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Research Article

Glucan and Vitamin D supplementation showed synergy in improvements of the immune response against an influenza challenge in mice

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Abstract

Influenza infection remains a serious health problem throughout the world. Unfortunately, current medicine offers no real treatment or protection, moving our attention to alternative options. In this study we aimed to evaluate the possible effects of a combination of glucan and vitamin C on immunosuppression caused by influenza infection. We found that supplementation with this combination significantly improved overall survival and selected immune mechanisms such as phagocytosis, NK cell activity, production of some cytokines and antibody response. Based on our data we can conclude that the positive effects of the glucan-vitamin D combination are based on stimulation of both humoral and cellular immune reaction and result in significant lowering of the viral load.

Keywords: Glucan; influenza; immune system; virus

Introduction

β1,3-D-glucan (hereafter referred to as "glucan") is a member of a group of natural materials generally called biological response modifiers. Glucan is conserved carbohydrate forming structural components of cell walls of yeast, fungi, grains, and seaweed. Glucan consists of various numbers of glucose molecules bound together in various types of linkages.

Glucan was found to have pleiotropic effects on various biological processes, most of all on several aspects of immunity. Among these well-established effects are stimulation of anti-infectious immunity, inhibition of cancer growth, reduction of stress, reduction of cholesterol level or improvements of vaccination (for review, see [1,2]). So far, glucan's effects include both branches of immune reactions and was found effective against every type of infectious agent tested. However, the effects against viral infections have been studied only recently. In a chicken model, glucan supplementation stimulated bone marrow-derived dendritic cells and $CD4^+$ T cell response to bronchitis virus [3]. Glucan treatment has been found to be beneficial in Herpes Simplex Virus infection, where the positive effects were found particularly on respiratory tract [4].

Vitamin D is a member of the steroid superfamily of hormones. The targets of this vitamin include dendritic cells, macrophages and T lymphocytes. Its beneficiary effects include promoting health of bones, regulation of insulin levels, support of lung function, regulation of gut microbiome [5] and reduction of colitis-associated colorectal cancer [6]. Recently, some studies suggested potential role of vitamin D in infections resulting from its effects on the innate and adaptive immune responses. A significant effect is also the suppression of inflammatory processes. Recent review of the possible effects of vitamin D on influenza described some positive effects, but concluded that more randomized controlled trials with effective, large populations are needed to explore the preventive effect of vitamin D supplementation on viral influenza infections [7].

Present study was based on two preliminary results. First, we found in clinical settings that a combination of glucan and vitamin D supplementation improved NK cell activity in patients with diabetic retinopathy [8] and that the same combination changed levels of CRP and leptin [9]. Second, glucan supplementation was found to enhance immune response against influenza [10], which was

further improved by a glucan-sulforaphane combination [11].

Material and Methods

Animals

Female, 8-week old BALB/c mice were purchased from the Jackson Laboratory (Bar Harbor, ME). The protocol for the research project has been approved by the University of Louisville IACUC Committee and it conforms to the provisions of the Declaration of Helsinki (as revised in Edinburgh 2000). Animals were sacrificed by CO2 asphyxiation followed by cervical dislocation.

Material

Yeast-derived insoluble Glucan #300 was purchased from Transfer Point (Columbia, SC). The purity is over 85%. An oral dose of 100 μg/mouse was used. Vitamin D (cholecalciferol, D3) was manufactured by Merck (Darmstadt, Germany). One ml of solution contains 20,000 IU of vitamin D3, one drop contains 500 IU. In our experiments we used a 0.25 IU/mouse dose, vitamin D was dissolved in olive oil.

Cells

Human cell line K562 (ATCC, Manassas, VA), was used in NK cell activity experiments. Cells maintained in culture at 37°C in a humidified atmosphere supplemented with 5% CO_2 in RPMI 1640 medium supplemented with 10% FCS.

Phagocytosis

Plaque assay

Plaque assay for monitoring virus titers of lung homogenates was performed as described previously [12]. Briefly, 10% suspensions of the lung homogenates were examined. Serial dilutions of the samples were inoculated on Madin-Darby canine kidney cells, overlaid with RPMI 1640 medium containing 1% Bacto Agar, incubated for 48 hrs and enumerated.

Antibody titer

Anti-influenza hemagglutination-specific antibodies in serum were measured by ELISA following a previously described protocol [13]. A purified hemagglutination protein was used for plate coating at 2 mg/L concentration.

The virus challenge to mice

Mice were orally treated with the glucan mixture or PBS once a day for 14 days by gavage. At day 14, the same mice were intranasally challenged with the H5N1 A/HK/483 influenza virus (1,000 50% mouse infectious dose diluted in PBS to a 50μl volume) as described previously [14]. Mice were monitored daily for morbidity and measured for survival and body weight changes. The samples were immediately frozen and stored at -80°C for subsequent determination.

Quantification of cytokines

Tissue homogenates were analyzed for the levels of IL-1β, TNF-α and IFN-γ by use of ELISA kits (R&D Systems, Minneapolis, MN) according to the manufacturer's instructions.

In vitro cytotoxicity assay

Assay used to evaluate the NK cell activity was described earlier [15].

Results

By day 12 post-infection, all PBS-treated mice had succumbed, while 50% of glucan-treated mice, 30% of vitamin D-treated and 70% of glucan+vitamin D-treated animals survived (Figure 1). Furthermore, control mice started to regain weight by day 7, but at day 12 (when all died) they still did not reach the weight of the treated groups, which achieved normal weight around day 14 (Figure 2). These differences were, however, not significant.

Glucan is known to improve immune reactions, most of all cellular immunity. We started with evaluation of the effects on phagocytosis. We used a well-established model of 2-hydroxyethylmethacrylate microparticles known for minimal nonspecific adherence to the cell membrane and found that influenza challenge significantly lowered phagocytic activity of peripheral blood neutrophils (Figure 3). All three supplemented groups showed significant improvements of phagocytosis, with the supplementation with glucan+vitamin D combination has the strongest effects.

When we tested the activity of NK cells, we found that both glucan and glucan+vitamin D combination returned the NK cell activity to normal levels, vitamin D alone had no significant activity (Figure 4). Data are from an effector/target ratio of 1:50, but it is important to note that two additional ratios (1:10 and 1:100) offered similar results (data not shown), proving that these effects are not based on a specific effector/target ratio.

In next part of the study we focused on proinflammatory cytokines. We collected lungs following influenza infection and measured IL-1β, TNF-α and IFN-γ levels by ELISA in homogenates on days 1, 3 and 5. We found that in all cases the infection caused an increase in levels of tested cytokines, which reflects the immune reaction to infection. In case of IL-1β, glucan supplementation significantly increased the cytokine reaction in all three tested intervals. The effects of the glucan-vitamin D combo were even stronger, but the differences between glucan and glucan-vitamin D supplemented groups were not statistically significant (Figure 5). Vitamin D alone had no effects. In case of TNF-α, only the glucan-vitamin D combination significantly increased the levels of this cytokines (Figure 6). When we measured the levels of IFN-γ, both glucan and glucan-vitamin D supplementation significantly improved the secretion, and on day 3 and ay 5 the glucan-vitamin D combination was even significantly higher than in glucan-supplemented group (Figure 7).

Not surprisingly, we found robust levels of specific antibodies in the influenza-challenged group. Both glucan and glucan+vitamin D supplementation significantly improved the antibody response (Figure 8). These findings were further supported by evaluation of the dietary supplementation on the viral replication. When we measured the virus titers in the lung, we found significant reduction of virus titers in glucan and glucan-vitamin D supplemented groups starting at day 3. Supplementation vitamin D showed only small nonsignificant improvements (Figure 9).

Data shown in Figure 8 revealed that addition of glucan or glucan-vitamin D combination significantly lowered the virus titer in the thymus. Different situation was found in the heart, where all three supplementation resulted in significant improvements. In the spleen, however, no differences have been found (Figure 10).

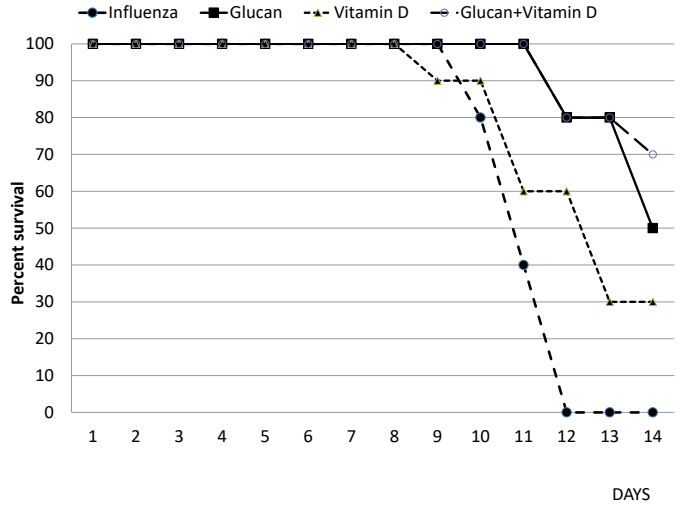

Figure 1: The oral supplementation of tested samples protects mice from lethal infection. All mice were infected with influenza, influenza group was fed with PBS. Ten mice/group.

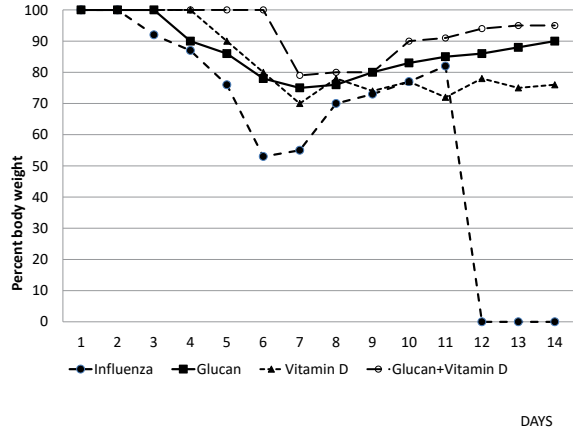

Figure 2: Effects of oral supplementation of tested samples on body weight. All mice were infected with influenza. Ten mice/group.

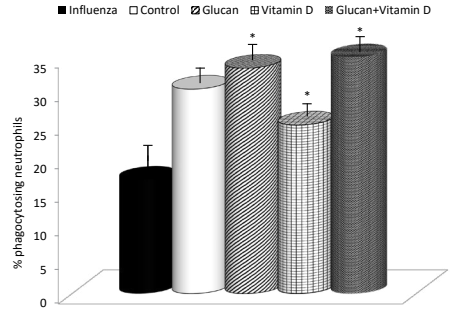

Figure 3: Effects of oral supplementation of tested samples on phagocytosis of synthetic particles by mouse peripheral blood neutrophils. Influenza-treated (Influenza), Influenza + glucan (Glucan), Glucan (Control). Data represents mean ± SD. *Significant differences between control group and experimental groups at P<0.05 level.

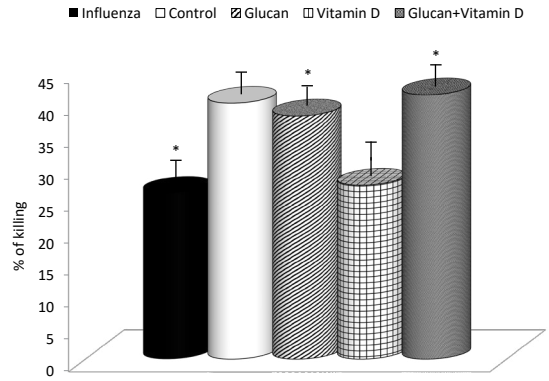

Figure 4: Effects of the glucan treatment on NK cell activity of mouse splenocytes. Influenza-treated (Influenza), Influenza + glucan (Glucan), Influenza + vitamin D (Vitamin D), Influenza + glucan + vitamin D (Glucan+vitamin D) or PBS (Control). Data represents mean ± SD. *Significant differences between Influenza and experimental groups at P<0.05 level.

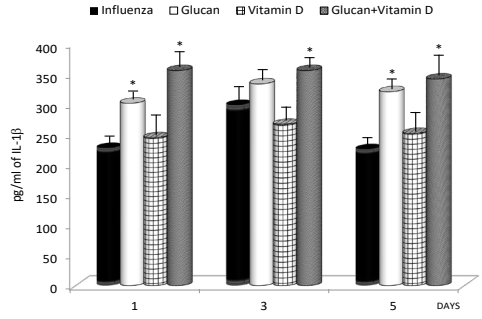

Figure 5: Evaluation of IL-1β levels in lungs. Influenza-treated (Influenza), Influenza + glucan (Glucan), Influenza + vitamin D (Vitamin D), Influenza + glucan + vitamin D (Glucan+vitamin D) or PBS (Control). Data represents mean ± SD. *Significant differences between individual supplemented groups and Influenza group at P<0.05 level.

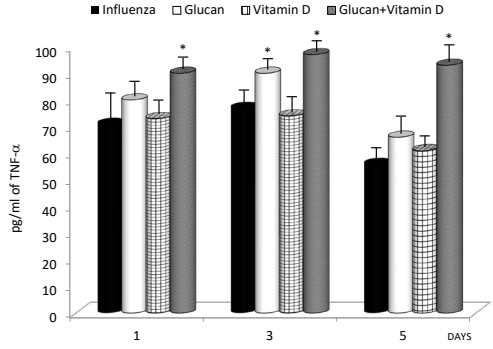

Figure 6: Evaluation of TNF-α levels in lungs. Influenza-treated (Influenza), Influenza + glucan (Glucan), Influenza + vitamin D (Vitamin D), Influenza + glucan + vitamin D (Glucan+vitamin D) or PBS (Control). Data represents mean ± SD. *Significant differences between individual supplemented groups and Influenza group at P<0.05 level.

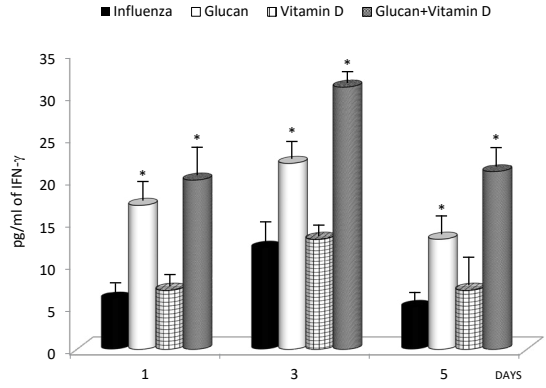

Figure 7: Evaluation of IFN-γ levels in lungs. Influenza-treated (Influenza), Influenza + glucan (Glucan), Influenza + vitamin D (Vitamin D), Influenza + glucan + vitamin D (Glucan+vitamin D) or PBS (Control). Data represents mean ± SD. *Significant differences between individual supplemented groups and Influenza group at P<0.05 level.

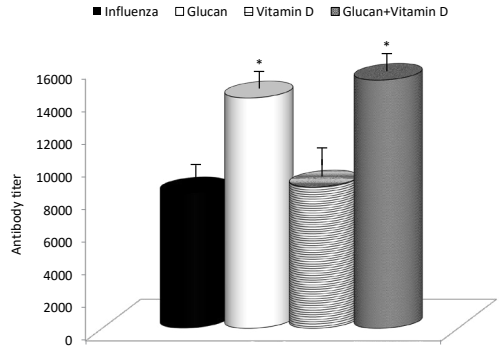

Figure 8: Dietary glucan (14 days) potentiated the antibody response induced by influenza infection. Influenza group was fed with PBS. Ten mice/group. Data represents mean ± SD. *Significant differences between individual supplemented groups and Influenza group at P<0.05 level.

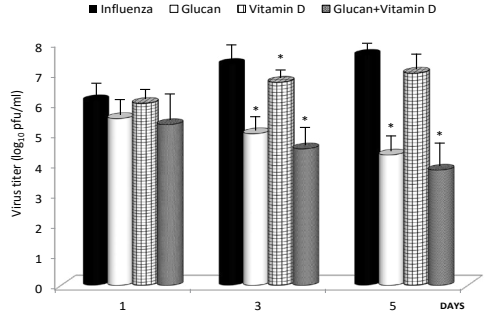

Figure 9: Effects of glucan on virus titers in lung. Ten mice/group. Data represents mean ± SD. *Significant differences between individual supplemented groups and Influenza group at P<0.05 level.

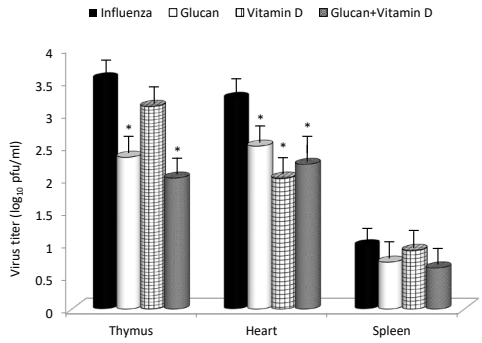

Figure 10: Effects of glucan on virus titers in thymus, heart and spleen measured at day 5 after infection. Ten mice/group. Data represents mean ± SD. *Significant differences between individual supplemented groups and Influenza group at P<0.05 level.

Discussion

Influenza epidemics occur every year in the United States and worldwide with app. 500,000 people dying every year [16]. In addition, the current coronavirus infection focused out attention on possible ways how to improve the immune system even more than before. Various immunomodulators have been studies, partly alone, partly in combination with vaccines. Results often shown improvements of the antiviral response [17,18].

Biological effects of glucan are well established. Immune system is one of the biological systems which benefits most from glucan actions. The antiviral activity of glucan is much less studied and our knowledge is limited. However, some studies exist. One study suggested that glucan treatment might be effective for the prevention of influenza in animals [19]. Strong antiviral effects of glucan on same type of infection were confirmed on swine model [20]. Our own studies found similar effects, both when the mice were supplemented by glucan alone or in combination with sulforaphane [10,11].

Vitamin D has been implicated in the pathophysiology of numerous inflammatory diseases such as rheumatoid arthritis of Crohn disease. Supplementation with vitamin D lowers the risk of development of diabetes mellitus [21]. In addition, vitamin D mitigates dangerous effects of bacterial infection on the mucosa [22]. Deficiency in vitamin D results in impaired colonic antibacterial activity [23].

As we previously observed the synergistic effects of glucan and vitamin D supplementation on various aspects of immune reactions [8,9], we decided to evaluate the effects of glucan and vitamin D combination on stimulation of immune responses to influenza challenge.

Influenza infections triggers a whole cascade of immune reactions, including changes in phagocytosis, antibody formation and secretion of various cytokines, both with immunomodulatory effects and closely associated with pathology of infection. The increased production of cytokines occurs in the spleen, lymph nodes, heart, and lungs [24,25].

Our finding showing that supplementation with glucan and/or glucan and vitamin D returned the virus-mediated depression of phagocytic activity to normal levels supported the older data showing glucan improving innate immune reactions depressed by various toxins [2]. Similar changes were found in case of NK cell activation and production of specific antibodies. The increased antibody levels are consistent with the smaller weight loss and lower morbidity in animals supplemented with glucan and particularly with glucan and vitamin D combo.

Changes in cytokine production are more difficult to interpret, as the full contribution of individual cytokines produces as a result of viral infection is unknown. However, IFN-γ has protective effects against infections; some others (such as IL-1, IL-6, and TNF-α) are more involved in inflammatory phase of infection. Our data showed significant improvements by glucan and vitamin D combinations of the already elevated levels of IL-1β, IFN-γ and TNF-α. The kinetics of time changes corresponds to the older data [14].

Our results confirmed our previous studies on glucan and influenza infection. As the previous experiments used different types of glucan, we can conclude that these glucan effects are general and not dependent on a special type of glucan. On the other hand, it is still true that individual glucans differ in the strength of their activities. Based on our data we can conclude that the positive effects of the glucan-vitamin D combination are based on stimulation of both humoral and cellular immune reaction and result in significant lowering of the viral load.

References

1. Vetvicka V. β-Glucans as Natural Biological Response Modifiers. Nova Science Publishers, Incorporated; 2013.
2. Vetvicka V, Novak M, editors. Biology and Chemistry of Beta Glucan. Bentham Science Publishers; 2013 May 24.
3. Larsen FT, Guldbrandtsen B, Christensen D, Pitcovski J, Kjærup RB, Dalgaard TS. Pustulan activates chicken bone marrow-derived dendritic cells in vitro and promotes ex vivo CD4+ T cell recall response to infectious bronchitis virus. Vaccines. 2020 Jun;8(2):226.
4. Urbancikova I, Hudackova D, Majtan J, Rennerova Z, Banovcin P, Jesenak M. Efficacy of Pleuran (β-Glucan from Pleurotus ostreatus) in the Management of Herpes Simplex Virus Type 1 Infection. Evidence-Based Complementary and Alternative Medicine. 2020 Apr 13;2020.
5. Ooi JH, Li Y, Rogers CJ, Cantorna MT. Vitamin D regulates the gut

microbiome and protects mice from dextran sodium sulfate–induced colitis. The Journal of Nutrition. 2013 Oct 1;143(10):1679-86.

6. Elimrani I, Koenekoop J, Dionne S, Marcil V, Delvin E, Levy E, Seidman EG. Vitamin D reduces colitis-and inflammation-associated colorectal cancer in mice independent of NOD2. Nutrition and Cancer. 2017 Feb 17;69(2):276-88.
7. Gruber-Bzura BM. Vitamin D and influenza—prevention or therapy? International Journal of Molecular Sciences. 2018 Aug;19(8):2419.
8. Josef R, Jitka P, Martina Z, Vlastimil K, Ivana S, Lucie DR, Vaclav V. Concentration of NK cells after β-glucan and vitamin D supplementation in patients with diabetic retinopathy. Folia Microbiologica. 2020 Apr 4:1-7.
9. Richter J, Závorková M, Vetvicka V, Liehneová I, Kral V, Rajnohova Dobiasova L. Effects of β-glucan and Vitamin D Supplementation on Inflammatory Parameters in Patients with Diabetic Retinopathy. Journal of dietary supplements. 2019 Jul 4;16(4):369-78.
10. Vetvicka V, Vetvickova J. Glucan supplementation enhances the immune response against an influenza challenge in mice. Annals of Translational Medicine. 2015 Feb;3(2).
11. Vaclav V, Jana V. A Novel Glucan-Sulforaphane Combination Stimulates Immune Response to Influenza in Mouse Model. American Journal of Immunology. 2016; 12:20-8.
12. Takada A, Matsushita S, Ninomiya A, Kawaoka Y, Kida H. Intranasal immunization with formalin-inactivated virus vaccine induces a broad spectrum of heterosubtypic immunity against influenza A virus infection in mice. Vaccine. 2003 Jul 4;21(23):3212-8.
13. Wen Z, Ye L, Gao Y, Pan L, Dong K, Bu Z, et al. Immunization by influenza virus-like particles protects aged mice against lethal influenza virus challenge. Antiviral Research. 2009 Dec 1;84(3):215-24.
14. Szretter KJ, Gangappa S, Lu X, Smith C, Shieh WJ, Zaki SR, et al. Role of host cytokine responses in the pathogenesis of avian H5N1 influenza viruses in mice. Journal of virology. 2007 Mar 15;81(6):2736-44.
15. Vetvicka V, Vetvickova J. Fucoidans stimulate immune reaction and suppress cancer growth. Anticancer Research. 2017 Nov 1;37(11):6041-6.
16. Stohr K. Influenza—WHO cares. The Lancet Infectious Diseases. 2002 Sep 1;2(9):517.
17. Zheng BJ, Chan KW, Lin YP, Zhao GY, Chan C, Zhang HJ, et al. Delayed antiviral plus immunomodulator treatment still reduces mortality in mice infected by high inoculum of influenza A/H5N1 virus. Proceedings of the National Academy of Sciences. 2008 Jun 10;105(23):8091-6.
18. Norton EB, Clements JD, Voss TG, Cárdenas-Freytag L. Prophylactic administration of bacterially derived immunomodulators improves the outcome of influenza virus infection in a murine model. Journal of Virology. 2010 Mar 15;84(6):2983-95.
19. Muramatsu D, Iwai A, Aoki S, Uchiyama H, Kawata K, Nakayama Y, et al. β-glucan derived from *Aureobasidium pullulans* is effective for the prevention of influenza in mice. PLoS One. 2012 Jul 23;7(7):e41399.
20. Jung K, Ha Y, Ha SK, Han DU, Kim DW, Moon WK, et al. Antiviral Effect of *Saccharomyces cerevisiae* β-glucan to Swine Influenza Virus by Increased Production of Interferon-γ and Nitric Oxide. Journal of Veterinary Medicine, Series B. 2004 Mar;51(2):72-6.
21. Pickup JC. Inflammation and activated innate immunity in the pathogenesis of type 2 diabetes. Diabetes care. 2004 Mar 1;27(3):813-23.
22. Assa A, Vong L, Pinnell LJ, Rautava J, Avitzur N, Johnson-Henry KC, et al. Vitamin D deficiency predisposes to adherent-invasive *Escherichia coli*-induced barrier dysfunction and experimental colonic injury. Inflammatory bowel diseases. 2015 Feb 1;21(2):297-306.
23. Lagishetty V, Misharin AV, Liu NQ, Lisse TS, Chun RF, Ouyang Y, et al. Vitamin D deficiency in mice impairs colonic antibacterial activity and predisposes to colitis. Endocrinology. 2010 Jun 1;151(6):2423-32.
24. Hoeve MA, Nash AA, Jackson D, Randall RE, Dransfield I. Influenza virus A infection of human monocyte and macrophage subpopulations reveals increased susceptibility associated with cell differentiation. PloS One. 2012 Jan 4;7(1):e29443.
25. Han SN, Meydani SN. Antioxidants, cytokines, and influenza infection in aged mice and elderly humans. The Journal of infectious diseases. 2000 Sep 1;182(Supplement_1):S74-80.