Does vitamin D have protective effect on human nasal polyposis: histological and immunohistochemical study

Eman M. Faruk1*, Mohamed M. Yousef1 and Taha Mohamed2

*Correspondence: mza3za32001@yahoo.com

1Department of Histology and Cell Biology, Benha Faculty of Medicine, Egypt.
2Department of Oto Rhino Laryngology, Benha Faculty of Medicine, Egypt.

Abstract

**Background**: Nasal polyps (NPs) are benign pedicled mucosal protrusions into the nasal cavity of multifactorial origin. Vitamin D has been demonstrated as having potential immunomodulatory activity and act as an antiproliferative agents.

**Aim of the work**: To determine the possible antiproliferative effect and immunomodulatory activity of VD on human nasal polyposis.

**Material and methods**: Based on thirty patients and divided equally into 3 groups. Group I (healthy subjects). Group II were received low daily oral dose of VD(1000 IU) for 4 weeks. Group III were received high daily oral dose of VD(4000 IU) for 4 weeks. Each group I and III divided into 2 subgroups; group b: patients with NP before taking VD and group a: patients with NP after taking.

Nasal biopsies were obtained of all groups for histological examination and immunohistochemical detection of Toll-like receptors 9 expression.

**Results**: patients of nasal polyps before VD taking (groups IIb and IIIb) presented with symptoms of Visual Analogue Scale, VAS score (facial pain, headache, nasal blockage, nasal discharge, post-nasal drip and olfactory disturbance) and endoscopic appearance of Lund and Mackey score (polyp, edema and discharge), damage of respiratory epithelium, extensive accumulation of collagen fibers in lamina propria and highly expressed TLR-9. The high dose VD group (IIIa) showed near normal respiratory epithelium, significant decrease (P<0.05) in all symptoms of VAS score, endoscopic appearance of Lund and Mackey score. The mean area % of submucosal accumulation of collagen fibers and TLR-9 expression were also significant decreased but decrease was insignificant (P<0.05) in the low dose VD group (IIa).

**Conclusion**: VD participate significantly in protection against human nasal polyposis when used by high therapeutic dose, by reducing the size of nasal polyps, relieving the symptoms and signs of nasal polyposis.

**Keywords**: Nasal polyposis, vitamin D, TLR-9 expression, visual analogue scale

Introduction

Chronic rhinosinusitis (CRS) is a chronic disease characterized by inflammation of the sinonasal mucosa. Symptoms of CRS include anterior and/or posterior rhinorrhea, nasal obstruction, decreased sense of smell, and nasal pressure, at least 2 of which persist for 12 weeks despite medical management. The pathogenesis of CRS is not fully understood at this time; some plants but largely in fungi. Cholecalciferol (vitamin D3) is sourced from sun exposure, while Ergocalciferol (vitamin D2) is sourced from plant-based food sources available. Vitamin D is synthesized via the UV irradiation of 7-dehydrocholesterol in the skin of animals with a further thermal growth of these polyps.

Vitamin D (VD) and its different analogues, besides their classic role as regulators of calcium and phosphor homeostasis, have emerged as a large family of antiproliferative agents. Such properties suggested VD potential as a therapy for chronic inflammatory diseases.

Compared with the other known vitamins essential to health, vitamin D is unique in its role because of the diverse sources available. Ergocalciferol (vitamin D2) is sourced from the UV irradiation of ergosterol, which is a steroid found in some plants but largely in fungi. Cholecalciferol (vitamin D3) is synthesized via the UV irradiation of 7-dehydrocholesterol to previtamin D3 in the skin of animals with a further thermal
Activation of Toll-like receptors (TLRs) results in initiation of signalling pathways that culminate in rapid induction of pro-inflammatory cytokines and chemokines [13]. specifically by TLR9, can play an important role in the pathogenesis of autoimmune diseases, such as SLE [14].

The aim of this study was to evaluate the possible anti-proliferative effect and immunomodulatory activity of VD on human nasal polyps.

Patients and methods

The present study has been conducted on thirty patients (21 males and 9 females) attended Oto-Rhino-Laryngology (ORL) Department, Benha University Hospitals from September 2012 to May 2013. The study was approved by the Local Ethics Committee of Benha Faculty of Medicine.

Vitamin D (D2, D3, or both) deficiency is an international health concern that has been associated with rickets, osteomalacia, muscle weakness, osteoporosis, and an increased risk of wheezing diseases, autoimmune diseases (eg, type 1 diabetes, multiple sclerosis, rheumatoid arthritis, and Crohn's disease), and cancer, such as of the prostate, breast, and colon [10]. Vitamin D has been demonstrated as having potential immunomodulatory activity; vitamin D analogues are effective in the treatment of psoriasis [11].

Objective assessment

1-General examination.
2-Full ORL examination.
3-Anterior rhinoscopic examination.
4-Diagnostic nasal endoscopy: was performed using 0 and 30 degree.

Evaluation was done according to Lund-Mackay endoscopic appearance score [15]. The evaluated endoscopic findings were (polyp, edema and discharge) as in (Table 1).

Table 1. Lund-Mackay endoscopic appearance score [15].

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Score</th>
<th>Endoscopic findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyps</td>
<td>0</td>
<td>absence of polyps</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>polyps in middle meatus (MM) only</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>polyps beyond the MM, but not completely obstructing the nose</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>polyps completely obstructing the nose</td>
</tr>
<tr>
<td>Edema</td>
<td>0</td>
<td>absent</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>mild</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>severe</td>
</tr>
<tr>
<td>Discharge</td>
<td>0</td>
<td>no discharge</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>clear, thin discharge</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>thick purulent discharge</td>
</tr>
</tbody>
</table>

Regarding group I, tissue samples were taken from the nasal mucosa (inferior turbinate mucosa) of all patients during otoplasty surgery at the start of the study by nasal endoscopy. Regarding group II and III, tissue samples were taken from the nasal polyps of all patients at the start of the study (before VD therapy) under local anesthesia and again after 4 weeks of daily oral dose of VD during Functional Endoscopic Sinus Surgery (FESS).

Tissue samples were immediately disinfected with Betadine, rinsed in phosphate-buffered saline (PBS), cut into small fragments and placed into a sample tube containing 1 ml PBS. A part of each sample was fixed in 10% buffered neutral formalin, processed routinely, and embedded in paraffin wax for subsequent histological; H&E and Masson's trichrome [16] staining. For immunohistochemistry (Toll-like receptors 9; TLR-9), a labeled streptavidin-biotin method was used in which deparaffinized sections were heated for 5 min at 120°C in a pressure cooker to reactivate the antigen. Sections were blocked and incubated with an anti-TLR-9 antibody (Imgenex, San Diego, CA, USA) overnight at 4°C. Sections were then incubated with a second biotinylated
antibody, followed by the avidin-biotin-peroxidase complex (Vector Laboratories, Burlingame, CA, USA) [17]. The tissue was counterstained with 10% hematoxylin [18].

Statistical analysis
Statistical analyses were carried out using Microsoft excel 2010 (Microsoft Egypt, Cairo, Egypt). Clinical data (VAS and endoscopic Lund-Mackay scores) are expressed as mean and standard deviation (SD). The mean area percentage of collagen fibers accumulation (in Masson's trichrome images) and TLR-9 expression were quantified in 10 images for each group using Image-Pro Plus program version 6.0 (Media Cybernetics Inc., Bethesda, Maryland, USA). T-test was used to determine statistical differences. A p-value <0.05 was considered statistically significant.

Ethical considerations
All patients were informed and a written consent was obtained.

Results
Clinical results
General data
The mean age of all patients was 28.2 (range 18-50 years). Of the 30 patients, 21 were men and 9 were women. The mean, standard deviation (SD), P value of different symptoms (Score by VAS) of the patients of groups II (b and a) and III (b and a) summarized in (Table 2 and Histogram 1). It showed significant decrease (S) in all VAS symptoms score in group IIIa compared with group IIIb (P<0.05) but this decrease was not significant in group II.

Endoscopic
The mean, standard deviation (SD), P value of Lund Mackay score of endoscopic appearance (polypi, edema, discharge) for the patients of groups II (b and a) and III (b and a) presented in (Table 3) (Figure 1 and Histogram 2). It showed significant decrease (S) in group IIIa score compared with group IIIb (P <0.05). Group IIa showed significant decrease (S) in edema but the decrease was not significant in polypi and discharge compared with group IIIb.

Histological results
H&E
In control group (group I), it showed normal nasal cavity mucosa with respiratory epithelium (psudostratified columnar ciliated with goblet cells) rests on thin basal lamina and lamina propria of loose connective tissue containing multiple blood vessels (Figure 2a). Low dose VD group before taking VD (group IIb) showed damage of respiratory epithelium rests on apparently thick basal lamina and lamina propria with numerous cells infiltration (lymphocytes, eosinophils and...
Table 3. Showing the mean, SD and P value of Lund Mackay score in groups II (b and a) and III (b and a). (a) compared with (b) in each group.

<table>
<thead>
<tr>
<th>Endoscopic appearance (Lund Mackay score)</th>
<th>Low vit. D dose group (III)</th>
<th>High vit. D dose group (IV)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD± P value</td>
</tr>
<tr>
<td></td>
<td>b a</td>
<td>b a</td>
</tr>
<tr>
<td>Polypi</td>
<td>1.5</td>
<td>1.3 0.527</td>
</tr>
<tr>
<td></td>
<td>1.7</td>
<td>0.8 0.483</td>
</tr>
<tr>
<td>Edema</td>
<td>1.6</td>
<td>1.1 0.516</td>
</tr>
<tr>
<td></td>
<td>1.4</td>
<td>0.5 0.516</td>
</tr>
<tr>
<td>Discharge</td>
<td>1.5</td>
<td>1.2 0.527</td>
</tr>
<tr>
<td></td>
<td>1.3</td>
<td>0.2 0.483</td>
</tr>
</tbody>
</table>

Figure 1. (a) A photograph of an endoscopic view for right nasal cavity of control group showing septum (s), middle turbinate (MT), uncinate process (U) and inferior turbinate (IT). (b) A photograph of an endoscopic view for left nasal cavity in low dose VD group (IIb) showing large nasal polyps (P) at middle meatus septum (S) and middle turbinate (MT). (c) A photograph of an endoscopic view for left nasal cavity of the previous same patient in low dose VD group (IIa) showing small nasal polyp (P) at middle meatus septum (S) and middle turbinate (MT) noticed. (d) A photograph of an endoscopic view for left nasal cavity of high dose vitamin D group (IIIb) showing multiple nasal polyps (P) filling the nasal cavity. Middle turbinate (MT) noticed. (e) A photograph of an endoscopic view for left nasal cavity of the previous same patient in high dose vitamin D group (IIIa) showing small nasal polyp (P) between the middle turbinate (MT) and inferior turbinate (IT).

Figure 2. (a) A photomicrograph of a section in the nasal mucosa of the control group showing normal nasal cavity mucosa with respiratory epithelium (E) rests on thin basal lamina (arrow) and lamina propria of loose connective tissue with plasma cells) and few blood vessels (Figure 2b). After taking VD (group IIa), it showed partial damage of respiratory epithelium rests on thick basal lamina and lamina propria with numerous cells infiltration and few blood vessels (Figure 2c). High dose VD group before taking VD (group IIIb) showed the same features of group IIb (Figure 2d). After taking VD (group IIIa), it showed near normal nasal mucosa with overcrowded respiratory epithelium rests on thin basal lamina and lamina propria of loose connective tissue with few blood vessels (Figure 2e).
few cells infiltration and many blood vessels (Figure 2e).

**Masson’s trichrome**

In control group, it showed faint and minimal collagen fibers in the lamina propria of nasal mucosa (Figure 3a). Low dose VD group before taking VD (group IIb) showed marked accumulation of collagen fibers at the basal lamina and lamina propria (Figure 3b). After taking VD (group Ila), it showed that accumulation of collagen fibers at basal lamina and lamina propria decreased (Figure 3c). High dose VD group before taking VD (group IIIb) showed accumulation of collagen fibers as in group IIb (Figure 3d). After taking VD (group IIIa), it showed minimal collagen fibers in the lamina propria (Figure 3e).

The mean area percentage of collagen fibers accumulation for all groups presented in (Table 4 and Histogram 3). There was a significant decrease (P < 0.05) in collagen fibers accumulation in group IIIa compared with group IIIb while the decrease in collagen fibers accumulation was insignificant in group Ila compared with group IIb.

**Immunohistochemical**

Positive immunohistochemical staining of TLR-9 demonstrated brown cytoplasmic and nuclear staining. Control group showed mild cytoplasmic expression for TLR-9 in apical epithelial surface (Figure 4a). Low dose VD group before taking VD (group IIb) showed highly expressed TLR-9 in epithelium and lamina propria (Figure 4b). After taking VD (group Ila), it showed moderately expressed TLR-9 in epithelium and lamina propria (Figure 4c). High dose VD group before taking VD (group IIIb) showed TLR-9 expression as in group IIb (Figure 4d). After taking VD (group IIIa), TLR-9 expression was weak (Figure 4e). The mean area percentage of TLR-9 expression for all groups presented in (Table 5 and Histogram 4). There was a significant decrease (P < 0.05) in TLR-9 expression in groups IIIa compared with group IIIb while the decrease in TLR-9 expression was insignificant in group Ila compared with group IIb.

**Discussion**

Nasal polyposis is a chronic inflammatory disease of the sinunasal mucosa frequently associated with asthma [19]. The infiltration of various inflammatory cells, epithelial damage and expression of the cytokines are similar to the pathological profile of asthma. From this perspective, NP could therefore be regarded as a paradigm of chronic airway inflammation [20]. Nasal polyposis is a common nasal disease with a high rate of recurrence [21].

The patients of nasal polyps before VD taking (groups IIb and IIIb) in our study presented with symptoms of VAS score (facial pain, headache, nasal blockage, nasal discharge, post-nasal drip and olfactory disturbance) and endoscopic appearance of Lund and Mackey score (polypi, edema and discharge) and this agreed with [22] and [23] who reported that nasal polyps are benign edematous masses in the nasal cavities, paranasal cavities, or both that can cause nasal obstruction, rhinorrhea, postnasal drip, loss of smell and facial pain and...
avascular edematous stroma with submucosal fibrosis and an often prominent mixed inflammatory infiltrate. On the other hand [26] stated that the histopathologic features of a nasal polyp include hyperplasia of surface epithelium and goblet cells but [27] explain this as he reported that in NP the epithelial damage followed by aberrant tissue repair and structural changes (remodeling).

The management of nasal polyposis has been the subject of frequent controversial debates for many decades [20]. Surgical or medical treatment or both have been recommended as the treatment of choice. Medical treatment should be used for at least 1 month before surgery is contemplated in patients with typical nasal polyposis because some studies have indicated that in those patients who respond to medical treatment, no additional treatment is necessary [28].

Despite up-to date pharmacological treatment, in most cases the outcomes are unsatisfactory and recurrences require surgery. At present, steroids in a long term topical and oral forms are the primary in the therapy for NP. Due to well-known side effects related to steroid intake, this therapeutic option is often rejected. All of these factors indicate the need for the investigation of new agents suitable for treating NP [29].

The active hormonal metabolite of VD has a broad spectrum of biological actions [30]. The discovery of the systemic role of VD opened a new area of research on the role of this vitamin in the modulation of physiological and pathological processes, as well as the prevention and treatment of many diseases as cancer disease, diabetes or multiple sclerosis [31].

VD used in the present study as two daily therapeutic doses; low (1000 IU) in group IIa and high (4000 IU) in group IIIa according to [32] who stated that six studies preferred daily oral supplementation strategies by using dosages between 1000 and 4000 IU and this agreed with [33,34] who stated that the supplementation was within the range of 1000–4000 IU/day of VD often nowadays considered as the daily physiological requirement. Glendenning et al., and Holick et al., used VD in a daily dose 1000 IU in their studies while [37] and [38] used VD in a daily dose 4000 IU.

The high dose VD group (IIIa) of the present study showed...
near normal nasal mucosa with overcrowded respiratory epithelium rests on apparently thin basal lamina and lamina propria of loose connective tissue with few cells infiltration and many blood vessels and showed significant decrease (P<0.05) in all symptoms of VAS score and endoscopic appearance of Lund and Mackey score and the mean area % of submucosal accumulation of collagen fibers and TLR-9 expression while this decrease was insignificant (P < 0.05) in the low dose VD group (IIa). These results agreed with [39] who reported that the antiproliferative effects of VD were dose- and time-dependent, the lower concentration used was mostly without any effect and [40] stated that in the overall study population of Finnish VD Study, clinical outcomes appeared to favor the high-dose VD group. Also Allen et al., reported that high dose VD (5000–10,000 IU/day) has immunomodulatory and anti-inflammatory effects through increasing interleukin-10 (IL-10) production by peripheral blood mononuclear cells and a reducing frequency of T helper 17 cells (Th17) cells and Kimball et al., reported that high doses of VD may be required for therapeutic efficacy and tolerability.

Apuhan et al., and Rostkowska-Nadolska et al., reported that VD is involved in essential cell regulatory processes such as proliferation, differentiation, apoptosis, angiogenesis and inhibition of pro-growth/pro-survival signalling pathways in a wide variety of cell types and these observations suggest the potential of active VD and its different derivatives as a therapy for chronic inflammatory diseases, including nasal polyposis. Also, Frączek et al., stated that the antiproliferative effect of VD derivatives could potentially be harnessed therapeutically as a supplementary method in rhinosinusitis, targeting not only the fibroblasts but also a wide range of inflammatory cells like the eosinophils and T lymphocytes that infiltrate polyps. McCarty et al., reported that low VD levels were documented in urban-dwelling African-American children with chronic rhinosinusitis, and VD was shown to inhibit in vitro proliferation of nasal-polyp fibroblasts and Rostkowska-Nadolska et al., reported that the pathogenesis of NP involves IL-6 secretion by fibroblasts which modulate the activation of immune response, synthesis of stroma components and tissue remodeling and adding VD diminished expression of IL-6 and IL-8 in fibroblast cultures derived from nasal polyps that may be a cause of diminished growth in the number of cultured cells and may confirm their anti-inflammatory effect.

Conclusion
The present study suggests that VD when used by high therapeutic dose, it is effective in reducing the size of nasal polyps, relieving the symptoms and signs of nasal polyposis and restore the nasal mucosa near its normal state. Therefore, these findings raise possibilities for the development of more efficient and specific types of therapy in nasal polyposis especially in patients unfit or refuse surgery and in recurrent cases. Due to the very limited published information on similar studies further researches are mandatory to fulfill the efficacy of different doses and routs of VD on nasal polyposis.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions

<table>
<thead>
<tr>
<th>Authors’ contributions</th>
<th>EMF</th>
<th>MMY</th>
<th>TM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Research concept and design</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Collection and/or assembly of data</td>
<td>✓</td>
<td>--</td>
<td>✓</td>
</tr>
<tr>
<td>Data analysis and interpretation</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Writing the article</td>
<td>✓</td>
<td>✓</td>
<td>--</td>
</tr>
<tr>
<td>Critical revision of the article</td>
<td>✓</td>
<td>✓</td>
<td>--</td>
</tr>
<tr>
<td>Final approval of article</td>
<td>✓</td>
<td>✓</td>
<td>--</td>
</tr>
<tr>
<td>Statistical analysis</td>
<td>--</td>
<td>✓</td>
<td>--</td>
</tr>
</tbody>
</table>

Acknowledgement
We thank prof Omayma Kamal Helal for her critical review and helpful comments.

Publication history
Editor: Gjumrakch Aliev, GALLY International Biomedical Research & Consulting LLC, USA.
Received: 27-Feb-2014 Final Revised: 06-Mar-2014
Accepted: 10-Mar-2014 Published: 05-Apr-2014

References


immunological effects of high-dose vitamin D in healthy volunteers. 
*Mult Scler.* 2012; **18**:1797-800. | Article | PubMed


43. McCarty DE, Chesson AL, Jr, Jain SK and Marino AA. *The link between vitamin D metabolism and sleep medicine.* *Sleep Med Rev.* 2013. | Article | PubMed


**Citation:**