sistem is being studied profoundly. In recent years, vitD3 deficiency and insufficiency have been found to be associated with many chronic diseases including metabolic syndrome, cardiovascular diseases, cancer, infectious and autoimmune diseases (1 - 3).

Vitamin D3 acts as a transcription factor in its biological activities, acting on the transcription of many different genes. It performs its function by binding to the nuclear receptor (VDR: vitamin D receptor) (4). The effects of vitD3 on the innate and adaptive immune system gained importance with the detection of the presence of VDR in immune system cells (1). In studies examining the relationship between vitD3 level and infections, especially tuberculosis, upper and lower respiratory infection; a positive correlation has been reported between low vitD3 levels and increased susceptibility to infections and the severity of infection (5 - 8).

The role of vitD3 in infection control is related to its effects on the innate and adaptive immune system and is mainly related to mechanisms mediated by innate immunity (1, 4, 9). Cationic antimicrobial peptides (CAMP), one of the most important elements of the innate immune system, disrupt the membrane integrity of pathogenic microorganisms such as H.pylori (10, 11). In cell culture studies by Gombart et al. showed that the CAMP and defensin ß 2 genes have VDR in the promoter region (12). It has been found that β-Defensins with known antibacterial effects inhibit H.pylori (12- 14). Vitamine D3 has inhibitor affect on adaptive immune system. This helps supression of inflamation (4, 15).
World Health Organization defines range of sufficient, insufficient, deficient, severe deficient vitD3 plasma levels are 20-100 ng/mL (50-250mmol/L), 15-20 ng/mL (22.5-50mmol/L), 5-15ng/mL (12.5-37.5mmol/L), 5ng/mL(12.5mmol/L) respectively (16 - 17).

Helicobacter pylori is gram negative pathogen infected half of the world population. Infection with H.pylori during childhood causes different clinical spectrum of diseases like asemptomatic carriers, chronic gastritis, peptic ulsers (PU), mucosa associated lymphoid tissue lymphoma, and gastric cancer (18, 19). Incidence and severity of disease related to H.pylori changes in different geographical regions even in children living in the the same regions. Chronic inflamation causes asemptomatic gastritis, PU or gastric cancer. Course of inflamation is related to interaction between virulance factors of H.pylori, host and environmental factors (20, 21).

Although many studies on host factors effecting immune resposes in H.pylori infection, to date complete host factors are not clearly defined. In this study probable relation between H.pylori and vitD3 which has important role in immune response is evaluated.

MATERIAL AND METHODS

In this cross sectional study patients age of 3-18 years underwent upper gastrointestinal endoscopy because of gastrointestinal symptoms and iron deficiency anemia refractory to medical therapy between March 2010 to March 2011 at the pediatric gastroenterology clinics of Celal Bayar University Hospital, Manisa, Turkey were enrolled. Patients' clinical findings, histopathologic diagnosis of biopsy speciments of the patients and plasma vitD3 levels were evaluated.

Exculation Criteria

Those who have received anti-acid, non-steroidal anti-inflammatory drugs and antibiotic treatment in the last 3 months; patients who have had H.pyori eradication in the last one year; children with a diagnosis of systemic disease and those younger than 3 years old were not included in the study.

In the study during upper gastrointestinal endoscopy two antral and two corpus biopsy specimen were taken from four different locations for each patient. Fragments fixed in Hollande solution and stained with hematoxylin eosin and Toluidin blue for histopathological examination and H.pylori evaluation according to 1994 updated Sydney scoring system for each antral and corpus biopsy specimens. For evaluation of tissue atrophy in biopsy specimens 2000 Atrophy Club criteria used and each biopsy material defined as atrop-

hy positive and negative. Presence of intestinal metaplasia is evaluated for each patient.

Plasma vitD3 Level Analysis

Plasma vitD3 level measured using HPLC method with vitD3 kit RECIPE Chemicals+Instruments GMBH, Labortechnik Dessauerstrabe 3, mD-80992 München/Germany (internet:www.recipe.de). Samples were separated chromatographicly and analysis performed with UV detector. After samples were precipitated with precipitant P. internal standart were added. After each sample were mixed with vortex for 30 seconds and santrifujed at 10000Xg for 5 minutes, obtained supernatants were used for HPLC analysis. Measurements calculated according to "İnternal standart-Via peak" area method.

Consensus values is used for classification of plasma vitD3 level (severe deficiency ≤ 12. 5 (5) nmol/L (ng/m), deficiency ≤ 37. 5 (15) nmol/L (ng/m), insufficient 37. 5–50.0 (15–20) nmol/L (ng/m), sufficient 50–250 (20–100) nmol/L (ng/m), high >250 (100) nmol/L (ng/m), toksik >375 (150nmol/L(ng/mL)).

Ethical Committee

In this study written informed concent were obtained from all patients and parents. All procedures performed in this study were approved by Ethics Committee of Celal Bayar University (protocol number 0046/2010).

Statistical Analysis

Statistical analyses to compare these parameters between groups were performed using the Statistical Package for the Social Sciences (SPSS) (Version 16.0; SPSS, Inc., Chicago, IL, USA). Means were compared between two groups by Student's t tests. Pevelance rates were compared between groups by the chi square test. A value of $p<0.05$ was considered statistically significant.

RESULTS

In the study a total of 201 patients consists of H.pylori positive 98 (48.7%) children 43 (43.1%) males and 55 (56.9%) females), H.pylori negative 103 (51.3%) children, 33 (32%) males and 70 (68%) females), were enrolled. The mean age of H.pylori negative and positive groups were 11.6 ± 3.81, 12.4 ± 3.37. years respectively. No significant statistical difference in sex and mean age were found between H.pylori negative and positive groups ($p>0.05$).

Mean plasma vitD3 level of H.pylori negative, H.pylori positive were 16.36±11.35 ng/mL, 15.64±8.91 ng/Ml respectively. There was no significant statistical difference between H.pylori positive and negative groups ($p>0.05$).

Vitamin D3 deficiency was detected in 76 (73%) and 80 (81.6%) of H.pylori negative and positive children respectively. There is no statistical difference between percentage of vitD3 deficiency in H.pylori positive and negative groups. The H.pylori positive and negative groups consists of 6 (6.1%) 6 (5.8%) patients with severe deficient plasma level of vitD3 (<5ng/mL), 49 (50%), 41 (39.8%) patients with deficient plasma level of vitD3 (<15ng/mL), 25 (25.6%), 29 (28.2%) patients with insufficient plasma level of vitD3 (15-20ng/mL) and 18 (18.3%), 27 (26.2%) patients with sufficient plasma level of vitD3 respectively. Plasma level of vitD3 deficiency was more frequent in H.pylori positive group than H.pylori negative group. Statistical difference was significant between groups ($p<0.05$) **(Table 1)**.

Table1: Frequencies and percentage of degree of severity of 25 OH Vit D deficiency in H.pylori positive and negative groups

25OH Vit D Level	H.pylori (-) N (%)	H.pylori(+) N (%)	Total N (%)
Severe Deficiency	6 (5.8)	6 (6.1)	12(6)
Deficiency	41 (39.8)	49 (50)	90(44.8)
Insufficiency	29 (28.2)	25 (25.6)	54(27)
Sufficiency	27 (26.2)	18 (18.3)	45(22)
Total	103 (100)	98 (100)	201(100)

Chi square test $P<0.05$, $p=0.04$,

When the severity of gastric mucosal H.pylori activity and plasma level vitD3 were compared in H.pylori positive cases, there was statistical significant relation between decrease in vitD3 level and severity of gastric mucosal H.pylori activity ($p<0.05$) **(Table 2)**. When the plasma vitD3 level of the cases and the severity of chronic inflammation were compared, gastric chronic inflamation increases when mean plasma vitD3 level of H.pylori positive patients decreases. This was statistically significant ($p<0.05$)**(Table 3)**.

Table 2: Association of mean plasma 25OH vitamin D plasma level with H.pylori activity

H.pylori Activity	n	25OH Vit D Level(ng/ml) (Mean±SD)
Mild	27	21.48±9.36
Moderate	31	17.64±9.28
High	40	12,72±6.30
Total	98	15.64±8.91

Annova test, p<0.05,p= 0.031

Table 3: Association of mean plasma 25 OH vitamin D level with chronic inflamation in H.pylori cases

Chronic Inflamation	n	25OH Vit D level (Mean± SD)
None	32	18.58±6.71
Mild	23	15.68±8.4
Moderate	35	15.54±5.71
Severe	8	7.12±3.56
Total	98	15.64±8.91

nova test p<0.05, p=0.04

DISCUSSION

In this study mean plasma mean vitD3 level were found not sufficent in both H.pylori positive and negative groups. Vitamin D3 plasma level was not statistically different between H.pylori positive (15.64±8.91 ng/mL) and negative (16.36±11.35 ng/mL) groups. Maternal vitD3 related rickets and subclinic viD3 deficiency is common in pediatric and adolescent age groups in different regions of Turkey in different seasons (22 - 24). Vitamin D3 deficiency is worldwide common public health problem specially in northern hemisphere. Andersen et al. reported during winter plasma vitD3 level almost all the children is under 10ng/mL in which 30 percent of the children is lower than 10ng/mL in northern Europe (25). In a study reported from Uganda, 38.5% of children were found to have low vitD3, and 2.7% of them had vitD3 deficiency (26). An other study conducted in Ireland showed 70% of children of 2 years of age were vitD3 deficient (27). In subgroup analysis of mean plasma level of vitD3 deficiency subgroup, H.pylori positivity statistically higher than other subgroups. The lack of difference in mean vitD3 levels between H.pylori positive and H.pylori negative groups in our study can be explained by low vitD3 levels in our region.

Kawaura reported that H.pylori infection was less common in elderly women who took vitD3 supplements compared to those who did not (28). In the study of Antico et al. vit D3 levels, which were found to be 11.3 ± 8.4 ng/mL in H.pylori gastritis, were found to be statistically significantly lower than in healthy individuals (29). In children there are limited number of studies related to H.pylori and vitD3 level in literature. Gao et al found the prevalence of vitD3 deficiency in H.pylori seropositive and seronegative groups was 20.7% and 12.1%, respectively in children (30). This study is different from our study because of no hystopathologic H.pylori verification.

In this study, there was no significant difference between vitD3 levels and H.pylori positive or H.pylori negative groups, but when the H.pylori positive groups were divided into subgroups as severe deficient, deficienct, insufficient and sufficient vitD3 level, there was significant difference between the deficiency of vitD3 level and the presence of H.pylori infection. In the study of Shafri et al. H.pylori positivity was found to be 31% higher when patients with vitD3 levels <20 ng/mL were compared with patients with vitD3 levels ≥20 ng/mL. In the same study, vitD3 level was found to be moderately higher in the group with H.pylori positive in which eradication was successful, compared to the group in which eradication treatment failed (19.34 ± 9.55 vs 18.64 ± 9.61) (31). In our study, unlike the study of Shafri et al. the presence of H.pylori infection was confirmed by gastric biopsy samples.

Helicobacter pylori triggers inflammation in two ways which are by secreting specific toxins or other aggressive factors; the latter by stimulating the natural and acquired immune response of the host (20, 21). Despite the strong immune response in H.pylori infection, clearance of the infection is often not possible (19, 32, 33). Vitamin D3, has an important role in signaling pathways that play a role in the antimicrobial effect of innate immunity. It has been shown that vitD3 (found in macrophages, monocytes, epithelial cells) stimulates the expression of genes encoding the antimicrobial peptide (CAMP and DEFB2) (34, 35). Guo, L et al reported that vitD3 increases CAMP expression and decreases cytokine activation in gastric epithelial cells, and it has been suggested to play a role in the inhibition of H.pylori (36). In the study of Hosada et al. VDPs (vitamin D3 decomposition products) and H.pylori were shown to have a bactericidal effect by providing membrane solubility and cell ly-

sis (37). In the study of Zhou et al. it was shown that VitD3 inhibited H.pylori infection by increasing CAMP secretion in vivo in mice (38). In our study, the increase in gastric mucosal H.pylori activity correlated with the severity of low vitD3 supports the importance of the antimicrobial effect of vitD3. A strong local immune response develops initially in a host infected with H.pylori. Neutrophils, macrophages, monocytes, and dendritic cells are the first cells to aggregate in the gastric mucosa (20). Histopathologically, chronic gastric inflammation is characteristic in the majority of cases (39). The production of almost all cytokines is increased in the inflammatory response against H.pylori. In H.pylori infection, damage to the gastric mucosa by indirect means caused by the host's immune response, as well as by various cytotoxins and enzymes such as urease, is responsible for the destructive effect (40). Compared with adults, decreased Th1 and Th17 response, increased TGF-β1, IL-10 secretion, resulting in less gastric inflammatory response and neutrophil infiltration have been shown in children (19, 41). Active vitD3 increases the release of the anti-inflammatory cytokine IL-10 and shifts the balance towards Th2. Vitamin D3 achieves this direct effect by binding to the NFAT (nuclear factor activated T cell) and IFγ promoter regions that cause the Th1 response and inhibiting synthesis (9). In vitD3 deficiency, there is a defect in T cell maturation (42). IL-8, which plays an important role in the pathogenesis of H.pylori-induced diseases, is a potent chemoattractant for neutrophils and lymphocytes. It also has effects on cell proliferation, migration and tumor angiogenesis. A correlation has been established between IL-1, IL8, and the severity of inflammation (43). It has been reported that vitD3 reduces the expression of IL-1, IL-6, IL-8 and TNF-α in different cell types (9, 44). Proinflammatory cytokines secreted in increased inflammatory reactions in the gastric mucosa in H.pylori infection result in intense infiltration by increasing chemotaxis of mononuclear cells and neutrophils (45). In this study, increase in gastric mucosal bacterial activity and the severity of chronic inflammation compare with the vitD3 levels of H.pylori positive cases the difference between the groups was statistically significant. Low vitD3 level causes increase in the severity of inflammation and gastric mucosal bacterial activity. These molecular mechanism supports increase in gastric mucosal H.pylori activity and severity of chronic inflamation in this study. To our knowledge, this study is the first report of mean vitD3 level inversely correlated with severity of H.pylori activity and chronic inflamation in children with H.pylori infection to date. These results reveals the importance of adequate vitD3 supplementation in preventing inflammation in addition to antimicrobial effect in H.pylori infection.

Vitamin D3 deficiency is a risk factor for H.pylori infection. The findings of this study shows vitD3 increasing antibacterial effect and reducing the severity of inflammation in H.pylori infection. These results supports during childhood and adolescence period providing sufficient vitD3 supplementation is important in H.pylori infection control and prevention of H.pylori related early and late complication.

Acknowledgement

This project was funded by the Scientific Research department of Celal Bayar University, Manisa, under grant number (332/140/1431). The authors, therefore, acknowledge with thanks research center for technical and financial support.

REFERENCES

1. Zhang S, Miller DD, Li W. Non-Musculoskeletal Benefits of Vitamin D beyond the Musculoskeletal System. Int J Mol Sci. 2021;22 (4):2128.

2. Bouillon R, Marcocci C, Carmeliet G, et al. Skeletal and Extraskeletal Actions of Vitamin D: Current Evidence and Outstanding Questions. Endocr Rev. 2019;40(4):1109–51.

3. Beckett E. More Than Bone Health: The Many Roles for Vitamin D. Nutrients. 2020;12(8):2388.

4. Vyas SP, Hansda AK, Kaplan MH, Goswami R. Calcitriol regulates the differentiation of IL-9-secreting Th9 cells by modulating the transcription factor PU.1. J Immunol. 2020;204(5):1201-13.

5. Wilkinson RJ, Liewelyn M, Toossi Z, et al. Influence of vitamin D deficiency and vitamin D polimorfisms on tuberculosis among Gujarati asians in west London: a case-control study. Lancet. 2000;355:618-21.

6. Gough ME, Graviss EdA, May EE. The dynamic immunomodulatory effects of vitamin D3 during Mycobacterium infection. Innate Immunity. 2017;23:(6)506–23.

7. Martineau AR, JolliffeDA, Greenberg L, et al. Vitamin D supplementation to prevent acute respiratory infections: individual participant data meta-analysis. Health Technol Assess. 2019;23:(2)1–44.

8. Laplana M, Royo JL, Fibla J. Vitamin D Receptor polymorphisms and risk of enveloped virus infection: A meta-analysis. Gene. 2018;678:384-94.

9. Ismailova A, White JH. Vitamin D, infections and immunity. Rev Endocr Metab Disord. 2022;23(2):265-77.

10. Hafeez AB, Jiang X, Bergen PJ, Zhu Y. Antimicrobial Peptides: An Update on Classifications and Databases. Int J Mol Sci. 2021;22(21):11691.

11. Wehkamp J, Schauber J, Stange EF. Defensins and cathelicidins in gastrointestinal infections. Curr Opin Gastroenterol. 2007;23:32–38.

12. Gombart AF, Borregaard N, Koeffler HP. Human cathelicidin antimicrobial peptide (CAMP) gene is a direct target of the vitamin D receptor and is strongly up-regulated in myeloid cells by 1,25-dihydroxyvitamin D3. FASEB J. 2005;19(9):1067-77.

13. O'Neil DA, Cole, SP, Martin-Porter E, et al. Regulation of human β-defensins by gastric epithelial cells in response to infection with Helicobacter pylori or stimulation with interleukin-1. Infect Immun. 2000;9:5412–15.

14. Uehara N, Yagihashi, A Kondoh K, Tsuj N, et al. Human β-defensin-2. induction in Helicobacter infected gastric mucosal tissues: Antimicrobial effect of overexpression. J Med Microbiol. 2003;52:41–45.

15. Hu W, Zhang L, Li MX, et al. Vitamin D3 activates the autolysosomal degradation function against Helicobacter pylori through the PDIA3 receptor in gastric epithelial cells.. Autophagy.2019;15:707–25.

16. Holick MF. High prevalence of vitamin D inadequacy and implications for health. Mayo Clin Proc. 2006;81(3): 353–73.

17. Misra M, Pacaud D, Petryk A, Collett-Solberg PF, Kappy M. Vitamin D deficiency in children and its management: review of current knowledge and recommendations. Pediatrics. 2008;122(2):398-17.

18. Ailloud F, Didelot X, Woltemate S, et al. Within-host evolution of Helicobacter pylori shaped by niche-specific adaptation, intragastric migrations and selective sweeps. Nat Commun. 2019;10:2273.

19. Seo JH, Bortolin K, Jones NL. Review: Helicobacter pylori infection in children. Helicobacter. 2020;25(1): e12742.

20. Blosse A, Lehours P, Wilson KT, Alain P, Gobert AP. Helicobacter: Inflammation, immunology, and vaccines. Helicobacter. 2018;23:e12517.

21. Săsăran MO, Meliț LE, Dobru ED. MicroRNA Modulation of Host Immune Response and Inflammation Triggered by Helicobacter pylori. Int J Mol Sci. 2021;22(3):1406.

22. Doğan N, Çolak AA, Güden N, Üstüner F. Vitamin D deficiency in children in Aegean Region in Turkey. Cumhuriyet Medical Journal. 2015;37:17-22.

23. Özdemir AA, Gündemir YE, Küçük M et al. Vitamin D Deficiency in Pregnant Women and Their Infants. J Clin Res Pediatr Endocrinol. 2018;10(1):44–50.

24. Alpdemir M, Alpdemir MF. Vitamin D deficiency status in Turkey: A meta-analysis. Int J Med Biochem. 2019;(3):118-31.

25. Andersen R, Molgaard C, Skovgaard LT, et al. Teenage girls and elderly women living in northern europe have low winter vitamin D status. Eur J Clin Nutr. 2005;59:533-41.

26. Mutua AM, Nampijja M, Elliott AM, et al. Vitamin D status is not associated with cognitive or motor function in preschool Ugandan children. Nutrients. 2020;12:1662.

27. Ní Chaoimh C, McCarthy EK, Hourihane JO, et al. Low vitamin D deficiency in Irish toddlers despite northerly latitude and a high prevalence of inadequate intakes. Eur J Nutr. 2018;57:783–94.

28. Kawaura A, Takeda E, Tanida N, et al. Inhibitory effect of long term 1alpha-hydroxyvitamin D3 administration on Helicobacter pylori infection. J Clin Biochem Nutr. 2006;38:103–06.

29. Antico A, Tozzoli R, Giavarina D, Tonutti E, Bizzaro N. Hypovitaminosis D as predisposing factor for atrop-hic type A gastritis: a case-control study and review of the literature on the interaction of Vitamin D with the immune system. Clin Rev Allergy Immunol. 2012;42:355–64.

30. Gao T, Zhao M, Zhang C, et al. Association of Helicobacter pylori infection with vitamine D deficiency in infants and toddlers. Am J Trop Med Hyg. 2020;102:541–46.

31. Shafri A. The Association between Serum Vitamin D Levels and Helicobacter pylori Presence and Eradication. Nutrients. 2021;13(1):278.

32. Gobert AP, Wilson KT. Induction and Regulation of the Innate Immune Response in Helicobacter pylori Infection. Cell Mol Gastroenterol Hepatol. 2022;13(5):1347–63.

33. Mărginean MO, Mărginean CO, Meli LE, et al. The impact of host's genetic susceptibility on Helicobacter pylori infection in children. Medicine (Baltimore). 2017;96(30):e7612.

34. Pero R, Coretti L, Nigro E, et al . β-Defensins in the Fight against Helicobacter pylori. Molecules. 2017;22(3):424.

35. Wang TT, Nestel FP, Bourdeau V. Cutting Edge: 1,25 dihydroxyvitamin D3 is a direct inducer of antimic-robial peptide gene expression. J Immunol. 2004;173:2909-12.

36. Guo L, Chen, W, Huatuo ZH, et al. Helicobacter pylori induces increased expression of the vitamin d receptor in immune responses. Helicobacter. 2014;19(1):37–47.

37. Hosoda K, Shimomura H, Wanibuchi K, et al. Identi-fication and characterization of a vitamin D_3 decomposition product bactericidal against Helicobacter pylori. Sci Rep. 2015;5:8860.

38. Zhou A, Li L, Zhaom G, et al. Vitamin D3 Inhibits Helicobacter pylori Infection by Activating the VitD3/ VDR-CAMP Pathway in Mice. Front Cell Infect Microbiol. 2020;23;10:566730.

39. Lai LH, Sung JJY. Helicobacter pylori and benign upper digestive disease. Best Pract Res Clin Gastroenterol. 2007;21:261-79.

40. Pachathundikandi SK, Müller A. Backert S. Inflammasome Activation by Helicobacter pylori and Its Implications for Persistence and Immunity. Curr Top Microbiol Immunol. 2016;397:117-31.

41. Serrano C, Wright HW, Bimczok D, et al. Th17 responses are associated with reduced gastritis in Helicobacter pylori-infected children. Mucosal Immunol. 2013;6(5):950-59.

42. Mullin GE, Dobs A. Vitamin D and its role in cancer and immunity: A prescription for sunlight. Nutr in Clinical Prac. 2007;22:305-22.

43. Tran LS, Chonwerawong M, Ferrero RC. Regulation and functions of inflammasome-mediated cytokines in Helicobacter pylori infection. Microbes Infect. 2017;19:449-58.

44. Kamen DL, Tangpricha V. Vitamin D and molecular actions on the immune system: modulation of innate and autoimmunity. A recent review on Vitamin D and immunoregulation. J Mol Med. 2010;88:441–50.

45. Martins DJ, Matos GCB, Loiola RSP, et al. Relationship of vitamin D receptor gene polymorphisms in Helicobacter pylori gastric patients. Clin Exp Gastroenterol. 2018;11:19–27.