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Original Article

Role of vitamin D3 on IL-17 expression in colon and improvement of colonic mucosa in an inflammatory bowel disease mice model

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Abstract

Background Inflammatory bowel disease (IBD) is an inflammation due to a Th1/ Th2 regulatory imbalance and a Th17/Treg transformation imbalance which then releases inflammatory mediators, such as interleukin-17. The administration of vitamin D has the potential to prevent the inflammation in IBD.

Objective To evaluate a possible role of vitamin D3 in reducing IL-17 expression and colonic mucosal repair in an IBD mice model.

Methods The study used male BALB/c mice, 8-10 weeks old, weighing 20-25 grams, divided randomly into five groups with 8 mices in each group. The experimental mice were given 5% dextran sulfate sodium (DSS) on days 1-7 to induce colitis, and then were given vitamin D3 on days 8-14. Group 1 was the control group; Group 2 was given 5% DSS; Group 3 was given 5% DSS and vitamin D3 0.2 mcg/25 g body weight; Group 4 was given 5% DSS and vitamin D3 0.4 mcg/25 g body weight; and Group 5 was given 5% DSS and vitamin D3 0.6 mcg/25 g body weight. On day 15, the mice underwent euthanasia and colonic retrieval. Parameters assessed were IL-17 expression (immunohistochemical, with monoclonal antibody against IL-17) and colonic histology improvement, using the mouse colitis histology index (MCHI) score.

Results The IL-17 expression measured by immunohistochemistry increased significantly in only 5% DSS group. There was a significant decrease in MCHI scores in the groups given vitamin D3, where the greater the dose of vitamin D3 given, the lower the MCHI score. Interleukin-17 expression had positive strong correlation with MCHI ($r=0.985$; $P=0.002$)

Conclusion The improvement of colonic mucosal damage based on MCHI score is significant in groups given vitamin D3. There is a significant correlation between IL-17 reduction and colonic mucosal repair in IBD mice. **[Paediatr Indones. 2023;63:1-7; DOI: https://doi.org/10.14238/pi63.1sup.2023.1-7**].

Keywords: *inflammatory bowel disease; interleukin-17; T helper 17; vitamin D3*

Inflammatory bowel disease (IBD) is a chronic inflammatory disease of the gastrointestinal tract that is either remitting or relapsing/recurring, the exact cause of which is unclear.[1] Recent epidemiological studies report that the incidence of IBD has increased in some continents, such as North America, Asia, Africa, and Eastern Europe. Environmental factors influence the occurrence of chronic inflammation. Inflammatory bowel disease often occurs in adolescents and young adults. Approximately 25% of patients develop IBD before the age of 20. Among children with IBD, 4% occurred before 5 years of age and 18% at 10 years of age, with the highest onset in adolescents.[2]

Vitamin D is important for regulating intestinal mucosal immunity.[3] Several studies have shown that vitamin D can affect intestinal epithelial integrity, innate immune defense function, as well as T cell development and function.[4-6] Vitamin D deficiency is common in IBD patients. A systematic review of 14

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observational studies consisting of 938 patients with IBD showed vitamin D deficiency (serum 25(OH)D < 25 ng/mL) prevalences of 38.1% in Crohn's disease and 31.6% in ulcerative colitis.[7]

Vitamin D and the vitamin D receptor (VDR) are important regulators of the immune system. In particular, vitamin D deficiency and VDR trigger autoimmune diseases, such as IBD, which develops due to an immune-mediated attack by pathogenic T-cells that produce IL-17 and IFN-α in excess and several other regulatory cells.[8]

Interleukin-17 has an important role in disorders of the immune system. The IL-17 expression regulates innate immune defenses, and it plays a role in several cell types including neutrophils, lymphocytes, macrophages, fibroblasts, keratinocytes, epithelial cells, endothelial cells, and dendritic cells.[9] Interleukin-17 stimulates the secretion of IL-6 and IL-8 from epithelial cells and myofibroblasts, which release inflammatory mediators that contribute to epithelial cell damage in IBD.[10] Inflammatory bowel disease is characterized by inflammation due to a Th1/Th2 regulatory imbalance and a Th17/Treg transformation imbalance, leading to release of inflammatory mediators. One such inflammatory mediator is IL-17 which can be reduced by vitamin D. Thus, the administration of vitamin D in IBD has the potential to prevent IBD.[11] We aimed to study the repair of colonic mucosal damage with regards to IL-17 and vitamin D3.

Methods

This experimental study was conducted in February 2020. The adaptation phase of new environment was carried out for 1 week, while the treatment and symptom observation phases were 14 days. The mice adapted to the temperature, humidity, light, and other cage conditions. Each cage (40cms x 50cms x 15cms) contained one male mice which has been given the treatment. Each mice received food with a composition of 6-18% protein, 5-12% fat, and 60-70% carbohydrates ad libitum. Drinking mineral water given by drops of libitum to avoid contamination of feces. Male BALB/c mice aged 8-10 weeks with a body weight of 20-25 grams were divided randomly into five groups with 8 mice in each group. Group 1 was the untreated/control group. Groups 2, 3, 4, and 5 were given 5% dextran sulfate sodium (DSS) on days 1 to 7 to induce colitis. On days 8 to 14, groups 3, 4, and 5 were given vitamin D3 at doses of 0.2 mcg/25 g body weight (equivalent to 8 IU), 0.4 mcg/25 g body weight (equivalent to 16 IU), and 0.6 mcg/25 g body weight (equivalent to 24 IU), respectively. On day 15, the mice were euthanized with ketamin intravenous and their colons were dissected. We excluded mice that appeared unwell (changes in eating/drinking patterns) and other clinical signs (weight loss, breathing patterns, diarrhea, vomiting), death during the observation period, or organ/tissue damage during preparation of the histopathology/immunohistochemical examinations.

The experimental animals were maintained at the Experimental Animal Research Laboratory, Universitas Brawijaya Medical School. Tissue processing was done at the Anatomical Pathology Laboratory of Universitas Brawijaya and light microscopy readings were carried out at the Light Microscopy Unit of Universitas Brawijaya Medical School.

The parameters studied were IL-17 expression by immunohistochemical examination with monoclonal antibodies against IL-17, and a picture of colonic mucosal histology improvement using the mouse colitis histology index (MCHI) score.[12] Data were analyzed by the homogeneity test with ANOVA using *SPSS 23* software. Data normality was tested by Kolmogorof frequency histogram and normal probability plot, followed by testing the mean difference in each exposure group. The ANOVA test was used for normally distributed data and Pearson's correlation test was used to to measure the strength and direction of the linear relationship of two variables. The Committee for Medical Research Ethics of Universitas Brawijaya Medical School approved this study.

Results

The use of 5% DSS for 7 days caused colitis in mice as shown in Figure 1. In our study, histopathological examination with HE staining revealed features of goblet cell loss, decreased density, crypt hyperplasia, and submucosal infiltration. Greater the MCHI

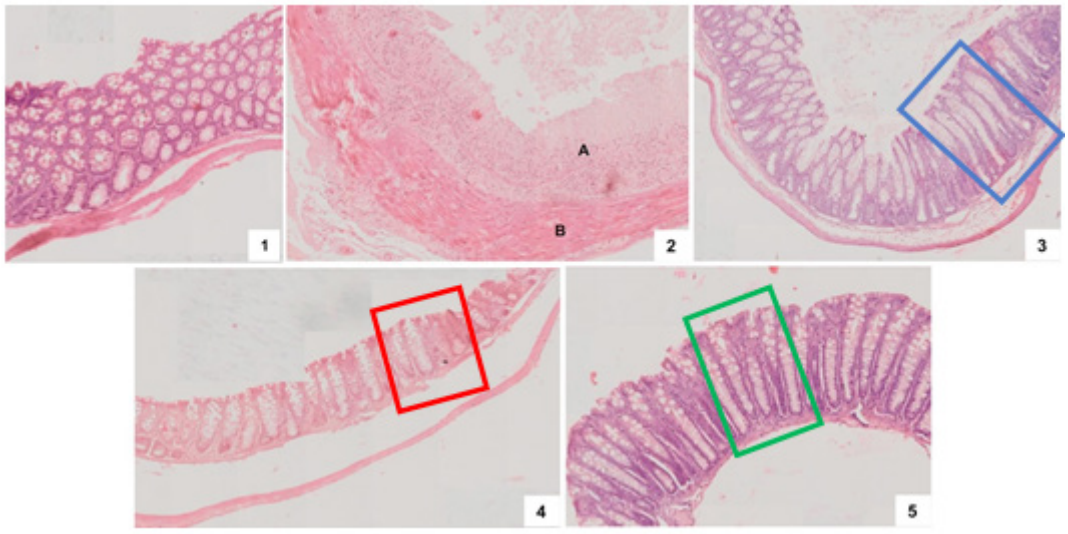

Figure 1. Histopathology of the colonic mucosal with hematoxylin eosin staining. (1) Group 1 was untreatment group with the normal histopathology of the colonic mucosal; no inflammatory cells were found and had a complete epithelial structure; (2) Group 2 (only DSS 5%): (A). Mucosal damage in the form of loss of goblet cells, decreased density, and hyperplasia of crypts, (B) Infiltration of inflammatory cells in the submucosal layer; (3) Group 3 (DSS 5% + vitamin D3 0.2 mcg/25 grams): mucosal damage was found in the form of goblet cell loss and crypt hyperplasia (blue box); (4) Group 4 (DSS 5% + vitamin D3 0.4 mcg / 25 grams): the mucosal damage began to improve with the increase in the number of goblet cells but there was still hyperplasia of the crypts (red box); (5) Group 5 (DSS 5% + vitamin D3 0.6 mcg/25 grams), there was an increase in the number of goblet cells, crypt hyperplasia was still found (green box).

score indicated the severity of inflammation of the colonic mucosa. The mean MCHI score of the colon histopathology tissue specimens was significantly higher in Group 2, which received only 5% DSS [18.63 (SD 3.08)], compared to Group 1, the untreated control [0.17 (SD 0.41)]. Groups 3 [12.6 (SD 3.31)], 4 [8.14 (SD 2.73)], and 5 [3.17 (SD 2.27)] had significantly decreased MCHI scores compared to Group 2, which received only 5% DSS ($P<0.001$), as shown in **Table 1**.

However, post hoc least significance different (LSD) test results shown in **Table 2** revealed no significant difference between Group 1 (control) and Group 5, which received the highest dose of vitamin D3 (0.6 mcg/25 g body weight) ($P>0.05$).

Pearson's correlation test revealed a dose-dependent relationship between vitamin D3 and decrease in MCHI score. **Figure 2** shows a very strong negative correlation, namely, the greater the dose of vitamin D3 given, the lower the MCHI score.

Interleukin-17 expression was calculated from immunohistochemical staining of tissue specimens from each group. Based on statistical analysis with one-way ANOVA test, test revealed that the IL-17 expression data were homogeneous and normally distributed, so a comparison test was done using one-way ANOVA test. The results in **Figure 3** show an effect of giving vitamin D3 on reducing of IL-17 expression in the mouse colitis model ($P<0.05$).

Tukey's post-hoc test was carried out to determine the effect of the difference in each group. There were significant differences between Groups 2 compared to Groups 3 and 4 (vitamin D3 doses of 0.2 and 0.4 mcg/25g BW, respectively) ($P<0.001$), as shown in

Table 1. MCHI score in each group of mice

Group	Mean MCHI score (SD)	P value*
1	0.17 (0.41)	
2	18.63 (3.08)	
3	12.6 (3.31)	0.000
4	8.14 (2.73)	
5	3.17 (2.27)	

*one way ANOVA, significant if P< 0.05

Table 2. Analysis of MCHI score by group

Group	Group 1	Group 2	Group 3	Group 4	Group 5
Group 1	-	0.000*	0.000*	0.000*	0.291
Group 2	0.000*	-	0.003*	0.000*	0.000*
Group 3	0.000*	0.003*	-	0.048*	0.000*
Group 4	0.000*	0.000*	0.048*	-	0.015*
Group 5	0.291	0.000*	0.000*	0.015*	-

*Post hoc LSD, significant if P< 0.05

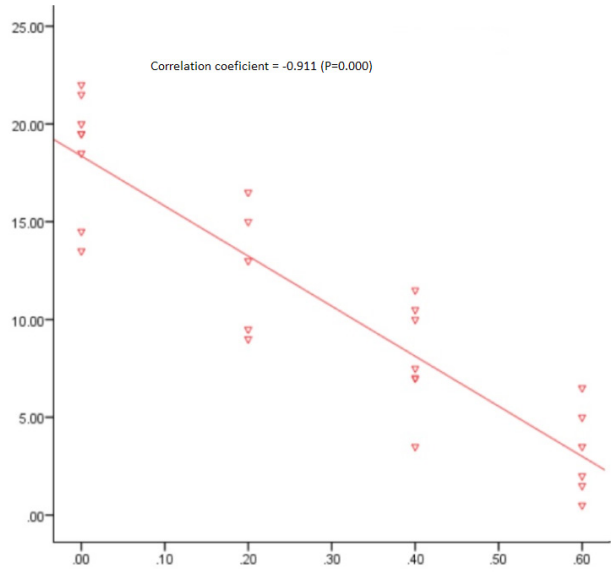

Figure 2. The relationship between vitamin D3 dose and decrease in MCHI

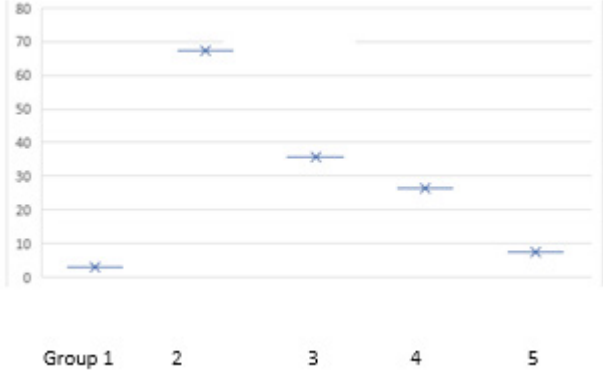

Figure 3. The IL-17 expression in each group (Oneway ANOVA, significant if P< 0.05)

Table 3. However, there was no significant difference between Group 1 (untreatment group) and Group 5 (vitamin D3 dose of 0.6 mcg/25g body weight).

Pearson's correlation test revealed a very strong positive correlation between IL-17 and MCHI score with correlation coeficient r=0.985 (P=0.002), indicating that higher IL-17 expression was associated with higher MCHI score (**Figure 4**).

Table 3. Interleukin-17 expression in each group

Group	Group 1	Group 2	Group 3	Group 4	Group 5
Group 1	-	0.000*	0.000*	0.000*	0.184
Group 2	0.000*	-	0.000*	0.000*	0.000*
Group 3	0.000*	0.000*	-	0.000*	0.000*
Group 4	0.000*	0.000*	0.000*	-	0.000*
Group 5	0.184	0.000*	0.000*	0.000*	-

*Post-hoc Tukey, *significant if P< 0.05

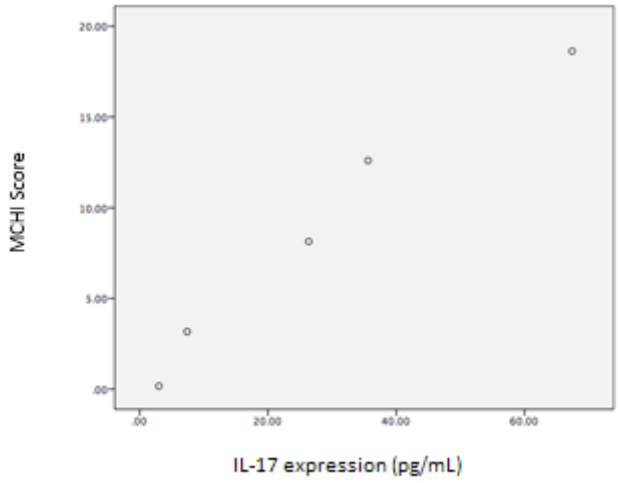

Figure 4. Pearson's correlation analysis of IL-17 expression and MCHI score

Discussion

The MCHI score was significantly higher in Group 2 than in Group 1, the 5% DSS group compared to the control group, indicating that clinical changes in mice that received 5% DSS corresponded to colonic mucosal damage. Similarly, previous studies showed that administration of DSS (40-50 kDa) can cause colitis[13] and there is a significant difference in the damage to the colonic mucosal structure based on the MCHI score between the group given 5% DSS and the control group.[14,15] In various study protocols, DSS (40-50 kDa) added to sterilized drinking water at various concentrations can produce the desired inflammatory effect. At this molecular weight, DSS penetrates the intestinal mucous membrane, as indicated by tissue distribution.[11]

Induction of DSS results in histopathological changes in the form of goblet cell loss, ulceration of the epithelial mucosa, and infiltration of neutrophil granulocytes into the lamina propria and submucosa. Infiltration of inflammatory cells, including neutrophils and macrophages, is a hallmark of the pathophysiology of IBD.[14]

The MCHI scores in the vitamin D3 Groups (3, 4, and 5) were significantly lower than its value in Group 2 (5% DSS alone). There was also a negative correlation between vitamin D3 dose and the MCHI score, in a dose-dependent fashion. Our results were in agreement with several previous studies. A study reported an inhibitory effect of the intestinal vitamin D signaling pathway on DSS-induced necroptosis colitis in IBD patients,[16] while another in-vitro experimental study reported that DSS-induced colonic mucosal damage was more severe in mice with vitamin D receptor (VDR) deletion of the colonic epithelium than in the control group.[17] Moreover, another study showed that vitamin D deficiency in experimental

models caused colitis with clinical symptoms and severe colonic mucosal damage.[18]

In our study, IL-17 expression in Group 2 (5% DSS alone) was significantly higher than in Group 1 (control), indicating that IL-17 expression increases in colitis conditions. The increase in IL-17, which is a pro-inflammatory marker, occurs in the inflammatory process. In this study, the inflammation referred to colonic mucosal inflammation (IBD) induced by 5% DSS. Similarly, two other animal model studies showed significantly higher IL-17 levels in DSS-induced colitis rats than in controls.[19,20] Also, a study reported that the IL-17 levels in peripheral blood mononuclear cells (PBMCs) were significantly higher in rat models of colitis induced with 2,4,6-trinitrobenzenesulfonic acid (TNBS) compared to control.[21]

We also noted a significant correlation between decreased IL-17 and improved MCHI scores in our vitamin D3-administered mice model groups. Previous studies reported that the disease activity index score was significantly lower in the mice groups receiving low and high doses of vitamin D3 compared to the control group, and significantly lower in mice given high doses of vitamin D3 compared to the control group.[22] The IL-17 and IL-17R levels were significantly lower in mice given high doses of vitamin D3 compared to the low dose group.[23] This findings suggest that vitamin D may increase the chemotaxis and differentiation of Th1 cells by inhibiting IL-17/IL-17R signaling, thereby increasing immune function, and reducing IBD disease severity.[24] Mice given vitamin D3 in our study has reduced IL-17 expression and colonic mucosal repair in IBD mice model.

Conflict of interest

None declared.

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References

1. Hegar B, Ananta Y, Rini D. Inflammatory bowel disease in Indonesian children. Paediatr Indones. 2007;47:307-12. DOI: https://doi.org/10.14238/pi47.6.2007.307-12.
2. Rosen MJ, Dhawan A, Saeed SA. 2015. Inflammatory bowel disease in children and adolescent. JAMA Pediatric. 2015;169:1053-60. DOI: https://doi.org/10.1001/jamapediatrics.2015.1982.
3. de Souza WN, Martini LA. The role of vitamin D in obesity and inflammation at adipose tissue. J Obes Metabolic Res. 2015;2;161-6. DOI: https://doi.org/10.4103/2347-9906.162350.
4. Cantorna MT, Mahon BD. Mounting evidence for vitamin D as an environmental factor affecting autoimmune disease prevalence. Exp Biol Med. 2004;229:1136-42. DOI: https://doi.org/10.1177/153537020422901108.
5. Raman M, Milestone AN, Walters JRF, Hart AL, Ghosh S. Vitamin D and gastrointestinal diseases: inflammatory bowel disease and colorectal cancer. Therap Adv Gastroenterol. 2011;4:49-62. DOI: https://doi.org/10.1177/1756283X10377820.
6. Fronczuk M, Raftery AE, Yeung KY. CyNetworkBMA: a Cytoscape app for inferring gene regulatory networks. Source Code Biol Med. 2015;10:11. DOI: https://doi.org/10.1186/s13029-015-0043-5.
7. Del Pinto R, Pietropaoli D, Chandar AK, Ferri C, Cominelli F. Association between inflammatory bowel disease and vitamin D deficiency: a systematic review and meta-analysis. Inflamm Bowel Dis. 2015;21:2708-71. DOI: https://doi.org/10.1097/MIB.0000000000000546.
8. Cantorna MT. Mechanisms underlying the effect of vitamin D on the immune system. Proc Nutr Soc. 2010;69:286-9. DOI: https://doi.org/10.1017/S0029665110001722.
9. Van den Berg WB, Mc Innes IB. 2013. Th17 cells and IL-17 a focus on immunopatogenesis and immunotherapeutics. Semin Arthritis Rheum. 2013;43:158-70. DOI: https://doi.org/10.1016/j.semarthrit.2013.04.006.
10. Fitzpatrick LR. 2012. Novel pharmacological approaches for inflammatory bowel disease: targeting key intracellular pathways and the IL-23/IL-17 axis. Int J Inflam. 2012:389404. DOI: https://doi.org/10.1155/2012/389404.

11. Eichele DD, Kharbanda KK. Dextran sodium sulfate colitis murine model: An indispensable tool for advancing our understanding of inflammatory bowel diseases pathogenesis. World J Gastroenterol. 2017; 23: 6016-29. DOI: https://doi.org/10.3748/wjg.v23.i33.6016.
12. Koelink PJ, Wildenberg ME, Stitt LW, Feagan BG, Koldijk M, van't Wout AB, van den Brink GR. Development of reliable, valid and responsive scoring systems for endoscopy and histology in animal models for inflammatory bowel disease. Journal of Crohn's and Colitis. 2018;12:794-803. DOI: https://doi.org/10.1093/ecco-jcc/jjy035.
13. Kiesler P, Fuss IJ, Strober W. Experimental Models of Inflammatory Bowel Diseases. Cell Mol Gastro-enterol Hepatol. 2015;1:154-70. DOI: https://doi.org/10.1016/j.jcmgh.2015.01.006.
14. Perse M, Cerar A. Dextran sodium sulphate colitis mouse model: traps and tricks. J Biomed Biotechnol, 2012;2012:718617. DOI: https://doi.org/10.1155/2012/718617.
15. Chassaing B, Aitken JD, Malleshappa M, Vijay-Kumar M. Dextran sulfate sodium (DSS)-induced colitis in mice. Curr Protoc Immunol. 2014; 104: Unit-15.25. DOI: https://doi.org/10.1002/0471142735.im1525s104.
16. Abdelmegid AM, Abdo FK, Ahmed FE, Kattaia A. Therapeutic effect of gold nanoparticles on DSS-induced ulcerative colitis in mice with reference to interleukin-17 expression. Sci Rep. 2019 9:10176. DOI: https://doi.org/10.1038/s41598-019-46671-1.
17. Sun Y, Zhao Q, Liu T. Intestinal vitamin D receptor signaling ameliorates dextran sulfate sodium-induced colitis by suppressing necroptosis of intestinal epithelial cells. The FASEB Journal. 2020;34:13494-506. DOI: https://doi.org/10.1096/fj.202000143RRR .
18. Wang H, Chen W, Li D, Yin X, Zhang X, Olsen N, Zheng SG. Vitamin D and Chronic Diseases. Aging Dis, 2017;8:346-53. DOI: https://doi.org/10.14336/AD.2016.1021.
19. Cantorna, Margheritta T, Lin YD, Yang L. Vitamin D and 1,25 (OH)2D regulation of T cells. Nutrients. 2015;7:3011-21. DOI: https://doi.org/10.3390/nu7043011.
20. Singh K, Coburn LA, Barry DP, Asim M, Scull BP, Allaman MM, Lewis ND Washington MK, Rosen MJ, Williams CS, Chaturvedi R, Wilson KT. Deletion of cationic amino acid transporter 2 exacerbates dextran sulfate sodium colitis and leads to an IL-17-predominant T cell response. Am J Physiol Gastrointest Liver Physiol. 2013; 305: G225-40. DOI: https://doi.org/10.1152/ajpgi.00091.2013.
21. He Y, Lin LJ, Zheng CQ, Jin Y, Lin Y. Cytokine expression and the role of Th17 cells in a mouse model of colitis. Molecular Medicine Report. 2012;6:1438-42. DOI: https://doi.org/10.3892/mmr.2012.1111.
22. Nobile S, Tenace MA, Pappa HM. The Role of Vitamin D in the Pathogenesis of Inflammatory Bowel Disease. Gastrointest Disord. 2019;1:231-40. DOI: https://doi.org/10.3390/gidisord1010018.
23. Randhawa PK, Singh K, Singh N, Jaggi AS. A review on chemical-induced in ammatory bowel disease models in rodents. Korean J Physiol Pharmacol. 2014;18:279-88. DOI: https://doi.org/10.4196/kjpp.2014.18.4.279.
24. Saleh MM, Frisbee AL, Leslie JL, Scully KW, Abhyankar MM, Petri WA. Colitis-induced Th17 cells increase the risk for severe subsequent clostridium difficile infection. Cell Host & Microbe. 2019;25:756-65.e5. DOI: https://doi.org/10.1016/j.chom.2019.03.003.