ORIGINAL RESEARCH PAPER

Endocrinology

VITAMIN D THERAPY IMPROVES THYROID FUNCTION AND AUTOIMMUNITY IN HASHIMOTO THYROIDITIS - ONE OF THE FIRST DOUBLE-BLIND PLACEBO-CONTROLLED RCT IN A TERTIARY CARE HOSPITAL IN SOUTH ASIA

KEY WORDS: Hashimoto disease, thyrotropin, hypothyroidism, vitamin D deficiency, vitamin D

Debarati Bhar — Post-Doctoral Trainee, Department of Endocrinology and Metabolism, Institute of Post Graduate Medical Education and Research and Seth Sukhlal Karnani Memorial Hospital, Kolkata, West Bengal, India

Chiranjit Bose — Junior Research Fellow, Department of Endocrinology and Metabolism, Institute of Post Graduate Medical Education and Research and Seth Sukhlal Karnani Memorial Hospital, Kolkata, West Bengal, India

Nihar Ranjan Sarkar — Associate Professor, Department of Radiodiagnosis, Institute of Post Graduate Medical Education and Research and Seth Sukhlal Karnani Memorial Hospital, Kolkata, West Bengal, India

Subhankar Chowdhury — Professor and Head of the Department, Department of Endocrinology and Metabolism, Institute of Post Graduate Medical Education and Research and Seth Sukhlal Karnani Memorial Hospital, Kolkata, West Bengal, India

Satinath Mukhopadhyay* — Professor, Department of Endocrinology and Metabolism, Institute of Post Graduate Medical Education and Research and Seth Sukhlal Karnani Memorial Hospital, Kolkata, West Bengal, India *Corresponding Author

ABSTRACT

Objective: Extra-skeletal benefits of 25hydroxy vitamin D in other autoimmune diseases are well known, but its role in autoimmune thyroiditis is yet to be proved in vivo, though an association was suggested by different studies. We aimed to show the beneficial impact of vitamin D therapy in the pathogenesis of this disease even without levothyroxine supplementation.

Design: A randomized double-blinded placebo-controlled trial.

Patients: 140 patients of subclinical autoimmune thyroiditis chosen by purposive sampling randomized in two groups: intervention and placebo, were weekly given 60000 units of cholecalciferol or placebo for eight weeks, followed by monthly for six months.

Measurements: Done as follows: 25 hydroxy vitamin D (ng/ml), free tetraiodothyronine (ng/dl), thyroid stimulating hormone (mIU/ml), thyroid peroxidase, thyroglobulin antibodies (IU/l).

Results: Decline in thyroid stimulating hormone (mean ± standard error 6.26 ± 0.39 mIU/ml to 3.23 ± 0.24 mIU/ml), thyroglobulin antibody (mean ± standard error 246.2 ± 124.3 IU/l to 69.74 ± 27.22 IU/l), rise in free tetraiodothyronine (mean ± standard error 1.039 ± 0.02 ng/dl to 1.243 ± 0.05 ng/dl) at end of six months of cholecalciferol therapy observed in a part of the intervention group (named 'responders') compared to placebo.

Conclusion: One of the earliest studies in South East Asia that unveiled a robust association of 25OH vitamin D therapy and thyroid function. The overall intervention group did not reach statistical significance. The reason why some patients are more likely to respond to cholecalciferol therapy while some do not, is unknown and needs further research. Overt cases of hypothyroidism were excluded.

The RCT is registered with www.ctri.nic.in, registration number CTRI/2017/05/008596.

INTRODUCTION

Hashimoto disease or chronic autoimmune thyroiditis (AITD) is the commonest cause of hypothyroidism worldwide. It may manifest as a subclinical or overt disease. Pathologically, there is infiltration of thyroid follicles by T cells that leads to inflammation and fibrosis. It is serologically characterized by circulating autoantibodies to thyroid peroxidase (TPOAb) and thyroglobulin (TgAb).[1] Levothyroxine supplementation is the only treatment for this condition and no therapy to modify its pathogenesis is available till date. Studies have shown a prevalence of 3.9% of overt and 11.7% of subclinical disease in an Asian population with a female: male ratio of 3.7:1.[2,3] Deficiency of 25OH vitamin D is rampant, especially in Southeast Asia even in tropical latitude.[4-6] Vitamin D may have a role to play in modifying the autoimmune state of this common thyroid disease. Several extraskeletal benefits of vitamin D have been exploited including suppression of autoimmune conditions. However, its use in AITD is not yet validated by well-designed randomized controlled trials (RCTs), though many in vitro and observational studies have shown its efficacy.[7] A previous study in India by S. Choudhary et.al *IJEM* in 2016 showed a definitive decline in autoantibody titers after 25OH vitamin D supplementation in newly diagnosed AITD. It was an open-label study without any evaluation of thyroid function.[8] Other similar studies of shorter duration were carried out by Talei A. et.al on 201 patients and Vahabi A.P.et al. on 56 patients in Iran.[9,10] Our study is a placebo-controlled double-blinded RCT, one of the first few studies in South Asia, wherein thyroid function and antibody titers against both thyroid peroxidase(TPO Ab) and thyroglobulin (Tg Ab) have been evaluated following a 6 month course of 60000 units of cholecalciferol on weekly basis for first 6 weeks followed by monthly basis for next 6 months vis a vis placebo.

The **hypothesis** behind the study is that standard therapeutic doses of vitamin D therapy can restore the altered thyroid function, in treatment-naive cases of subclinical hypothyroidism due to AITD, especially when used in long term over 6 months. It can halt the underlying autoimmune processes by suppressing innate immunity, decrease proliferation and activity of antigen presenting cells, and prevent migration of harmful helper T cell and memory cells, polarize the adaptive immune system from pro-inflammatory toward the anti-inflammatory state. This, in turn, restores thyroid follicle back to normal hormone production and lowers thyrotropin (TSH) by negative feedback.

Overall prevalence of 25OH D deficiency (<20ng/ml) in the study is 98.6% (71.4% have moderate deficiency (12-20

ng/ml) and 27.1% severe deficiency (<12 ng/ml); the findings are similar to Tamer et.al and Taalei et.al (92%) Iran, Halder et al (78%), Chaudhary et al(74%) in India.[9-13] Our study depicted a statistically significant 48% fall in mean TSH and 21.6% rise of mean free tetraiodothyronine (FT4)levels that the 'responder arm' in group A (who received six months of cholecalciferol therapy). Similar results have been found in other studies as well, viz.66.6% rise in free T4 (Simcek et.al.); 15.4% and 14.6% fall in TSH (Taalei, et.al, Simcek et.al. respectively.)[9,14]

MATERIALS AND METHODS

The study was done over a period of about 2 years from 2017 to 2019 in a tertiary care hospital in Kolkata in eastern India. Consecutive patients aged 18 years and above of either genders, positive for TPO (> 35 IU/l upper normal lab limit) or Tg antibody (> 40 IU/l upper normal lab limit) with normal free T4 (lab reference normal range 0.8-1.9 ng/dl) and any TSH value were screened in OPD and indoor of Endocrinology and Medicine departments of our hospital. Over 300 were screened by above criteria, excluding those suffering from diseases like chronic renal or liver diseases, tuberculosis, drugs like phenytoin, phenobarbital, rifampin, orlistat, steroid; calcium, levothyroxine recipients in last 6 months; pregnancy and lactation.[1, 15] After randomization in two groups of 70 each, each person was asked to drink sachets of cholecalciferol (group A) or placebo (group B), dissolved in milk by a blinded pharmacist weekly for initial eight weeks, then monthly for next six months. 118 persons completed the trial, unfortunately, twenty-two persons were lost during follow up (ten in group A and twelve in group B). A formal clinical history and physical examination were recorded in a case based proforma; anthropometric variables like weight and height were measured by standard electronic weighing sale and stature meter respectively; plasma total 25(OH)D was measured by EUROIMMUN ELISA (analyte sensitivity 1.6 ng/ml; range 1.6–99.8 ng/ml; intra- and inter-assay coefficient of variation [CV] 3.2-4.9% and 7.8-8.6%, respectively); TPOAb, TgAb, TSH by Immulite-1000 (two-site immunometric assay); free T4 by chemiluminescent, competitive immunoassay; (analyte sensitivity, range, intra-and inter-assay CV for respective analytes-- TSH: 0.004 micro IU/ml, (range: 0.004–75 IU/mL, CV 4.5–13.8% and 8–17.5%); free T4: 0.3 ng/dl (range 0.3- 6ng/dl, CV 4.1–9.8% and 5.2–12.1%); TPO-Ab: 7 IU/mL, (range 7-1000 IU/L, CV 3.5–5.6% and 7.8–10.5%); Tg-Ab: 10 IU/ml (range 10-3000IU/ml, CV 2.3-3.9% and 6.9-8.1%). Sonographic evaluation of thyroid volume was performed by a single blinded operator using a Philips make ultasound (USG) machine, 7.5–12 MHz transducer; volume of the gland was calculated using anteroposterior(AP), width(W) and length (D) of each lobe (volume V= 0.479X AP x W x D)[15] with a mention of any diffuse hypo-echogenicity of the gland with respect to surrounding neck muscles.[8,16,17]

Serum total calcium was measured by arsenazo method (analyte sensitivity, intra assay sensitivity, inter-assay sensitivity-0.6 mg/dl, range 8.6-10.2 mg/dl, CV 0.89-2.05% and 1.6-1.7% respectively); serum albumin by bromocresol green method (analytical sensitivity, range, intra assay sensitivity, inter-assay sensitivity-0.1 g/dl, range3.5-5.2 mg/dl, 0.51-1.29% respectively). Since sunlight exposure could vary among two study groups, patient self-reported data were obtained based on a simple questionnaire about sunlight exposure in order to gain a simplistic idea of matching sun exposure in between the groups.

At end of first 6 months, samples of study subjects of both groups were re-analyzed using the same methodology as described above for parameters-25 OH D status, thyroid function (free T4, TSH, autoantibodies (TPOAb, TgAb) and thyroid sonographic volume. Standard statistical data analysis was done by the mean, standard error of the mean (SEM) for normally distributed numerical variables, the median and inter-quartile range for skewed numerical variables, inter group comparison by student's independent sample '*t*' test, counts by Chi-square test; p-value <0.05 was regarded as statistically significant. Microsoft SPSS version 21.0 and Graph Pad Prism 6.0 software were used for computation.

RESULTS

Power analysis: Sample size was calculated based on the anticipated difference in TSH levels that can be brought about by cholecalciferol supplementation as projected from earlier studies. It was estimated that 63 subjects will be required per group to detect a difference of 1.5IU/ml in TSH with 80% power and 5% probability of Type 1 error. Assuming a further 10% dropout rate, the recruitment target was 140 overall (assuming an SD of 3IU/ml and 2-sided testing; sample size calculation was done using n-Master 2.0(Department of Biostatistics, Christian Medical College, Vellore).

Demographic factors:- Mean age was 34.6 years; female (84.3 %) versus male (15%), ratio of 5.6:1. Rural preponderance (84.3%) in our study population was striking even though based on a tertiary care hospital in a metro city and disproves the so-called urban bias. Baseline demographic, thyroid function, autoimmunity were matched in between two groups (intervention (A) and placebo (B) after randomization (Table 1). At end of the study, 62 out of 65 patients in group A (95.4%) achieved a serum 25OH vitamin D level of 20—50 ng/ml which was adequate[11], signifying satisfactory compliance to therapy. Out of 140 patients, 118 completed the study and rest were lost during follow up. No significant adverse effect of therapy was noted in either group.

Thyroid function tests:- Serum free T4, TSH, TPO and Tg antibody levels in group A at end of six months did not show a statistically significant change after six months of cholecalciferol therapy compared to the placebo group. Group A was further stratified based on the change in TSH levels as either a 'responder' whose serum TSH declined at the end of first six months of cholecalciferol therapy or 'non-responder' whose TSH remained same or increased. In the sub-group analysis, the 'responder' arm showed a statistically significant increment of free T4 and fall in TSH and TgAb levels {mean FT4: 1.243 ± 0.05 ng/dl at end, 1.039 ± 0.02 ng/dl at start; p-value 0.002; figure 1A}, {mean TSH: 3.23 ± 0.24 mIU/ml at end, 6.26 ± 0.39 mIU/ml at start; p-value 0.004; figure 1B}, {mean TgAb: 69.74 ± 27.22 IU/l at end, 246.2 ± 124.3 IU/l at start; p-value 0.04; figure 1C; Table 2). Unfortunately, TPOAb failed to reach statistical significance. There was no significant change in the mean volume of either thyroid lobes assessed sonographically. TgAb showed moderate negative correlation with 25OHD, TPOAb did not show any correlation (r = -0.209 for Tg & -0.03 for TPO; p = ns; figure 2B). As expected, Tg antibodies positively correlated with TPOAb (r = 0.25; p < 0.01, figure 2A). All results were done from a single lab of our hospital to minimize inter-assay variability, and listed as mean +/- standard error (SEM).

DISCUSSION

To the best of our knowledge, this is one of the first placebo-controlled RCT designed to investigate the impact of 25OH vitamin D therapy in subclinical Hashimoto's thyroiditis in South Asia. The primary objective of this study is to investigate any improvement in thyroid function in subjects of subclinical autoimmune hypothyroidism after cholecalciferol 60000 units weekly therapy for eight weeks and monthly therapy to six months. A previous open labelled study by Chaudhary, et al. had already demonstrated a fall of TPO and Tg titers with cholecalciferol therapy in a similar population group without measuring its impact on thyroid function.[8] Prevalence of vitamin D deficiency in our population probably relates to a sedentary lifestyle, with limited, indoor physical activity (81.4% occupied in desk/household work; use of traditional clothing and very hot summers). Mean serum 25OHD levels (32.18±0.78 ng/ml) at six months has been satisfactory

without any adverse reaction, toxic levels or significant malabsorption, suggesting such therapy is potentially safe.

Tetraiodothyronine (T4) is the major circulating thyroid hormone in plasma (0.02%-free, rest plasma protein bound). It is a sensitive barometer of thyroid function, especially in the background of subclinical hypothyroidism. A falling level may indicate gland failure and future progression to overt disease. The normal range for free T4 (FT4) is 9 to 30 pmol/L (0.7 to 2.5 ng/dl).[18] Greater rise of FT4 in other studies compared to ours may be explained from the inclusion of both overt and subclinical cases. Greater reduction of TSH in our study may be explained from longer periods of cholecalciferol therapy (six months) compared to other studies (four to twelve weeks). TSH or thyrotropin is secreted by pituitary thyrotrophs. It is the principal stimulant for thyroid hormonogenesis and trophic factor. The rate of TSH secretion follows exquisitely sensitive inverse log-linear relationship to plasma concentrations of FT4. The normal range of serum TSH by immunometric assay is stable-0.4 to 4.2 micro IU/ml that includes 96% of disease and risk-free population.[18] In the placebo group at end of six months, the occurrence of a 20.9% rise in TSH (mean 7.57±0.32 ng/dl) and fall in FT4 reflects the tendency in the natural history of Hashimoto's to progress to overt disease with time in absence of intervention. TPOAb (thyroid peroxidase) titers do not change significantly at the end of six months.

A significant 31% drop in TgAb (thyroglobulin) titers is demonstrable at the end of six months of therapy in 'responder arm' of group 'A'(intervention). Other studies, viz. Simcek et.al[29] have shown a similar drop in TPO-Ab (before therapy- 278.3 ± 218.4 IU/ml, after therapy-267.9 ± 200.7 IU/ml) and Tg-Ab (before- 331.9 ± 268.1 IU/ml and after-275.4 ± 187.3 IU/ml); Chaudhary et.al[8] - TPO-Ab {before therapy - 832 ±260 IU/ml and after therapy -739 ±343 IU/ml}. Compared to the general population (8-27%), the prevalence of these antibodies in AITD (autoimmune thyroiditis) is almost 95-100%. Hence, higher initial autoantibody titers with a declining trend post 25OHD replacement likely suggest a waning of the autoimmune process, especially in the context of a robust change in thyroid function in the responder arm of our intervention group. Confounding factors like non-autoimmune thyroid disease, polyclonality, variable epitope recognition and affinity, etc. affect Tg-Ab and TPO-Ab. They exhibit a secondary immunoglobulin response to T cell-mediated thyroid injury and chronicity and are not directly linked to causality.[18]

Sonographic changes: - Baseline echogenic pattern is found to be heterogeneous in 70.7% persons in our study; at end of first 6 months, there is no significant change in either lobar volume or echogenicity in both groups (Anderson et.al.–similar USG pattern in 43% of patients.)[19] The higher prevalence of heterogeneous echotexture in our study cohort may relate to a lesser number of newly diagnosed cases and longer disease duration at the inclusion point.[20]

A plausible mechanism of action of 25OH vitamin D on thyroid in AITD is in amelioration of the autoimmune process-> restoration of function and activity of thyroid follicle to normalcy normalization of FT4 negative feedback on hypothalamo-pituitary axis decline in TSH to normal range.

The exact mechanism of autoimmune modulation of 25OHD is not fully elucidated. Calcitriol, the active form of vitamin D, acts by binding to vitamin D receptors (VDRs) that are present in almost all the organs of the body including activated T and B cells and monocytes that invade the thyroid and prevent them from triggering autoimmune destruction of the follicular cells. It possibly modifies adaptive immunity by decreasing growth and proliferation of activated CD4+ helper T cells, creating anergy, decreasing expression of co-stimulatory molecules, down-regulate receptors for cytokines- IFN-gamma, TNFα, IL-2, and prevents the generation of a pro-inflammatory signal and favours an inhibitory signal to shut down the vicious cascade. It also prevents growth and proliferation of B cells from progenitor cells by its influence on brake-points in the cell cycle, prevents antibody formation and complement-mediated cell destruction. $1,25(OH)_2D$ (calcitriol) shifts polarization of T cells from a Th1 and Th17pro-inflammatory phenotype to a Th2 anti-inflammatory phenotype.[21] It decreases the CD4: CD8 ratio.[22] It modulates innate immunity by decreasing expression of MHC-class II (major histocompatibility complex) and co-stimulatory molecules on the surface of intra-thyroidal macrophages, dendritic and other antigen presenting cells. It prevents differentiation and maturation and survival of dendritic cell (DC), eliminating initial trigger for exaggerated maladaptive T cell response. Moreover, $1,25(OH)_2D$ (calcitriol) also modulates DC-derived cytokine expression by inhibiting the production of interleukin (IL)-12 and IL-23 (major cytokines that drive Th1 differentiation) and IL-17 producing T helper (Th17) differentiation, respectively. It enhances the release of anti-inflammatory cytokine IL-10.[6] Finally, $1,25(OH)_2D$ inhibits B cell proliferation and differentiation into plasma cells, immunoglobulin secretion (IgG and IgM), memory B cell generation, and induces B cell apoptosis.[21,23-27]

Though our study conclusively reveals attainment of normalization of thyroid function in autoimmune thyroiditis among a subset of subclinical hypothyroid patients (defined as responders) by using six months of cholecalciferol therapy alone, without the need of hormonal (levothyroxine) supplementation, there are some obvious limitations. Other confounders like parathormone, vitamin D binding protein, etc. are not assessed. No quantitative measure of sunlight exposure can be documented. It is a hospital-based and not community study, so the study population may not be truly representative and hospital referral bias may have been present. No head to head comparison has been done between cholecalciferol and levothyroxine therapy as patients of overt hypothyroid on hormone therapy are excluded from the study. The dropout rate is 15.7% and the size of the study population is not large enough. It demands further research into the insights of molecular mechanisms and the clinico-biochemical relationship of this sunshine vitamin in thyroid autoimmunity that may explain certain enigmas in our study like why some people tend to respond better to cholecalciferol therapy than others.

REFERENCES

1. Jeffrey, R., et al. "ATA/AACE Guidelines for Hypothyroidism in Adults." Endocr Pract 2012;18(6):6-42.
2. Unnikrishnan, A. G., & Menon, U. V. Thyroid disorders in India: An epidemiological perspective. Indian journal of endocrinology and metabolism 2011;15(Supple2),S78:78-81
3. Deshmukh, V., Behl, A., Iyer, V., Joshi, H., Dholye, J. P., & Varthakavi, P. K. Prevalence, clinical and biochemical profile of subclinical hypothyroidism in normal population in Mumbai. Indian journal of endocrinology and metabolism 2013;17(3):454.
4. Harinarayan, C. V., & Joshi, S. R. Vitamin D status in India–its implications and remedial measures. JAPI 2009;57:40-48.
5. Marwaha, R. K., & Sripathy, G. Vitamin D & bone mineral density of healthy school children in northern India. Indian Journal of Medical Research 2008;127(3):239-244
6. Harinarayan, C. V. Prevalence of vitamin D insufficiency in postmenopausal south Indian women. Osteoporosis International 2005;16(4):397-402.
7. Consolini, R., Pala, S., Legitimo, A., Crimaldi, G., Ferrari, S., & Ferrari, S. Effects of vitamin D on the growth of normal and malignant B-cell progenitors. Clinical & Experimental Immunology 2001;126(2):214-219.
8. Chaudhary, S., Dutta, D., Kumar, M., Saha, S., Mondal, S. A., Kumar, A., & Mukhopadhyay, S. Vitamin D supplementation reduces thyroid peroxidase antibody levels in patients with autoimmune thyroid disease: An open-labeled randomized controlled trial. Indian journal of endocrinology and metabolism 2016;20(3):391-8.
9. Talaei, A., Ghorbani, F., & Asemi, Z. The effects of Vitamin D supplementation on thyroid function in hypothyroid patients: A randomized, double-blind, placebo-controlled trial. Indian journal of endocrinology and metabolism 2018;22(5):584-8.
10. Anaraki, P. V., Aminorroaya, A., Amini, M., Momeni, F., Feizi, A., Iraj, B., & Tabatabaei, A. Effect of Vitamin D deficiency treatment on thyroid function and autoimmunity markers in Hashimoto's thyroiditis: A double-blind randomized placebo-controlled clinical trial. Journal of research in medical sciences: the official journal of Isfahan University of Medical Sciences 2017;22:103.

11. Ross, A. C., Taylor, C. L., Yaktine, A. L., & Del Valle, H. B. Committee to Review Dietary Reference Intakes for Vitamin D and Calcium, Institute of Medicine. Dietary reference intakes for calcium and vitamin D 2011. Cited Jun 18, 2012. Available at National Academy Press, Washington, DC.
12. Tamer, G., Arik, S., Tamer, I., & Coksert, D. Relative vitamin D insufficiency in Hashimoto's thyroiditis. Thyroid 2011;21(8):891-896.
13. Halder, T., Dastidar, R., Bhattacharya, S., & Maji, D. Prevalence of Hashimoto's Thyroiditis and its association with Vitamin D deficiency in West Bengal, India. British Journal of Medicine and Medical Research 2016;12:1-10.
14. Simsek, Y., Cakır, I., Yetmis, M., Dizdar, O. S., Baspinar, O., & Gokay, F. Effects of Vitamin D treatment on thyroid autoimmunity. Journal of research in medical sciences: the official journal of Isfahan University of Medical Sciences 2016;21(1):85-92.
15. Gröber, U., & Kisters, K. Influence of drugs on vitamin D and calcium metabolism. Dermato-endocrinology 2012;4(2):158-166.
16. Raber, W., Gessl, A., Nowotny, P., & Vierhapper, H. (). Thyroid ultrasound versus antithyroid peroxidase antibody determination: a cohort study of four hundred fifty-one subjects. Thyroid 2002;12(8):725-731.
17. K.A. Robinson. Ultrasonographic Evaluation of the Thyroid. Ultrasound Clinics 2014 July; 9(3):325–337.
18. Melmed, S., Polonsky, K. S., Larsen, P. R., & Kronenberg, H. M. Williams textbook of endocrinology. Elsevier Health Sciences 2015;13:426-7.
19. Anderson, L., Middleton, W. D., Teefey, S. A., Reading, C. C., Langer, J. E., Desser, T., Cronan, J. J. Hashimoto thyroiditis: Part 1, sonographic analysis of the nodular form of Hashimoto thyroiditis. American Journal of Roentgenology 2010;195(1):208-215.
20. Chaudhary V, Bano S. Thyroid ultrasound in clinical practice. Indian J Endocrinol Metab. 2013;17(2):219-27.
21. Mathieu, C., & Adorini, L. The coming of age of 1, 25-dihydroxyvitamin D3 analogs as immunomodulatory agents. Trends in molecular medicine 2002;8(4):174-179.
22. Holick, M. F. Vitamin D: the underappreciated D-lightful hormone that is important for skeletal and cellular health. Current Opinion in Endocrinology, Diabetes and Obesity 2002;9(1):87-98.
23. D'Aurizio, F., Villalta, D., Metus, P., Doretto, P., & Tozzoli, R. Is vitamin D a player or not in the pathophysiology of autoimmune thyroid diseases? Autoimmunity reviews 2015;14(5):363-369.
24. Bikle D. Nonclassic actions of vitamin D. J. Clin. Endocrinol. Metab. 2009; 94:26–34.
25. Prietl, B., Treiber, G., Pieber, T. R., & Amrein, K. (). Vitamin D and Immune Function. Nutrients 2013;5:2502-2521.
26. Baeke F., Takiishi T., Korf H., Gysemans C., Mathieu C. Vitamin D: Modulator of the immune system. Curr. Opin. Pharmacol. 2010;10:482- 496.
27. Hewison M. "An update on vitamin D and human immunity". Clin. Endocrinol. 2012;76:315–325.