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ORIGINAL ARTICLE

Are Low Serum Vitamin D Levels a Risk Factor for Advent of COVID-19 Associated Rhinocerebral Mucormycosis: A Preliminary Case Control Study

Harsha Popli[1] · Ambika Gupta[1] · Virendra Singh[2] · Varsha Agarwal[1] · R. Akilan[2] · Adarsh Kumar[3]

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Abstract To determine whether low serum vitamin D level is a risk factor for development of Rhinocerebral mucormycosis in COVID-19 afflicted patients. A case control study was conducted in a tertiary care hospital utilizing the archived records of COVID-19 afflicted Rhinocerebral mucormycosis cases and age and gender matched controls. The mean value (± standard deviation) of vitamin D level in patients with Mucormycosis was 19.65 ± 13.07 ng/ml and in control subjects it was 27.88 ± 18.04 ng/ml.There was a significant difference between groups ($p = 0.02$). Thus, low Vitamin D level may be implicated as a risk factor for the advent of mucormycosis in a COVID-19 afflicted patient and therefore Vitamin D supplements may be provided to such patients to achieve normal serum levels.

Keywords Coronavirus disease (COVID-19) · Mucormycosis · Rhinocerebral Mucormycosis · Vitamin D(25-hydroxycholecalciferol)

Introduction

Mucormycosis is an infection caused by fungi of the order Mucorales belonging to the class Zygomycetes. The majority of human infections are caused by the genera *Rhizopus* followed by *Lichtheimia* and *Mucor* [1]. *Rhizopus oryzae* is the most frequently isolated organism from patients with mucormycosis [2].

On the basis of site(s) of involvement, it could be classified into 6 major clinical forms- rhinocerebral, pulmonary, cutaneous, gastrointestinal, disseminated and rare forms, such as endocarditis, osteomyelitis, peritonitis, and renal infection [1]. In the current era, Mucormycosis especially of the Rhinocerebral variety has incarcerated the individuals suffering and recovering from Coronavirus disease 2019 (COVID-19) [3, 4] making it important for us to dig deep into the disease. The etiopathogeneses that have been proposed for the development of this disease in COVID-19 patients include use of steroids, immune dysregulation by the virus, use of concurrent immunomodulatory drugs such as Tocilizumab and effect on CD4 + and CD8 + T-cells, but none of these stands with strong evidence [4, 5].

Vitamin D is one of the most popular vitamins in medical research. Its physiological role is to maintain calcium–phosphate homeostasis, proper functioning of musculoskeletal, immune, nervous, and cardiovascular systems [6]. Besides, it has a well proven role in immunity through stimulation of the function of macrophage, T cells, and activated B cells; maturation of dendritic cells; modulation of tumour necrosis factor expression and by production of neutral antibacterial peptides [7]. Its levels have been corelated with various infectious diseases such as tuberculosis, respiratory tract infections, influenza, chronic obstructive pulmonary disease exacerbations, cystic fibrosis and sepsis. Most studies state that vitamin D levels are directly related to the severity and morbidity of these diseases [8]. Researchers have also found out a link between COVID-19 infection, its severity and mortality with serum

✉ Ambika Gupta
drambika79@rediffmail.com

[1] Department of Oral Medicine and Radiology, Post Graduate Institute of Dental Sciences, Rohtak, Haryana, India

[2] Department of Oral and Maxillofacial Surgery, Post Graduate Institute of Dental Sciences, Rohtak, Haryana, India

[3] Department of Public Health Dentistry, Post Graduate Institute of Dental Sciences, Rohtak, Haryana, India

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levels of vitamin D, most studies yielding a positive correlation [9–12].

Rightly called as an epidemic within a pandemic after COVID-19, especially for countries like India, Mucormycosis requires a multifaceted treatment- surgical, sino-nasal drainage and debridement of orbital or cerebral disease along with a prolonged course of antifungal drugs. Some cases may even require craniectomy, lobectomy, and orbital exenteration [13]. Despite the treatment options available, the mortality rate is fairly high, ranging from 14.2% 83% [14]. Finding solid measures of prevention is therefore needed as there is still lack of sufficient evidence for a definitive treatment protocol and the prognosis is bleak.

There have been very few studies concerning fungal diseases and Vitamin D. Researchers have explored the role of vitamin D in Candida infection [15–17], *Aspergillus fumigatus* [18–20] and Cryptococcal meningitis [21] and the results are varied.

Association of vitamin D with Mucormycosis has not been explored yet. Thus, the aim of this case–control study was to determine whether vitamin D level is a risk factor for development of Rhinocerebral Mucormycosis in COVID-19 afflicted patients.

Materials and Methods

Study Setting

The current case control study was carried out in a tertiary care hospital in India. This study was performed in accordance with the principles of the Declaration of Helsinki and approved by the Biomedical and Health Research Ethics Committee. Informed consent was obtained from the patients.

Study Participants

All the COVID-19 afflicted patients with the diagnosis of Rhino-cerebral mucormycosis who reported to the OPD of our institute between July 2020 and June 2021 were screened. An equal number of matched controls were chosen from the archived database and their records were screened.

Cases

COVID-19 positive patients (or recent history of COVID-19 infection) diagnosed with rhino-cerebral mucormycosis through histopathology or culture.

Controls

COVID-19 positive patients (or recent history of COVID-19 infection) who were not diagnosed with rhino-cerebral mucormycosis.

Exclusion Criteria

1. Incomplete case records where the patient's Vitamin D level was not available
2. Patients on vitamin D supplementation currently or had taken them in the last 3 months [22] or status could not be ascertained.

After screening all the records of COVID-19 associated Mucormycosis (Rhinocerebral) patients, 23 met the inclusion criteria for cases. From the archived records, 23 controls were selected who had visited/were admitted to our institute for treatment of COVID-19 during the above-mentioned time period. Serum vitamin D levels of the two groups was the outcome measured in ng/ml. The status of Diabetes i.e., whether the patient is diabetic or not was also recorded as it is a potential confounder.

Data Collection

Demographics (sex and age) and history of COVID-19 and Diabetes were extracted from medical records. COVID-19 history was considered positive when either RT-PCR was positive or IgG against COVID-19 was positive, the latter valid only for non-vaccinated individuals. Diabetes was considered as present when either HbA1c levels were greater than 6.5% or fasting glucose greater than 126 mg/dL or random glucose over 200 mg/dL [23] or history of use of anti-diabetic medications.

Data Processing and Analysis

Descriptive analysis was performed where means and standard deviations values were presented for all continuous variables, whereas numbers and percentages were used for categorical variables. Pearson Chi-square or Fisher's exact test was used to examine the associations between categorical variables. Normality of data was assessed using Shapiro Wilk test and it was found to be not normally distributed. Therefore, Mann Whitney U test was used to compare the means between two groups. Significance level was set at 0.05. Odds ratio and relative risk were also calculated. We used IBM SPSS Statistics 21 software for statistical analysis.

Results

The demographic data and vitamin D levels of patients in each of the groups is shown in Table 1. There were no significant differences in the age, gender and diabetes status among the two groups.

The mean serum vitamin D levels in the sample (n = 46) was 23.76 ± 16.12 ng/ml. The mean value (± standard deviation) in patients with Mucormycosis was 19.65 ± 13.07 ng/ml and in control subjects it was 27.88 ± 18.04 ng/ml.There was a significant difference between groups (p = 0.02). Vitamin D deficiency was significantly associated with the occurrence of Mucormycosis [Odds ratio (OR) = 2.79 and Relative risk (RR) = 1.76].

Discussion

Vitamin D deficiency is associated with multiple adverse health outcomes such as rickets, osteopenia, hypertension, myocardial infarction, inflammatory bowel disease, diabetes mellitus Type I, leukemia, squamous cell carcinoma and infectious diseases among others [24].

Recently, researchers have attempted to corelate the incidence, severity and mortality of COVID-19 with serum Vitamin D levels [9–12]. Nimavat et al. [9], in their case control study comprising of 156 cases of COVID-19 and 204 controls, they found no statistical difference between mean Vitamin D levels among cases and controls (19.7 ± 8.4 ng/ml and 20.0 ± 11.7 ng/ml respectively) but the status of Vitamin D was positively associated with severity of the disease. Jain et al. [10] compared serum vitamin D levels among asymptomatic and severely ill COVID-19 patients wherein the levels were 27.89 ± 6.21 and 14.35 ± 5.79 ng/ml in the former and latter groups respectively, the difference being highly significant. Another study also showed a statistical difference in cases of COVID-19 and controls, mean(range) being 24 (19–29) and 26 (21–35) ng/ml, respectively [12]. Ye et al. [11] found out that the serum 25(OH)D levels in COVID-19 patients (22.4 ng/ml) were statistically lower than in healthy controls (28.72 ng/ml). So, it has been well established that serum vitamin D deficiency is associated with the incidence and severity of COVID-19 disease. The results of this study are in concordance with the previous studies as all the subjects included in the study had a deficiency of Vitamin D (cases 19.65 ± 13.07 ng/ml, controls 27.88 ± 18.04 ng/ml).

Table 1 Demographic data

Variables	Cases n = 23 n (%)	Controls n = 23 n (%)	p value
Age (years ± SD)	48 ± 11.84	48.43 ± 14.53	0.912
Males	13 (56.5)	12 (52.2)	0.205
Females	10 (43.5)	11 (47.8)	
Diabetic	18 (78.2)	16 (69.5)	0.062
Non-diabetic	05 (21.8)	7 (30.5)	

In fungal diseases there has not been much research and none of the studies in the literature have attempted to determine corelation between vitamin D and Mucormycosis and therefore it is tough to make direct comparisons with previous studies.

In an in vitro study by Bouzid et al. [15] to establish the relation of vitamin D and candida infection,100 mg/ml of vitamin D3 had a power inhibition in the growth of C. albicans with zone of inhibition 12.5 mm indicating good fungicidal activity. Sroussi et al. [25], through their retrospective study on HIV patients, concluded that vitamin D deficiency was a stronger predictor of oral candidiasis than other established factors such as CD4 count, tobacco usage, or HIV targeted treatment.

In yet another study on candida infection, a bimodal influence of vitamin D was discovered. Candida-infected mice treated with low-dose Vitamin D had reduced fungal burden and better survival relative to untreated mice. Conversely, higher doses led to poor outcomes. They found out that it was through potentiation of the proinflammatory cytokine response, among which IFN-γ, TNF-α, and IL-17, all of which are known to have important roles in antifungal host defense. Pathophysiology behind very high levels producing poor outcomes is unknown [16]. This lays impact on refraining from injudicious prescription of supplements during infection.

In a case control study on the effects of vitamin D level in Candida associated denture stomatitis, the vitamin D levels in the case group and control group was 54.68 ± 17.07 and 56.82 ± 17.75 nmol/L, respectively, with no significant difference between the groups [17]. In a study on HIV affected individuals, Vitamin D status was not found to be associated with disease severity of Cryptococcal meningitis, host immune response, or microbiological clearance [21].

Daily vitamin D_3 supplementation with 4000 IU over a 24-week period has been found to reduce *Aspergillus* induced IL-13 responses from peripheral CD4 + T cells and *Aspergillus*-specific IgE levels [18]. In an experimental study, two groups of mice were fed with Vitamin D sufficient and deficient diets followed by inoculation with A. fumigatus. The vitamin D deficient mice exhibited a higher rate of death, more fungal growth, and more weight loss than the Vitamin D sufficient group, while the viability of *A. fumigatus* conidia in sufficient mice was significantly

lower than that in deficient mice. Vitamin D here exhibited its effects by lowering autophagy levels and increasing activity of Treg cells(T regulatory cells which suppress immune response) [19]. Contrarily, a study showed that adjunctive vitamin D therapy has no beneficial or deleterious effects on systemic aspergillosis and also does not alter the efficacy of Amphotericin B in the treatment of this disease [20].

As proven by the previous literature on role of vitamin D in fungal diseases, this preliminary study also establishes an association between low vitamin D levels and occurrence of Mucormycosis.

The known risk factors for Mucormycosis are provided by COVID-19 infection which include hyperglycemia, raised ferritin, immunosuppressed condition, diabetes and endothelial damage [26]. Vitamin D exerts its immune effects due to the presence of vitamin D receptors (VDRs) and CYP27B1 on the surface of immune cells. In the presence of extracellular pathogens, there is upregulation of these cell surface receptors and binding of 1,25- dihydrocholecalciferol leads to the production of antimicrobial polypeptides such as cathelicidins, alfa defensins, beta defensins and LL-37 which act against bacteria, viruses and fungi [27]. Besides, patients with 25(OH) D levels less than 20 ng/mL are unable to fully express cathelicidin, which could be associated with increased susceptibility to fungal invasion [28]. Neutrophils produce reactive oxygen metabolites, perforin, cationic peptides, and enzymes, tumor necrosis factor-alpha (TNF-α), interferon-gamma (INF-γ), interleukin-1b (IL-1b) which fight infection, all of which is enhanced due to the expression of functional VDR on neutrophils [29].

Hyperglycemia, another risk factor is mitigated by Vitamin D as it plays a regulatory role in insulin secretion, calcium flux within β cell and β cell survival. Its deficiency has been shown to impair glucose-mediated insulin secretion in rat pancreatic beta cells, while vitamin D supplementation seems to restore such glucose-stimulated insulin secretion attributed to the presence of VDR on pancreatic cells, thus helping in maintenance of normoglycemic states [30]. Moreover, the constituent of cell membrane of Mucorales is ergosterol and Vitamin D being a sterol compound, its liposolibility hampers the integrity of cell membrane and causes disruption and death of the organism. Thus, it can be ascertained that vitamin D has a role in the preventing pathogenesis of Mucormycosis as suggested by our study.

To the best of our knowledge, this study gives the first insight into the association of vitamin D deficiency and Mucormycosis; with the advantage of using an age, gender and disease matched control group. Still, our findings must be interpreted warily because of our small sample size. More multicentric studies with a larger sample size corelating the severity of vitamin D deficiency with the occurrence as well as severity of Mucormycosis are required to further establish this co-relation. Conclusively, low serum vitamin D level may be implicated as a risk factor for the advent of mucormycosis in a COVID-19 afflicted patient and therefore Vitamin D supplements may be provided to such patients to achieve normal serum levels, at the same time being cautious of over prescription.

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Author Contribution All authors contributed significantly to the conception, design, data collection and writing the study.

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Declarations

Conflict of interest The authors have no conflicts of interest.

Research Involving Human Participants Ethical approval was obtained from the Biomedical and Health Research Ethics Committee of Post Graduate Institute of Dental sciences, Rohtak, vide approval number PGIDS/BHRC/21/45 and the study was performed in line with the Helsinki declaration.

Informed Consent Informed consent was obtained from the participants and confidentiality of the records was maintained throughout the study.

Ethical Approval Ethical approval was obtained from the Biomedical and Health Research Ethics Committee of Post Graduate Institute of Dental sciences, Rohtak, vide approval number PGIDS/BHRC/21/45 and the study was performed in line with the Helsinki declaration.

Consent to Publish All participants consented for publishing of their data and confidentiality was maintained.

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