To assess serum Vitamin D3 levels and inflammatory markers (IgE, IL-6) in patients with rhinosinusitis: An observational study

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ABSTRACT

Background: Rhinosinusitis or allergic rhinitis (AR) is the primary non-infectious type of rhinitis, marked by symptoms like sneezing, coughing, and flu-like signs. While the exact pathophysiology of AR remains unclear, vitamin D3 insufficiency is increasingly recognized as a potential contributor to allergic disorders due to its role in immunomodulation.

Aim & objectives: To assess serum Vitamin D3 levels and inflammatory markers (IgE, IL-6) in patients with allergic rhinitis and healthy controls among North Indian population group.

Materials and Methods: This observational study involved 200 participants, divided into two groups. The case group included 100 allergic rhinitis patients (aged 18-55), diagnosed through medical history, examination, and lab tests at SGT Hospital's ENT department. The control group comprised 100 healthy volunteers, matched for age and gender. Both groups were briefed on the study, and consent was obtained. A 5 ml blood sample was collected and processed for serum extraction, stored at -20°C. Vitamin D levels were measured using a Competitive ELISA assay, while Interleukin 6 and IgE were analyzed via Sandwich ELISA. The study was conducted at SGT Hospital and the Department of Pharmacology, SGT University, Gurugram, Haryana, India.

Results: The study compares gender distribution and participant numbers across age groups in 200 participants, with Control and Case groups. It also compares Vitamin D, IgE, and IL-6 levels. The Control group has a mean Vitamin D of 25.9 ng/ml, significantly higher than the Case group's 12.5 ng/ml ($p = 0.001$). IgE levels are 35.9 ng/ml for Control and 270 ng/ml for Case, but the difference is not significant ($p = 0.1$). IL-6 levels are 7.01 ng/ml for Control and 25.9 ng/ml for Case, showing borderline significance ($p = 0.05$).

Conclusion: Vitamin D deficiency was evident within the case group. Those diagnosed with Allergic Rhinitis exhibited markedly lower mean serum vitamin D levels than their counterparts in the control group. Moreover, upon deeper stratification of the data, the disparity in vitamin D concentrations became even more striking.

Introduction:

Rhinosinusitis or allergic rhinitis (AR), an inflammatory condition of the nasal membrane, manifests with symptoms like sneezing, rhinorrhea, nasal congestion, and itching. It affects over one-third of the global population, spanning all ages and regions. Triggers include respiratory allergens, food, skin allergens, and medications. The development, progression, and management of allergies involve genetic predisposition, environmental factors, nutritional status, and biochemical influences [1, 2].

Clemens von Pirquet coined the term "allergy" in 1906. Allergic rhinitis (AR) is a prevalent health issue triggered by inflammation following exposure to allergens, involving an immune response mediated by immunoglobulin E (IgE) [3]. Although allergic rhinitis is typically not life-threatening, it can lead to complications and significantly diminish quality of life. It manifests when an atopic individual encounters allergens, and can be either seasonal or perennial [4].

The treatment options for allergic rhinitis include allergen avoidance, pharmacotherapy, immunotherapy, and surgical intervention. Pharmacotherapy encompasses antihistamines, decongestants, steroid sprays, and leukotriene receptor antagonists. The prevalence of allergic rhinitis varies globally due to geographical and aeroallergen differences. It is the most common type of chronic rhinitis, impacting 10-20% of the population, with evidence indicating an increasing prevalence. Low- and middle-income countries generally report lower prevalence rates, though incidence is rising steadily. Allergic rhinitis affects both children and adults, and its prevalence varies due to differing definitions, study methodologies, and seasonal variations [5, 6, 7].

Results from the International Study of Allergy and Asthma in Children (ISAAC) indicated that Indian children aged 13-14 years had an overall 10% prevalence of allergic rhinitis, with a higher prevalence of 11.6% in Delhi. Among adults in Delhi, the prevalence was estimated to be 11.7% [8, 9].

In a European study of the general adult population using the Allergic Rhinitis and its Impact on Asthma (ARIA) definition for diagnosis, allergic rhinitis prevalence was reported to be approximately 25%, varying between 17% - 28.5%. In the Asia Pacific Region (2008), the prevalence of allergic rhinitis in adults ranged from 10% to 32% [9].

Several attempts have been made to prevent allergic diseases, yet most have not succeeded. Further research is needed in light of these inconsistent findings. Some parameters sensitive to allergic rhinitis can be measured as markers, including vitamin D3, calcium, trace elements (zinc, iron, magnesium), IL6, and IgE. Vitamin D is crucial for the body's absorption of dietary calcium and phosphate. It exists in two major forms: cholecalciferol (Vitamin D3) and ergocalciferol (Vitamin D2). While both forms can be obtained from foods or supplements, only vitamin D3 is synthesized in the skin. Numerous studies have recently suggested a potential link between vitamin D and the development of allergic rhinitis. Vitamin D deficiency is highly prevalent in India across all age groups and genders, ranging from 70-80% [9, 10].

Allergic rhinitis (AR) symptoms are caused by inflammatory mediators like histamine and leukotrienes, released due to increased production of immunoglobulin E (IgE) by plasma cells. This IgE production is stimulated by cytokines released from inflammatory T cells in the nasal mucosa, triggered by exposure to allergens. IgE production is further enhanced by allergens, leading to hypersensitivity reactions [11]. IgE levels in serum typically range from 150-300 IU/ml (or below 110 ng/dL) and play a crucial role in allergic responses and immune functions, including activation of Th2 cells and wound healing. During anaphylactic shock, hypotension serves as a protective mechanism to limit blood flow temporarily, aiding in toxin removal. The cytokine IL-6, produced by T cells and macrophages, has both pro-inflammatory and anti-inflammatory properties, influencing inflammation and acute phase

protein synthesis, though it's exact role in allergy development is not fully understood. Various strategies to prevent allergic rhinitis have been largely unsuccessful, prompting this study to explore the roles of serum Vitamin D3, Calcium, trace elements (Iron, Zinc, Magnesium), and inflammatory markers (IgE, IL-6) in allergic rhinitis [11, 12, 13].

Materials and Methods

The current observational study was conducted on a total of 200 individuals, comprising two distinct groups. The case group included 100 clinically diagnosed allergic rhinitis patients, aged between 18 and 55 years, who were evaluated in the ENT outpatient department at SGT Hospital. Diagnosis was established through a comprehensive approach, incorporating medical history, physical examination, and laboratory investigations.

To ensure a well-matched comparison, the control group consisted of 100 healthy volunteers from the general public, selected to align with the case group in terms of age and gender. The study was carried out at SGT Hospital and the Department of Pharmacology, faculty of medicine & health sciences, SGT University, Gurugram, Haryana, India.

Prior to initiating sample collection, ethical clearance was obtained from the Institutional Ethical Committee. Additionally, written informed consent was secured from all participants after thoroughly explaining the study's purpose and procedures to both groups.

Study Population: - According to convenient sampling and taking the value as reference according to Velankar et al., [14] the minimum number of sample size was calculated. Using this formula below sample size has been calculated. n= Z2 x P x (1-P)/e2 where: n= Sample size Z= Value from standard normal distribution, corresponding to desired confidence level (Z= 1.96 for 95% CI) P= prevalence of allergic rhinitis e= allowable error

Inclusion Criteria: The case group comprised individuals clinically diagnosed with allergic rhinitis, exhibiting characteristic signs and symptoms of nasal allergy. In contrast, the control group consisted of healthy volunteers, carefully matched for age and gender to ensure comparability were utilized for the study.

Exclusion criteria: Individuals with nasal pathologies, pregnant Women, habitual smokers, regular alcohol consumers, and those with asthma were not included in this study.

Methodology:

Both the control and case groups received comprehensive briefings on the study's purpose, ensuring informed participation. Consent was formally obtained before proceeding. A venous blood sample (5 ml) was meticulously drawn via venepuncture, employing a sterile, single-use syringe and needle under stringent aseptic conditions. To separate the serum, centrifugation was performed at 3500 RPM for 15 minutes. The extracted serum was then securely stored at -20°C to maintain integrity.

Serum Vitamin D levels were quantified through a Competitive ELISA kit-based assay, while Serum Interleukin 6 and IgE concentrations were assessed using a Sandwich ELISA kit-based technique, ensuring precision and reliability in biomarker analysis.

Statistical Analysis:

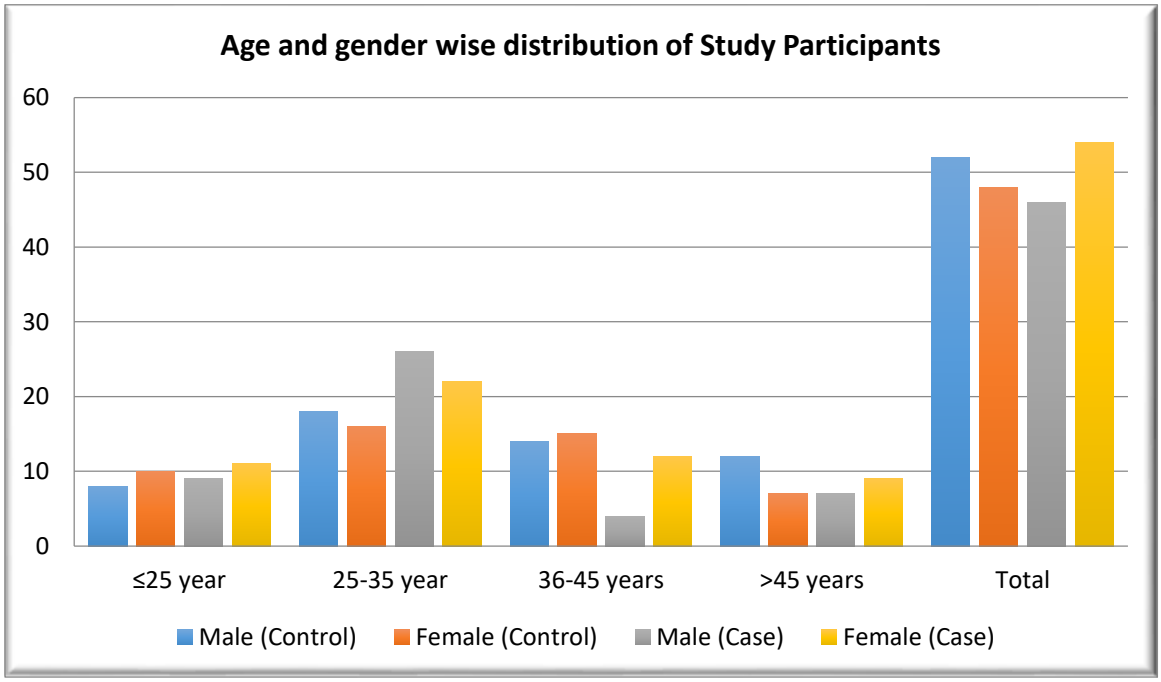
Statistical analysis was conducted using SPSS software (Version 23, USA Inc.) to evaluate Vitamin D levels, trace elements, calcium, and various other parameters. Mean values and standard deviations were calculated for all measured variables. Comparisons between two groups were performed using the Student's t-test, while the Pearson correlation coefficient was utilized to assess relationships between variables.

Results:

In the present observational study compares the gender distribution in two groups (Control and Case) across various age groups. Table 1 and graph 1 presents the number of males and females in each group, along with the total participants and the average number per age group. The data highlights differences in gender distribution and participant numbers between the groups across age ranges.

Age Group	Control Group		Case Group	
	Male	Female	Male	Female
≤25 year	8	10	9	11
25-35 year	18	16	26	22
36-45 years	14	15	4	12
>45 years	12	7	7	9
Total	52	48	46	54
Mean	13	12	11.5	13.5

Table 1: Showing age and gender wise distribution of Study Participants.



Graph 1: Showing Age and gender wise distribution of Study Participants.

Table 2. compares Vitamin D levels, IgE, and IL-6 between the Control and Case groups. The Control group has a mean Vitamin D of 25.9 ng/ml, while the Case group has 12.5

ng/ml, with a significant difference (p = 0.001). For IgE, the Control group averages 35.9 ng/ml, and the Case group has 270 ng/ml, but the difference is not statistically significant (p = 0.1). The Control group's IL-6 is 7.01 ng/ml, while the Case group's is 25.9 ng/ml, with a borderline significance (p = 0.05).

Parameters	Control group (ng/ml)		Case group (ng/ml)		t- value	p-value
	Range	Mean± SD	Range	Mean± SD		
Vitamin D level	6.08- 90.8	25.9 ± 14.9	4.1- 21	12.5 ± 3.7	3.01	0.001*
IGE	3.8- 81.9	35.9 ± 18.7	168- 501	270 ± 70.6	0.9	0.1
IL6	0.8- 41	7.01 ± 5.9	4.01- 90.1	25.9 ± 21.7	1.9	0.05*

Table 2: The range and average (ng/ml) on control group and case group; Paired student's t-test, *level of significant p-value <0.05.

Discussion:

Allergic rhinitis is a prevalent health condition that significantly affects daily life. It is triggered by various allergens, including domestic allergens (such as dust mites, pet dander, insects, and plant-based allergens), outdoor allergens (such as pollen and mold), and occupational allergens (such as latex). Other contributing factors include tobacco smoke, automobile emissions (containing ozone, nitrogen oxides, and sulfur dioxide), aspirin, and other non-steroidal anti-inflammatory drugs (NSAIDs) [14, 15].

The condition is characterized by four main symptoms: rhinorrhea, sneezing, nasal itching, and nasal congestion. These symptoms can lead to sleep disturbances, fatigue, low mood, irritability, and impaired cognitive function. Additionally, allergic rhinitis may be associated with other conditions such as conjunctivitis, postnasal drip, Eustachian tube dysfunction, otitis media, and sinusitis. In infants, it can also contribute to dental malocclusions and facial abnormalities [15].

Agarwal S et al. conducted a study in which participants were divided into two groups, each consisting of 40 individuals. During the subsequent investigation, two participants were excluded, leaving a total of 38 cases. Among the 80 participants, 50 were men and 28 were women, resulting in a male-to-female ratio of 1.78:1, indicating a higher proportion of males [16]. Additionally, a study by Gupta et al. in India in 2016 involved 27 individuals diagnosed with allergic rhinitis, with an average age of 26.47 ± 9.25 years [17].

Bhat VS et al. conducted a study comparing vitamin D3 levels between two groups. The findings revealed that patients in the AR group had an average blood vitamin D level of 15.8±7.4 ng/ml, while those without AR had an average level of 18.1±6.6 ng/ml. This difference was statistically significant, with a p-value of 0.003. Both groups consisted of individuals with insufficient or low vitamin D3 levels [18].

Similarly, a 2012 study by Hollams et al. in Australia investigated the relationship between blood vitamin D levels and allergic rhinitis. Their research confirmed a correlation between vitamin D insufficiency and allergic rhinitis [19].

In another related study by Agarwal S et al., researchers observed that the average blood vitamin D level in 38 patients at the start of the trial was 20.15 ± 10.26 ng/ml. After three months of supplementation, the average serum vitamin D level significantly increased to 38.05 ± 14.6 ng/ml (p = 0.0001). No notable changes were observed in the control group [17].

Present study assessed vitamin D3 levels in both groups. The results showed that patients in the AR group had an average blood vitamin D level of 12.5 ± 3.7 ng/ml, while individuals without AR had a level of 25.9 ± 14.9 ng/ml. This difference was statistically significant ($p = 0.001$). Notably, both groups exhibited insufficient or deficient vitamin D3 levels.

A study by Awan N. U. et al. reported that the average IgE levels were 378.36 ± 132.84 IU/ml, with a range of 103 to 740 IU/ml. In Group A, the mean serum IgE levels were 383.69 ± 154.86 IU/ml, while in Group B, they measured 373.03 ± 106.83 IU/ml ($p = 0.0001$). In contrast, the current study found that the average serum IgE levels in the control group were 35.9 ± 18.7 IU/ml, whereas in the case group, they were 270 ± 70.6 IU/ml ($p = 0.1$) [20].

The IL-6 gene is located on the short arm of chromosome 7, specifically within the p15–21 region. It spans approximately 5 kilobases and consists of four exons and six introns. Research on mite sensitivity has linked the phenotype to the 4q12 region in the British population and the 4q27 region in the German population, both of which are considered significant candidates for mite sensitivity traits. Additionally, a previous study identified an association between the 7q35 region and HDM allergens (Dermatophagoides farinae) in a subset of individuals with asthma [21].

Similarly, the IL-8 gene is located on chromosome 4, within the q13–q21 region. It consists of four exons, three introns, and a proximal promoter region, with a total length of 5.25 kilobase pairs (kbp). In the present study, the average serum IL-6 levels were found to be 7.01 ± 5.9 IU/ml in the control group, whereas in the case group, they were significantly elevated at 25.9 ± 21.7 IU/ml ($p < 0.05$). Consistently, Dey et al. reported that the mean serum IL-6 level in the affected group was 51.66 ± 12.09 pg/mL, compared to 4.65 ± 1.78 pg/mL in the control group ($t = 15.06$, $p < 0.0001$) [22].

Conclusion:

Vitamin D deficiency was evident within the case group. Those diagnosed with Allergic Rhinitis exhibited markedly lower mean serum vitamin D levels than their counterparts in the control group. Moreover, upon deeper stratification of the data, the disparity in vitamin D concentrations became even more striking.

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