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Evaluation of nasal mucociliary clearance time in patients with Vitamin-D deficiency

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Abstract

Objectives The main purpose of the current study was to investigate nasal mucociliary clearance time (NMC) in patients with Vitamin-D deficiency.

Methods A total of 55 patients with Vitamin-D deficiency and 32 controls were evaluated. NMC time was measured with subjective saccharine test and compared between study and control groups. In addition, NMC time was re-evaluated after Vitamin-D replacement protocol in patients with Vitamin-D deficiency.

Results The mean 25(HO)Vitamin-D levels were 14.32 ± 4.23 ng/mL (7–24.6) and 29.38 ± 7.05 ng/mL (25–53.8) in study and control groups, respectively ($p < 0.001$). The mean NMC time was 11.15 ± 3.05 (6.3–17.6) and 8.40 ± 2.33 (6–13.2) in study and control groups, respectively ($p < 0.001$). The mean 25(HO)Vitamin-D level after the replacement protocol was 33.38 ± 10.03 and the mean NMC time was 9.56 ± 2.54 ($p < 0.001$).

Conclusion The mean NMC time was significantly increased in patients with Vitamin-D deficiency which can be corrected after Vitamin-D replacement protocols. The prolonged mucociliary clearance might be one of the pathophysiologic pathways at increased upper respiratory tract infections, and sinonasal and ear infections in patients with Vitamin-D deficiency.

Keywords Vitamin-D · Nasal mucociliary clearance · Upper respiratory tract infection · Ear infection

Introduction

The removal of pathogens and foreign particles from surfaces of the airway mucosa and maintaining the moistness and freshness of the mucosal surfaces are dependent on an influential ciliary function and orderly renewal of airway liquids [1]. The ciliary function can be evaluated by measuring nasal mucociliary clearance (NMC) by determining the removal times of the molecules that have been inhaled [2]. NMC is one of the most important defense mechanisms of the airway and has a vital role in keeping the body safe against harmful materials that are being inhaled. Any failure or retardation in the NMC causes secondary infections due to stasis of secretions [3]. There are many studies on mucociliary clearance, and many factors that cause deterioration in NMC have been found [4–6].

Active Vitamin-D (25(HO)Vitamin-D) receptors have been detected in several tissues, namely pancreas, stomach, ovaries, kidney, lymphocytes, parathyroid glands, skin, and thymus. This evidence supports 25(HO)Vitamin-D has lots of activities alongside the calcium homeostasis. 25(HO) Vitamin-D receptors identified in all immune system cells, especially in antigen producing cells [7]. 25(HO)Vitamin-D has a substantial role in immunoregulation. An up-regulation of microbicide effects of macrophages and monocytes has been defined in the event of 25(HO)Vitamin-D enriched ambiance [8, 9]. The relationship between upper respiratory tract infections (URI) and 25(HO)Vitamin-D deficiency has been reported by different studies [10, 11]. However, the effect of 25(HO)Vitamin-D on the mucociliary function of upper respiratory tract is still unclear.

We aimed with the current study to demonstrate the association of 25(HO)Vitamin-D deficiency and NMC time.

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Materials and methods

Study design

This study was conducted at the otolaryngology and internal medicine clinics of Duzce University between October 2017 and May 2018. The local ethics committee approval was obtained. Informed consent was obtained from all subjects. We calculated the minimum sample size (30 patients/per group) for each group at a 95% significance level and 90% statistical power based on similar studies [5].

Study population

Fifty-five adult patients with 25(HO)Vitamin-D deficiency were referred to Ear Nose Throat Department for assessment. The majority of healthy adults with Vitamin-D deficiency do not require any additional evaluation if there is no clinical concern for a secondary cause of Vitamin-D deficiency (e.g., malabsorption, celiac disease, and liver or kidney pathologies) [12]. All participants included to the study were evaluated by internal medicine department. After exclusion of possible secondary reasons the deficiency was accepted as primary due to insufficient oral intake and reduced sun exposure. The evaluation of cases was reviewed with detailed history, physical examinations, and nasal endoscopy in every patient. The findings were recorded for age, sex, past medical and surgical history, medications, family history, and physical examination findings. These are accepted as exclusion criteria if patients have any of the following; nasal obstruction due to septum deviation, nasal polyposis; upper respiratory tract infection during the study; history of nasal operation; history of allergic diseases; smoking (active and/or passive) and systemic disease such as diabetes mellitus, hypertension, chronic renal failure, and menopause for women. Control group was conducted with voluntary participants with normal Vitamin-D levels who had been referred to our clinic from internal medicine and whom without rhinological and systemic diseases mentioned above.

None of the study and control group participants were using chronic medication. In addition, none of the patients were taking medication for infection or any other reason during NMC time measurement.

Study and control groups were assessed depending on the serum 25(HO)Vitamin-D levels; < 25 ng/mL was accepted as 25(HO)Vitamin-D deficiency. After NMC time measurements, In our clinical practice, we use "weekly 50,000 IU of Vitamin-D3 (cholecalciferol) capsule for a period of 8 weeks' protocol, and after 3 months, we repeat the Vitamin-D level. And then, 800 IU Vitamin-D3 daily thereafter [12]. In patients without any systemic diseases for every 100 IU of added Vitamin-D3, serum Vitamin-D concentrations increased by approximately 0.7 to 1.0 ng/ml [13]. We expect normalization of Vitamin-D levels after 3-month Vitamin-D replacement unless patient has an underlining pathology. NMC tests were repeated in patients who had 25(HO)Vitamin-D level normalized after 3 months. The NMC results measured before and after 25(HO)Vitamin-D replacement were compared with each other and with the control group.

Measurement of NMC

NMC time was evaluated with the sodium saccharine test by the same otolaryngologist. A standardized technique was used as previously described [5]. All patients were required to blow their nose and not to consume any food or drink an hour before the test. Patients were kept to rest for 30 min before the test. Patients were sitting an upright position during the test and instructed not to lean forward or lay down after saccharine particle placement. Approximately 1 mm sodium saccharine particle was placed on the medial surface of the inferior nasal turbinate with a bayonet forceps, 10 mm behind its head to avoid the area of squamous epithelium. Patients were instructed not to wipe or sniff their noses during the test. The time that from the placement of pieces to the perception of the sweet taste in the oropharynx was measured by a timekeeper and accepted as NMC time.

Statistical analysis

Distribution of continuous variables was analyzed with the Shapiro–Wilk test. Comparison of study and control groups for continuous data was done with independent sample *t* test, and paired sample t test were used to analyze the difference between pre- and post-treatment values of study groups. Pearson Chi-square test was used to analyze categorical variables. IBM SPSS v.22 was used for statistical analyses and the significance level was considered as 0.05.

Results

A total of 87 participants were included in the study. There were 55 and 32 patients in study and control groups, respectively. The mean ages of the study and control groups were 38.16 ± 10.99 and 35.31 ± 9.78, respectively, and ranging from 20 to 54. The mean 25(HO)Vitamin-D level in study and control group was 14.32 ± 4.23 ng/mL (7–24.6) and 29.38 ± 7.05 ng/mL (25–53.8), respectively, and the difference was statistically significant ($p < 0.001$). The mean NMC time in 25(HO)Vitamin-D deficient and control group was

11.15 ± 3.05 (6.3–17.6) and 8.40 ± 2.33 (6–13.2), respectively. The mean NMC time in patients with 25(HO)Vitamin-D deficiency was significantly longer than the control group ($p < 0.001$). Table 1 summarizes the demographics, 25(HO)Vitamin-D, and NMC levels of these groups.

25(HO)Vitamin-D replacement therapy was given to all the patients with deficiency. 25(HO)Vitamin-D level and NMC time were re-assessed 3 months after the replacement protocol. Thirty-nine patients completed the study after 3 months of follow-up; six participants did not want to repeat the NMC test at the end of 3 months follow-up after replacement therapy, five participants could not be reached at the end of 3 months, and the remaining five patients were unable to repeat the test due to upper respiratory tract infection at the time of control. Pre- and post-treatment values were compared with each other and also with the control group. The mean 25(HO)Vitamin-D level was 33.38 ± 10.03 in patients with Vitamin-D deficiency after replacement therapy and the difference was statistically significantly different from the pre-treatment levels ($p < 0.001$). The mean NMC time was 9.56 ± 2.54 after replacement protocol and the difference was statistically significant from the pre-treatment NMC time ($p < 0.001$). Although the NMC time after treatment was still longer than the control group, the difference was not statistically significant ($p = 0.051$). Table 2 and Figs. 1 and 2 summarize the demographics, 25(HO)Vitamin-D, and NMC levels of these three groups.

Table 1 Demographics and mean laboratory values of study and control groups

	Study ($n = 55$)	Control ($n = 32$)	p
Age	38.16 ± 10.99	35.31 ± 9.78	0.228
Sex			
Male	27 (49.1)	14 (43.8)	0.630
Female	28 (50.9)	18 (56.3)	
25(HO)Vitamin-D	14.32 ± 4.23	29.38 ± 7.05	***< 0.001***
NMC time*	11.15 ± 3.05	8.40 ± 2.33	***< 0.001***

P < 0.05 was considered statistically significant

The statistically significant data were specified with bold and italic

**NMC* nasal mucociliary clearance

Discussion

NMC is the main protection mechanism of the respiratory tract. Particles entering the nose by inhalation that may be detrimental are detained by the mucus layer and directed from the nasal cavity to the nasopharynx by the mucociliary clearance [14]. In humans, duration of the normal mucociliary transit is considered as 12–15 min. The NMC times measured more than this value are considered impaired NMC [3]. Deteriorated nasal mucociliary function can lead to respiratory, middle ear, and sinonasal infective disease [15]. There are several factors which affect NMC time such as smoking and various inhalation agents, nasal polyposis, adenoid hypertrophy, septum deviation, chronic renal failure, menopause, diabetes mellitus, temperature, and moisture [4, 5, 16, 17]. We excluded patients who have these described confounding factors. Along with many different methods such as stroboscopy, photon-electron techniques, and phase contrast microscopy used for NMC measurement, sodium saccharine test is the most preferred method because of its ease of implementation and cost [18].

The regulatory effect of 25(HO)Vitamin-D on the immune system is well known. Active 25(HO)Vitamin-D induces the synthesis of antimicrobial peptides from

Table 2 Comparison of post-treatment values with control group and also with pre-treatment values of the same patients

	Pre-treatment ($n = 39$)	Post-treatment ($n = 39$)	p* (Post vs pre)	Control ($n = 32$)	p^a (Post vs control)
Age	38.08 ± 10.75	38.33 ± 10.75	–[c]	35.31 ± 9.78	0.225
Sex					
Male	19 (48.7)	19 (48.7)	–	14 (43.8)	0.676
Female	20 (51.3)	20 (51.3)		18 (56.3)	
25(HO)Vitamin-D	14.76 ± 4.34	33.38 ± 10.03	***< 0.001***	29.38 ± 7.05	0.053
NMC time[b]	11.03 ± 3.23	9.56 ± 2.54	***< 0.001***	8.40 ± 2.33	0.051

$P < 0.05$ was considered statistically significant

The statistically significant data were specified with bold and italic

*Paired sample t test

[a]Independent sample t test

[b]Nasal mucociliary clearance

[c]Not compared, because the standard error of the difference is 0 due to the age increases equally for all patients

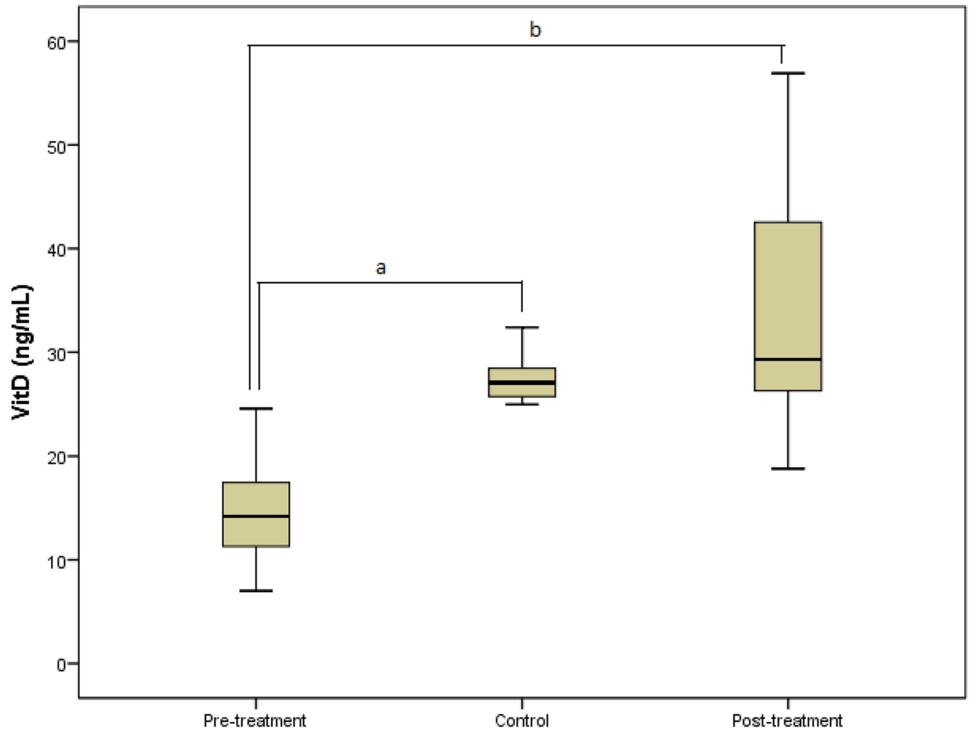

Fig. 1 Pre-treatment (study) and post-treatment values of deficient patients were compared with each other and also with control group. **a** Comparison of pre-treatment and control group values ($p < 0.001$). **b** Comparison of pre-treatment and post-treatment group values ($p < 0.001$). Paired sample *t* test was used for pre-treatment vs post-treatment comparison, while independent sample *t* test was used for pre-treatment vs control comparison

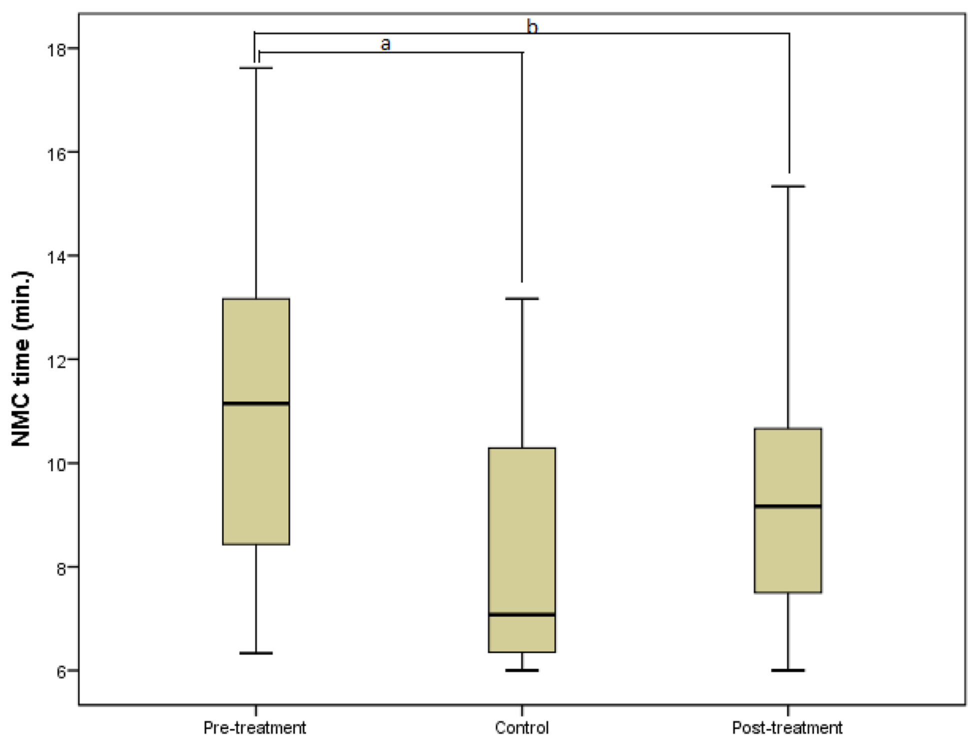

Fig. 2 Pre-treatment (study) and post-treatment NMC times were compared with each other and also with control group. **a** Comparison of pre-treatment and control group values ($p < 0.001$). **b** Comparison of pre-treatment and post-treatment group values ($p < 0.001$). Paired sample *t* test was used for pre-treatment vs post-treatment comparison, while independent sample *t* test was used for pre-treatment vs control comparison

the natural killer and respiratory tract epithelial cells [9, 19]. As well as, upregulated S100 protein and calprotectin levels were identified under the effect of active 25(HO) Vitamin-D [18]. In the event of a Vitamin-D deficiency, the immune response is disrupted and leukocyte chemotaxis is reduced [11, 20]. The rate of infection diseases rises due to disrupted immunity. The first prototype disease associated with 25(HO)Vitamin-D deficiency is tuberculosis, which has been studied over the years, and a strong correlation has been defined [21, 22]. Yim and colleagues revealed that cathelicidin synthesis was impaired in the bronchial epithelial cells in patients with frequent respiratory tract infection [23]. Deterioration of synthesis of the 25(HO)Vitamin-D-dependent immune regulator proteins such as cathelicidin, defending, S100, and calprotectin may clarify the increased URI in patients with 25(HO)Vitamin-D deficiency. Some studies propose that 25(HO)Vitamin-D may be effective in protecting against respiratory tract infections [19, 24–27]. Although the effect of 25(HO)Vitamin-D on the immune system is well known, there is no information about the association of Vitamin-D deficiency with mucociliary clearance. In addition to all these effects on the immune system, it is not known how the mucociliary clearance, which is the most important defense mechanism in the respiratory system, is affected by Vitamin-D deficiency. We have previously reported the association of Vitamin-D deficiency with otitis media with effusion [28, 29]. We have also inspected the increased rate of ventilation tube application in patients with Vitamin-D deficiency. However, we did not have enough data to clarify the pathophysiologic mechanism of the increased rate of otitis media with effusion and poor prognosis in patients with Vitamin-D deficiency.

Vitamin-D and its extra-skeletal functions are still unclear, and there is an ongoing debate about an association of Vitamin-D and the immune system. However, we know that about 3% of the human/mouse genome is under the control of active Vitamin-D and at least ten tissues rather than kidney express 1-alpha-hydroxylase enzyme which converts Vitamin-D to its active form [30, 31]. Therefore, the activity of Vitamin-D is much broader than calcium/bone metabolism. In a meta-analysis of 25 trials, Vitamin-D supplementation reduced the rate of acute respiratory tract infection [32]. In spite of the literature, the pathophysiologic pathway of the increased rate of URI still remains unclear. The most known hypothesis is an impaired immune response in patients with Vitamin-D deficiency. However, this is not well documented and not enough to explain pathophysiologic pathways. In the current study, we tried to investigate mucociliary functions in patients with Vitamin-D deficiency. Our results indicate the prolonged nasal mucociliary functions in patients with Vitamin-D deficiency. The NMC time is still in the normal range in patients with Vitamin-D deficiency, but it is significantly different from the control group. In addition, there is a significant change after Vitamin-D replacement. These two findings support that there is a strong association between Vitamin-D level and nasal mucosal clearance. This prolonged nasal mucociliary functions might be one of the contributing factors of an increased rate of URI in patients with Vitamin-D deficiency.

Pathophysiologic pathway of impaired NMC in patients with Vitamin-D deficiency has not been still clarified. We assert two probable hypotheses to explain this question: (1) the increased risk of upper respiratory tract infection and otitis media in patients with Vitamin-D deficiency is previously described [11, 28, 33, 34]. The previous studies highlight the increased squamous metaplasia rate on adenoid tissue in patients with otitis media with effusion [35]. The increased squamous metaplasia is associated with nasal mucociliary functions. Vitamin-D plays a prognostic role in patients with otitis media with effusion. The impaired mucociliary functions in patient with Vitamin-D deficiency might be associated with increased squamous metaplasia on upper respiratory tract. (2) Second hypothesis is decreased release of nitric oxide (NO) in patients with Vitamin-D deficiency. The previous studies indicated the impaired release of many bactericidal factors in case of Vitamin-D deficiency [9, 19, 20]. NO is one of important mediators released by inducible nitric oxide synthase from nasal-paranasal epithelia. NO has a role on nasal mucociliary clearance besides its bactericidal effects. Lungberg et al. reported the inverse correlation with nasal mucociliary functions and NO levels [36].

Limitations

The main limitation of the study is lack of biomolecular and electron microscopic findings in the study and control groups which might be useful to clarify the pathophysiologic pathway of prolonged mucociliary functions in patients with Vitamin-D deficiency. Another limitation is lack of data to evaluate the duration of Vitamin-D deficiency which might be associated with prolonged NMC.

Conclusion

This is the first study evaluating the association of mucociliary clearance with Vitamin-D deficiency. We are hereby reporting the impaired mucociliary activity in patients with Vitamin-D deficiency. These results are correlated with the published literature which shows the increased rate of upper respiratory tract infections in patients with Vitamin-D deficiency.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest regarding publication of this paper.

Ethical approval This article contains studies with human participants and local ethics committee approval was obtained before the study.

Informed consent Informed consent was obtained from all individual participants included in the study.

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