

## **Circulatory levels of 25-OHD and vitamin D binding protein in patients with chronic rhinosinusitis with polyposis: A case-control study**

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### **Abstract**

**Chronic rhinosinusitis (CRS) is a prevalent disease which is characterized by chronic inflammation of the nasal mucosa and paranasal sinuses. One of the most disabling subgroups of CRS is chronic rhinosinusitis with nasal polyposis (CRSwNP), which requires further investigation regarding its pathogenesis and management. The aim of this study was to compare the circulatory Levels of 25-OHD and Vitamin D Binding Protein (VDBP) in CRSwNP patients in healthy controls. This research is an age-gender matched case-control study. All participants were recruited from the Loghman-Hakim Hospital of Shahid Beheshti University of Medical Sciences, Tehran, Iran during June 2015 to June 2016. Plasma levels of VDBP and 25-OHD were measured using the ELISA method. Forty five CRSwNP patients and forty five healthy individuals (23 females and 22 males in both groups) participated in this study. The mean plasma levels of 25-OHD were significantly lower in CRSwNP patients compared to healthy controls (36.27%,  $P < 0.001$ ). However, VDBP serum levels were not significantly different between CRSwNP and control groups. VDBP levels were significantly higher ( $P = 0.017$ ) in women ( $274.29 \pm 72.30 \mu\text{g/ml}$ ) compared to men ( $234.73 \pm 80.04 \mu\text{g/ml}$ ). However, the mean serum levels of 25-OHD were not significantly different between men and women. CRSwNP patients have significantly lower serum levels of 25-OHD compared to healthy individuals, however, VDBP levels were not different between the CRSwNP and healthy individuals.**

**Keywords:** Vitamin D, Sinusitis, Nasal polyposis, Vitamin D-binding protein.

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### **Introduction**

Chronic rhinosinusitis (CRS) is a common disabling disease which is recognized by chronic inflammation of the nasal mucosa and paranasal sinuses [1]. The morbidity rate of CRS is significant which exerts a considerable negative impact on quality of life. Recent findings have revealed a substantial heterogeneity, with regard to the inflammatory subtypes, among CRS patients with nasal polyposis and CRS patients without nasal polyps (CRSsNP) [2]. CRS is yet to be effectively treated due to the heterogeneous nature of the disease and variable responses to the therapeutic strategies [1-4]. Etiology and pathogenesis of CRS and Acute rhinosinusitis (ARS) have not yet been addressed in the formulation of this concept by the experts of the field. Although ARS is widely considered as an infectious disorder, CRS is typically described more as an inflammatory disease; moreover, the role of specific microbial agents in triggering the process of developing CRS and ARS remains controversial [5].

Chronic sinus inflammation disrupts the normal sinus discharge and consequently disturbs the microbial growth. Because of the chronic inflammation in paranasal sinuses, sinus mucosal hyperplasia and its subsequent protrusion leads to polyposis [6]. In addition to chronic infection of sinuses, allergic sensitivities are one of the most common underlying causes of nasal polyposis [4,6,7]. However, the exact etiology of CRSwNP and the modality of its personalized management are yet to be determined. It has been found that the serum levels of 25-OHD are reduced in CRSwNP compared to CRSsNP. Moreover, the inverse relationship between the levels of 25-OHD and the grade of polyposis suggests that vitamin D deficiency/insufficiency might be a dominant etiologic cofactor which represents the severity of polyposis [8]. One of the factors that determines the circulatory levels of 25-OHD is vitamin D binding protein (VDBP) that also known as G-globulin. VDBP is an alpha-globulin which is produced in the liver and transfers about 85-90% of vitamin D from the liver to the target tissues [9]. Furthermore, VDBP affects the clearance

of vitamin D metabolites through renal reabsorption, which leads to the prevention of urinary excretion of vitamin D [9-11]. More importantly, VDBP plays important roles in innate immunity and inflammatory responses which have been demonstrated to be linked with CRS pathogenesis [7,12]. To the best of our knowledge, there have not been any studies examining whether the decreased levels of 25-OHD in CRSwNP are linked with the circulatory levels of VDBP. Therefore, the aim of this study was to determine the levels and correlations between VDBP and 25-OHD in CRSwNP patients and healthy controls.

## Methods and Materials

### Study design and Participants

This is an age-gender matched case-control study in which all the participants were recruited from the Loghman-Hakim Hospital of Shahid Beheshti University of Medical Sciences, Tehran, Iran, during June 2015 to June 2016. Ethical approve (IR.SBMU.RETECH.REC.1395.3) of this study was obtained from Shahid Beheshti University of Medical Sciences (according to the Declaration of Helsinki). Furthermore, patients were informed formally and signed consent prior to enrollment in the study. Forty five CRSwNP patients and forty five healthy subjects (23 females and 22 males in each group) with the age range of 18-65 years were enrolled in this study. The 2012 European position paper on rhinosinusitis and nasal polyps (EPOS 2012) definitions for inclusion criteria for adults [13] was used in the present study: presence of inflammation of the nose and the paranasal sinuses characterized by two or more symptoms, either nasal blockage/obstruction/congestion or anterior/posterior nasal discharge with or without facial pain/pressure and with or without reduction or loss of smell and either Endoscopic signs of Nasal polyps,  $\pm$  mucopurulent discharge primarily from middle meatus  $\pm$  edema/mucosal obstruction primarily in the middle meatus and/or CT changes that include: mucosal changes within the ostiomeatal complex  $\pm$  the sinuses.

**Table 1.** The circulatory levels of 25-OHD and Vitamin D Binding Protein (VDBP) in Patients with Chronic Rhinosinusitis with Polyposis (CRSwNP), as compared to healthy controls

Characteristics	Serum levels						P value
	CRSwNP (n=45)		Control (n=44)		Total (n=89)		
	Mean	SD	Mean	SD	Mean	SD	
25-OHD (ng/mL)	16.62	5.16	26.08	14.16	21.3	11.57	<0.001
VDBP ( $\mu$ g/mL)	258.27	75.51	252.67	81.44	255.41	78.2	0.739

VDBP: Vitamin D Binding Protein

The control group was recruited from individuals who were referred to the Ear Nose Throat clinics of the Loghman-Hakim Hospital, without complaint of sinonasal disease and patients without inflammatory disease in the head and neck region.

Exclusion criteria was CRS without polyps in nasal endoscopy and having other chronic diseases, such as liver or kidney disease, infectious diseases, malignancies, allergic and immunological diseases, using medications that suppress the immune system and eventually taking any supplements, including vitamin D or antioxidant supplements, and smoking.

**Table 2.** The association between CRSwNP and vitamin D deficiency.

Vitamin D Levels		Status		OR (95% CI)	P-value
		CRSwNP (N=46)	Control (N=45)		
25-OHD (ng/mL)	<20	33	22	2.654 (1.114-6.322)	0.026
	$\geq$ 20	13	23	referent	
25-OHD (ng/mL)	<12 (deficient)	11	1	-	0.003
	12-20 (insufficient)	22	21	-	
	20-50 (adequate)	13	20	-	
	$\geq$ 50 (adverse effects)	0	3	-	

### Measurements

Ten milliliter (mL) fasting blood samples were obtained from each participant. VDBP and 25-OHD levels were measured using the ELISA method. After separation of plasma, VDBP levels were determined using a Quantikine ELISA kit (R&D Systems, Inc. Minneapolis, USA), according to the manufacturer's instructions. The Intra-assay and inter-assay variabilities were 6.2 and 7.4, respectively. The circulatory levels of 25-OHD was measured using direct day ELISA (Immundiagnostik, K 2108, Bensheim, Germany). The Intra-assay and inter-assay variabilities were 5 and 8.6, respectively.

### Statistical analysis

Data are shown as means  $\pm$  standard deviation (SD) and were analyzed using SPSS 16 (SPSS, Chicago, IL, USA). Descriptive analysis was performed for demographics and clinical data. All the quantitative variables were tested for normality using the Shapiro-Wilk tests before analysis. Differences between groups were analyzed by Student's t-test or Mann-Whitney U test. Correlations between VDBP and 25-OHD were analyzed by Pearson's correlation test. P value of less than 0.05 was considered significant.

## Results

Forty five CRSwNP patients and forty five age-sex matched healthy subjects were enrolled in this study. The mean age of participants was 43.2 (SD: 6.9). The laboratory data of one case of -healthy controls were missing. The mean 25-OHD and VDBP levels are shown in Table 1. The mean serum levels of 25-OHD were significantly lower in CRSwNP patients compared to healthy controls (P<0.001). However, VDBP serum levels were not significantly different between the

groups. CRSwNP patients showed a significant 2.65 fold higher risk for vitamin D deficiency (<20 ng/mL 25-OHD, Table 2). The circulatory levels of 25-OHD were lower than 12 ng/mL in 11 CRSwNP patients. While, only one healthy control represented the same concentration of 25-OHD (Table 2).

**Table 3.** The circulatory levels of 25-OHD and Vitamin D Binding Protein (VDBP) in men and women

Variable	Serum level						P value
	Male (n=45)		Female (n=44)		Total (n=89)		
	Mean	SD	Mean	SD	Mean	SD	
25-OHD (ng/mL)	22.59	12.56	20.14	10.62	21.3	11.57	0.334
VDBP (µg/mL)	234.73	80.01	274.29	72.3	255.41	78.2	0.017

VDBP: Vitamin D Binding Protein

**Table 4.** The correlations between 25-OHD and vitamin D binding protein levels

Variables	Case				Control			
	25-OHD		VDBP		25-OHD		VDBP	
	r	P	r	P	r	P	r	P
25-OHD	-	-	0.159	0.31	-	-	-0.005	0.974
VDBP	0.159	0.31	-	-	-0.005	0.974	-	-

VDBP: Vitamin D Binding Protein

In terms of gender differences, VDBP levels were significantly higher ( $P=0.017$ ) in women ( $274.29 \pm 72.30$  µg/mL) compared to men ( $234.73 \pm 80.04$  µg/mL). However, the mean levels of serum 25-OHD were not significantly different between men and women (Table 3). In CRSwNP patients, there were no significant correlations between any of the studied variables (Table 4). Splitting the samples, based on gender, revealed no correlations between the studied variables.

## Discussion

This study aimed to compare the serum levels of VDBP and 25-OHD in patients with CRSwNP and healthy individuals. Based on our findings, serum levels of 25-OHD in CRSwNP patients were significantly lower compared to healthy subjects. However, no significant difference was observed in VDBP levels between both groups. Vitamin D emerges as one of the most promising immuno-modulators which plays pathogenic roles in various diseases [14-16]. Vitamin D deficiency has been demonstrated to be associated with an increased risk of inflammatory and non-inflammatory diseases, such as respiratory diseases [17-19]. It is well-known that the biologically active metabolite of vitamin D reduces the secretion of pro-inflammatory cytokines, such as interleukin-6, interleukin-8 and chemokine ligand 20 in sinus epithelia [20].

Furthermore, the aberrant activity of macrophages has critical roles in vitamin D deficiency [21].

Based on previous studies, 25-OHD levels are lower in CRS or CRSwNP patients compared with the healthy subjects [8, 22-25], which are consistent with our results. In this study, 25-OHD levels of CRSwNP patients were 36.27% lower than healthy controls. Moreover, CRSwNP patients showed an increased risk of vitamin D deficiency. Evidence supports that reduced serum levels of 25-OHD are associated with CRSwNP pathogenesis and severity [8,25]. Vitamin D metabolism in macrophages is associated with pathogen recognition, thus making it a paramount part of the innate immune response which is important in the pathogenesis of CRSwNP [7,26]. Vitamin D has been demonstrated to be able to inhibit both Th1- and Th2-type responses by suppressing both the production of IL-12-generated IFN-γ, IL-4 and IL-4-induced expression of IL-13 [27]. This is a crucial ability since it determines the Th1 and Th2 balance and consequently affects the pattern of immune response. Altogether, evidence supports the importance of vitamin D as an important immuno-modulator in pathways which are involved in human diseases, such as CRSwNP.

To the best of our knowledge, this is the first study that measures the VDBP levels in CRSwNP patients, which indicated no significant differences between CRSwNP patients and healthy individuals regarding the VDBP levels. Some studies have demonstrated that circulatory levels of VDBP are decreased in type 1 diabetes mellitus [28], chronic liver [29] and kidney diseases [30] while pregnancy and oral contraceptive pills have been found to increase VDBP levels [31,32]. However, the findings of the present study indicated that VDBP levels were higher in women than men, which might be justified by the effects of estrogen on VDBP [33]. We did not find a significant correlation between VDBP and 25-OHD circulatory levels. Consistent with the findings of this study, no significant correlations between VDBP and 25-OHD circulatory levels has been reported by some other studies [11,28]. However, a significant correlation between VDBP and 25-OHD levels has been reported in conditions other than CRSwNP [12,34,35], which is not in line with findings of this study. These differences might be justified by considering the fact that there are several components which are altered in the vitamin D metabolic pathway.

Some studies have shown that the polymorphisms in the gene encoding VDBP (GC gene) and the subsequent phenotypes of this protein could affect 25-OHD serum levels [36,37]. Among the three common phenotypes of VDBP (GC1F, GC1S and GC2), GC1F exerts the most affinity and capability of transporting vitamin D [36,37]. It has been reported that there are phenotypic differences between various ethnicities [37]. Therefore, the ethnic-based differences in polymorphisms of VDBP may be linked with the differences in serum levels of VDBP, which require further studies concerning the role of VDBP polymorphisms in CRSwNP. Lack of determining the genotypes/phenotypes of VDBP was one of the limitations of this study.

In conclusion, this study showed that CRSwNP patients have significantly lower serum levels of 25-OHD compared with healthy subjects. Moreover, the majority of individuals with deficient and insufficient vitamin D are more susceptible to CRSwNP. Furthermore, there were no associations between VDBP levels and CRSwNP. Moreover, no significant disease-specific correlations were found between 25-OHD and VDBP circulatory levels.

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