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Original Article

Study of Relation between Vitamin D Serum Level and Biomarkers of Human Sinonasal Fibroblast Proliferation in Patient with Chronic Rhinosinusitis with Nasal Polyposis

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ABSTRACT

Introduction: Chronic rhinosinusitis with nasal polyposis (CRSwNP) is an inflammatory disease of paranasal sinuses and nasal coverage, which reduces the quality of life and overall health. Vitamin D regulates many cellular functions, including anti-inflammatory and immune-modulating functions. There is an increasing evidence of the relative role of fibroblasts in the accumulation of inflammatory cells in chronic rhinosinusitis. The aim of this study was to investigate the relationship between vitamin D levels and the proliferation of sinonasal fibroblasts in patients with chronic rhinosinusitis with nasal polyposis and its therapeutic role. **Material and Method:** In this case-control study blood and tissue samples were extracted from patients with CRSwNP (n=30). Control subjects with non-inflammatory conditions underwent endoscopic and non-endoscopic surgery (n=30). The serum level of 1,25 dihydroxyvitamin D ($1,25(OH)_2D$) and proliferation of fibroblasts sampled from nasal and sinus tissue using ki 67 and vimentin were evaluated and analyzed using SPSS® 23 software. **Results:** The average level of vitamin D was 23.1±7.36 ng/ml in the case group and 31.19±7.02 ng/ml in control group, which was significantly lower in case group (P=0.008). The proliferation of sinonasal fibroblasts were positive in all cases patients and only in 20% of control patients using vimentin. The proliferation of sinonasal fibroblasts were negative in all control patients while only 10% of the case patients were negative using Ki 67. The level of vitamin D decreased with the increased rate of Ki 67. **Conclusion:** The results of this study indicate a reversed relation between vitamin D levels and the proliferation of sinonasal fibroblasts.Thus vitamin D has both prophylactic and therapeutic effects in chronic rhinosinusitis with nasal polyposis.

INTRODUCTION

Chronic rhinosinusitis (CRS) is an inflammatory disease of paranasal sinuses and upper airways for more than 12 weeks and requires mucosal inflammatory evidence for diagnostic establishment (1-2-3-4-5). The prevalence of this disease varies between 2-27% in different parts of the world (6-7-8).

It reduces the quality of life and overall physical health of involved patients (9-10-11). The disease is divided into three subgroups of CRS with NPs (CRSwNP), CRS without NPs (CRSsNP) and allergic fungi (12).

CRSwNP is defined as the presence of bilateral polyposis in both middle meatuses of the nose, mostly due to T2 helper cells, and it often involves patients with immune dysfunction (13-14). Mucosal tissue biopsy indicates eosinophilic infiltration in these patients (13-14-15).

Not only Vitamin D has a role in calcium and bone homeostasis, but also it regulates many cellular functions, including anti-inflammatory and immune-modulatory functions (16-17-18).

Vitamin D status is defined properly by evaluating the concentration of serum 25(OH)D level (19-20). The active form of vitamin D is 1,25-dihydroxyvitamin D. 1,25-dihydroxyvitamin D is an immune system modulator, and the lack of it increases the risk of autoimmune diseases. Many epidemiologic studies confirm that serum level of vitamin D less than 20 ng/ml increases the risk of cancers, infectious diseases, cardiovascular diseases and autoimmune diseases.

Fibroblast cells have a complex cytokinetic role in the regulation of the immune system. There is increasing evidence relative role of fibroblasts in the accumulation of inflammatory cells in chronic rhinosinusitis.

Ki67 is a protein that is related to cell cycle.this antigen is a nuclear antigen and is find only in prolifrating cells. Ki67 presents during specific stages of cell cycle. It is practical marker of cell proliferation.

Vimentin is a protein that is find in mesenchymal cells and is one of major cytoskeletal parts of mesenchymal cells. Epithelial cells can convert to fibroblasts under certain con-

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ditions and Vimentin is a indicator for this convertion. Also this protein is a marker for fibroblast prolifration.

In-vitro studies have shown 1,25VD3 reduces the release of eosinophilic recruiting chemokines from sinonasal fibroblasts in CRSwNP.

Regarding the fact that CRS reduces the quality of life and decreases the overall physical health of patients, and so far no study has been conducted in the region, we decided to investigate the relationship between serum vitamin D level with proliferation biomarkers of sinonasal fibroblasts in patients with CRSwNP and its therapeutic effect.

MATERIAL AND METHOD

This case-control study was approved by the ethic committee of Tabriz University of Medical Sciences and written informed consent were obtained from all enrolled subjects. The sinonasal tissue sample was obtained during surgery from control group and patient with chronic rhinosinusitis with nasal polyposis(inflammatory and allergic polyps).

This study was performed in ENT ward of Imam Reza educational hospital of Tabriz University of Medical Sciences during 2017.The case group was collected from patients with sinonasal polyposis admitted for surgery.The control group was collected from patients with non-inflammatory conditions admitted for other sinonasal surgeries such as CSF leak repair, septoplasty,and non-secretory tumors of hypophysis. Inclusion criteria for case group was patients with CRSwNP admitted for polypectomy and nasal surgery, and inclusion criteria for control group was patients with non-inflammatory conditions undergoing endoscopic and non-endoscopic surgery such as cerebrospinal fluid leakage repair and non-secreted tumors of the pituitary.

The exclusion criterias were use of systemic steroids (including long acting steroids), use of antibiotics and immune modulatory agents 14 days before surgery, consuming drugs that accelerate the metabolism of vitamin D, acute upper respiratory tract infections, kidney disease, asthmatic patients, patients with samter triad, digestive diseases, endocrine diseases, bone disease, idiopathic pulmonary fibrosis, cystic fibrosis, ciliary dyskinesia, immune system disorders (multiple myeloma, rheumatoid arthritis, Immune deficiency, ect.),pregnancy, obesity (BMI>30), non-sufficient tissue samples for microscopic evaluation or bleeding and necrosis and inappropriate lamellae quality (inappropriate staining and fixation). Local steroids was ceased at least 14 days before surgery.

Tissue Processing and Analyze

Sinonasal tissue biopsies were taken from sinus tissue and dissociated, prepared, and stored as defined by the EPOS-2012. Tissue from a total of 60 patients was used for flow cytometric analysis.

The proliferation of Human sinonasal fibroblast (HSNFs) was evaluated by Ki67 and Vimentin proliferation factors using immunohistochemistry (IHC) method. All blood and pathology samples were reviewed at Imam Reza Hospital's lab.

Immunohistochemistric study of these specimens was performed with two 3 micron cuts using Ki67 and Vimentin monoclonal antibodies. All sections were initially dewaxed and dehumidified in a fresh solution of Citrate HCl Buffer 10MM with pH of 6 for 10 minutes in the microwave.

After cooling at room temperature, they were washed with PHOSPHATE BUFFERED SALIN (PBS) and incubated with anti-Ki67 and anti-Vimentin antibodies at 10% dilution for 1 hour.

The sections were then washed with Pbs and incubated with Piotinylated antibodies for 30 minutes followed by Pbs washing and peroxidase-labeled streptavidin incubation for 30 minutes and then washed again with Pbs and adjacent with 3.3 diaminobenzidine hydrochloride chromogene, which results in a brown-colored reaction product. Then the sections were stained with ethyl-green and re-dehumidified and finally covered with lamell. independent sample t-test was utilized to determine significance. For nonparametric data, Kruskal-Wallis followed by Mann-Whitney U test was used. Correlations for parametric and nonparametric data were examined using a Pearson or Spearman correlation analysis, respectively. The p-values less than 0.05 were statistically considered significant. Plasma levels of vitamin D were simultaneously evaluated with other preoperative laboratory tests. Plasma $1,25(OH)_2D$ levels were measured using an enzyme immunoassay (RIA kit, IDS) using 0.5 mL of serum EDTA and heparin plasma, according to the manufacturer's instructions. The assay's range was 0 to 210 pg/mL. All data were collected and analyzed using IBM® SPSS® release 23 software.

RESULTS

All of 60 patients (30 control and 30 with CRSwNP) had sinonasal tissue biopsy available for fibroblast proliferation analysis. Thirty percent of each group werefemale (M:F ratio=7:3). The average age for control and CRSwNP patients were 29.8 and 44.9 years respectively. The average BMI of control subjects was 24.5 and 25.4 in the CRSwNP patient. The prevalence of smoking was 10% in the case and 16.7% in control group.

The average serum level of $1,25(OH)_2D$ was 23.1 ng/mL in CRSwNP patients while it was 31.2 ng/mL in control patients (P=0.008, t=-4.35).

The fibroblast proliferation determined by analysis of sinonasal tissue explants flow cytometry. The proliferation of HSNFs was identified by Vimentin and Ki67 staining.

We observed that patients in case group had a higher expression of Ki67 proliferation marker compared to controls, (90% vs 0%, P =0.007).

We also observed higher Vimentin expression (proliferation marker) compared to controls, (100% vs 20%, P=0.009).

we compared the differences in HSNF proliferation as a function of $1,25(OH)_2$ D.

An independent sample T-test was used to investigate the relationship between vitamin D level and the proliferation of HSNFs in CRSwNP patients by Vimentin proliferation marker. This indicated a low level of vitamin D in the Vimentin positive group (proliferation of fibroblasts) ($P<0.01$).

These findings are in line with the Ki67 results demonstrated an inverse correlation between vitamin D level and Ki67 positivity, with regard to One Way ANOVA test ($P < 0.01$).

DISCUSSION

Vitamin D deficiency is one of the major concerns in public health and is exacerbated by obesity, lack of adequate sunlight and excessive consumption of sunscreens. Recent study showed that patients with CRSwNP have lower levels of serum vitamin D than those with chronic rhinosinusitis without nasal polyposis. Comparing the results of this study with previous ones (21, 22, 23), it can be concluded that vitamin D levels have been reduced in patients with CRSwNP. This confirms the immunomodulatory role of vitamin D, which acts upon attachment to vitamin D receptors in the nucleus of cells such as monocytes, macrophages, dendritic cells, and immune cells including B and T cells. The reduction of this vitamin causes inflammation and pathogenesis of the CRSwNP.

Vitamin D deficiency is associated with many upper and lower respiratory diseases (25-26) such as chronic rhinosinusitis with nasal polyps (22-23-24-25-26- 27).

Variety of cells have VDR on their surface, including fibroblasts (28-29). The fibroblasts are responsible for the eosinophils recruitment, one of the dominant cells in the inflammatory response and the development of symptoms in patients with CRSwNP (30). The results indicate that these cells were elevated with respect to the Ki67 and Vimentin proliferation markers increase in patients with CRSwNP, consistent with Carroll et al. study, which showed high levels of fibroblasts in patients with CRSwNP (23). These results indicate an increase in fibroblasts in CRSwNP, which are the dominant cells responsible for the production of extracellular matrix in the connective tissue and asthma. These cells also play an important role in immune system regulation, and at least part of the inflammatory response in CRSwNP is due to them, and their number greatly adds to this response.

Further studies on the relationship between vitamin D levels and the proliferation of sinonasal fibroblasts should be undertaken. Therefore, this study evaluates the relationship between vitamin D levels and the proliferation of sinonasal fibroblasts in patients with CRSwNP. The results of this study showed an inverse relationship between sinonasal fibroblast proliferation and systemic vitamin D levels. These results are consistent with the results of studies performed by Rostkowska (34), Ling-Feng (35), Takahashi H (36), and Carroll (23), resulting in an increase Inflammatory response in CRSwNP. Further researches on the effects of HSNFs and Vitamin D on CRSwNP pathophysiology are necessary.

The proliferation of fibroblasts is just one way to increase the number of these cells in the target tissue. Fibrocytes are bone marrow-derived and blood-transfused cells that represent a modest mesenchymal differentiation and may migrate into tissues and form fibroblasts (31-32). Also, fibroblasts may be formed by epithelial-mesenchymal transfer (EMT), in which epithelial cells exhibit fibroblast features (33). Recent research suggests that EMT can also play an important role in the pathogenesis of CRS. Further studies are needed to understand all the mechanisms involved in increasing the number of fibroblasts in the CRS.

Limitations and Suggestions

There were some limitations in the current study, including the small sample size and the design of the study. Additionally, there may be changes in HSNF levels based on the anatomical site of the biopsy. Despite these limitations, the current study provides compelling data to evaluate the role of HSNF and Vitamin D in CRSwNP. Further microscopic studies are needed to fully understand the role of vitamin D and its supplementation benefit for patients with CRSwNP.

CONCLUSION

The results of this study showed that vitamin D deficiency is associated with the sinonasal fibroblasts proliferation and, consequently, the development of chronic rhinosinusitis with nasal polyposis. Therefore, vitamin D supplementation may have therapeutic and preventive effects in patients with chronic rhinosinusitis with nasal polyposis.

REFERENCES

1. Meltzer EO, Hamilos DL, Hadley JA, (2004). Rhinosinusitis: establishing definitions for clinical research and patient care. J Allergy Clin Immunol.114:155.
2. Scadding GK, Durham SR, Mirakian R, (2008). BSACI guidelines for the management of rhinosinusitis and nasal polyposis. Clin Exp Allergy.38:260.
3. Rosenfeld RM, Piccirillo JF, Chandrasekhar SS, (2015). Clinical practice guideline (update): adult sinusitis. Otolaryngol Head Neck Surg.152:S1.
4. Fokkens WJ, Lund VJ, Mullol J, (2012). EPOS 2012: European position paper on rhinosinusitis and nasal polyps 2012. A summary for otorhinolaryngologists. Rhinology. 50:1.
5. Slavin RG, Spector SL, Bernstein IL, (2005). The diagnosis and management of sinusitis: a practice parameter update. J Allergy Clin Immunol.116:S13.
6. Shashy RG, Moore EJ, Weaver A (2004). Prevalence of the chronic sinusitis diagnosis in Olmsted County, Minnesota. Arch Otolaryngol Head Neck Surg.130:320.
7. Blackwell DL, Collins JG, Coles R (1997). Summary health statistics for U.S. adults: National Health Interview Survey., Vital Health Stat 10 2002;:1.
8. Hastan D, Fokkens WJ, Bachert C, (2011). Chronic rhinosinusitis in Europe--an underestimated disease. A GA²LEN study. Allergy 2011; 66:1216.
9. Senior BA, Glaze C, Benninger MS (2001). Use of the Rhinosinusitis Disability Index (RSDI) in rhinologic disease. Am J Rhinol. 15:15.
10. Bhattacharyya N (2012). Functional limitations and workdays lost associated with chronic rhinosinusitis and allergic rhinitis. Am J Rhinol Allergy. 26:120.
11. Gliklich RE, Metson R (1995). The health impact of chronic sinusitis in patients seeking otolaryngologic care. Otolaryngol Head Neck Surg. 113:104.

12. Hamilos DL (2007). Chronic rhinosinusitis patterns of illness. Clin Allergy Immuno.20:1.
13. Klossek JM, Neukirch F, Pribil C, Prevalence of nasal polyposis in France: a cross-sectional, case-control study. Allergy 2005; 60:233.
14. Chang JE, White A, Simon RA, Stevenson DD (2012). Aspirin-exacerbated respiratory disease: burden of disease. Allergy Asthma Proc.33:117.
15. Ponikau JU, Sherris DA, Kephart GM, (2003). Features of airway remodeling and eosinophilic inflammation in chronic rhinosinusitis: is the histopathology similar to asthma? J Allergy Clin Immunol.112:877.
16. Deluca HF, Cantorna MT (2011). Vitamin D: its role and uses in immunology. FASEB J.15:2579–2585.
17. Cunningham FG, Leveno KJ, Bloom SL, Spong CY, Dashe JS, Hoffman BL. Williams obstetrics. 24th edition. ed. xviii, 1358 pages p.
18. Lieben L, Carmeliet G, Masuyama R (2012). Calcemic actions of vitamin D: effects on the intestine, kidney and bone. Best practice & research Clinical endocrinology & metabolism.25(4):561-72.
19. Holick MF. Vitamin D deficiency (2007). The New England journal of medicine.357(3):266-81.
20. Rosen CJ (2011). Clinical practice. Vitamin D insufficiency. The New England journal of medicine.364(3):248-54.
21. Mostafa BE, Taha MS, Abdel Hamid T, Omran A, Lotfi N. Evaluation of vitamin D levels in allergic fungal sinusitis, chronic rhinosinusitis, and chronic rhinosinusitis with polyposis. InInternational forum of allergy & rhinology 2016 Feb 1 (Vol. 6, No. 2, pp. 185-190).
22. Mulligan JK, White DR, Wang EW, Sansoni SR, Moses H, Yawn RJ, et al. Vitamin D3 deficiency increases sinus mucosa dendritic cells in pediatric chronic rhinosinusitis with nasal polyps. Otolaryngology--Head and Neck Surgery. 2012 Oct;147(4):773-81.
23. Carroll WW, Schlosser RJ, O'Connell BP, Soler ZM, Mulligan JK. Vitamin D deficiency is associated with increased human sinonasal fibroblast proliferation in chronic rhinosinusitis with nasal polyps. InInternational forum of allergy & rhinology 2016 Jun 1 (Vol. 6, No. 6, pp. 605-610).
24. Sansoni ER, Sautter NB, Mace JC, Smith TL, Yawn JR, Lawrence LA, et al. Vitamin D3 as a novel regulator of basic fibroblast growth factor in chronic rhinosinusitis with nasal polyposis. InInternational forum of allergy & rhinology 2015 Mar 1 (Vol. 5, No. 3, pp. 191-196).
25. Ginde AA, Mansbach JM, Camargo CA., Jr. Association between serum 25-hydroxyvitamin D level and upper respiratory tract infection in the Third National Health and Nutrition Examination Survey. Arch Intern Med. 2009;169(4):384–90.
26. Brehm JM, et al. Serum vitamin D levels and markers of severity of childhood asthma in Costa Rica. Am J Respir Crit Care Med. 2009;179(9):765–71.
27. Mulligan JK. Vitamin D3 correlates inversely with systemic dendritic cell numbers and bone erosion in chronic rhinosinusitis with nasal polyps and allergic fungal rhinosinusitis. Clin Exp Immunol. 2011;164(3):312–20.
28. Yawn J. Vitamin D for the treatment of respiratory diseases: is it the end or just the beginning? J Steroid Biochem Mol Biol. 2015; 148:326–37.
29. Stio M. Vitamin D receptor in IMR-90 human fibroblasts and antiproliferative effect of 1,25-dihydroxyvitamin D3. Biochem Mol Biol Int. 1997;43(6):1173–81.
30. Oyer SL, Nagel W, Mulligan JK. Differential expression of adhesion molecules by sinonasal fibroblasts among control and chronic rhinosinusitis patients. Am J Rhinol Allergy. 2013;27(5):381–6.
31. Bellini A, Mattoli S. The role of the fibrocyte, a bone marrow-derived mesenchymal progenitor, in reactive and reparative fibroses. Lab Invest. 2007;87(9):858–70.
32. Hong KM. Differentiation of human circulating fibrocytes as mediated by transforming growth factor-beta and peroxisome proliferator-activated receptor gamma. J Biol Chem. 2007;282(31):22910–20.
33. Kalluri R, Weinberg RA. The basics of epithelial-mesenchymal transition. J Clin Invest. 2009;119(6):1420–8.
34. Rostkowska-Nadolska B, Fraczek M, Gawron W, Latocha M. Influence of vitamin D(3) analogues in combination with budesonid R on proliferation of nasal polyp fibroblasts. Acta Biochim Pol. 2009;56:235–242.
35. Wang LF, Lee CH, Chien CY, Chen JY, Chiang FY, Tai CF. (2013). Serum 25-hydroxyvitamin D levels are lower in chronic rhinosinusitis with nasal polyposis and are correlated with disease severity in Taiwanese patients. American journal of rhinology & allergy, 27(6):e162-5.
36. Takahashi H, Ibe M, Kinouchi M, Ishida-Yamamoto A, Hashimoto Y, Iizuka H. (2003). Similarly potent action of 1, 25-dihydroxyvitamin D3 and its analogues, tacalcitol, calcipotriol, and maxacalcitol on normal human keratinocyte proliferation and differentiation. Journal of dermatological science, 31(1):21-8.