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Elevated White Blood Cell Subsets and Imbalances in Iron, Magnesium, and Vitamin D3 Are Linked To Severity of Pediatric Asthma

Shivani Singh[1,2], Manish Raj Kulshrestha[2], Aditi Singh[1], Shetanshu Srivastava[3], Vandana Tiwari[2*]

[1]Amity Institute of Biotechnology, Amity University Uttar Pradesh, Lucknow Campus
[2*]Department of Biochemistry, [3]Department of Pediatrics; Dr. Ram Manohar Lohia Institute of Medical Sciences, Lucknow

[*]**Corresponding author**: Prof. Vandana Tiwari, Professor, Department of Biochemistry, Dr. Ram Manohar Lohia Institute of Medical Sciences, Lucknow

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ABSTRACT:

Background: The diagnosis of asthma is dependent on the clinical presentation of bronchospasm, spirometry results, altered white blood cells, allergic variables, essential minerals (cofactors of enzymes), and VitaminD3.

Aim: This study is aimed to evaluate the hematological parameters and allergy markers. In addition, we studied the effect of essential elements and Vitamin D3 on asthma.

Method: In this study, 48 children diagnosed with asthma at the OPD of asthma clinic, Dr. RMLIMS, Lucknow, and 19 non-asthmatic controls age-sex-matched were enrolled. The hematological parameters were estimated by SysmexXT2000i. Calcium, Magnesium, Phosphorus, Iron, IgE, and hs-CRP were estimated by Beckman Coulter (AU480) automated analyzer and Vitamin D3 was analyzed by the immunochemilumine scent method.

Result: There were no significant differences in age and sex in asthma and the control group showed adequate matching (p=0.743 and p=0.462 respectively); however, FEV1% [asthmatic case: median, 75.8 (Q157.2- Q387.5)] vs [control: median, 95 (Q1:79.2- Q3: 89.5) was significantly associated with asthma (p<0.00001). The study revealed significant differences between asthmatic children and a control group. Asthmatic children had higher levels of leukocyte counts ((Median;9.01 ×103/µL, p<0.0001), neutrophil counts (Median: 54.5%, p<0.0001), lymphocyte counts (Median: 38.50%, p<0.0001), eosinophil counts (Median: 9.50%, p<0.0001). The IgE (median: 996.85IU/mL; Q1:331.06-Q3:1676.3 vs median: 57.32IU/mL; Q1:49.09-Q3:71.29) and hs-CRP (median: 3.10 mg/L; Q1:0.90- Q3:6.59 vs median: 0.81 mg/L; Q1:0.75- Q3:0.92) were significantly elevated in asthmatic cases as compared to control (p<0.05). However, iron, magnesium, and vitamin D3 were significantly lower in asthmatic cases than the control (p<0.0001). The univariate and multivariate regression analyses showed that iron (univariate: β=0.132, p=0.041; multivariate: β=0.112, p=0.049) and magnesium (univariate: β = 0.133, p=0.043; multivariate: β=0.114, p=0.048) showed a positive association with the FEV1/FVC ratio. Conversely, vitamin D3 was negatively associated (univariate: β=-0.165, p=0.012; multivariate: β =- 0.156, p=0.018).

Conclusions: Rise in WBCs subsets with IgE and hs-CRP in asthmatic children was observed. Essential elements and Vitamin D3 association indicate it may as a confounder of asthmatic components**.** Parental smoking, family history, and atopy are risk modifiers for asthma.

Keywords: Bronchial Asthma, FEV1, IgE, hs-CRP, Vitamin D3, Bronchospasm.

1. Introduction

In India, pediatric asthma is a significant health concern, affecting approximately 6% of children [1,2]. This chronic respiratory condition is characterized by hyperresponsive and inflammatory airways, with a multifaceted etiology involving both genetic and environmental factors. Essential minerals such as iron and magnesium play a role in immunological processes as enzyme cofactors, and their impact on asthma has been extensively studied [3-6]. Given that infants have developing immune systems and lungs, they are more susceptible to diseases like asthma [7,8].
In children with asthma, total and differential leucocyte counts are often elevated due to the chronic inflammatory nature of the disease [9]. The total leucocyte count increases as a general marker of inflammation and the immune response. Specifically, differential leucocyte counts show an increase in eosinophils, which are associated with allergic inflammation and are a hallmark of asthma [10]. Elevated eosinophils indicate heightened allergic responses and airway inflammation. Additionally, increased neutrophils may be observed, reflecting broader aspects of immune activation and inflammation [11].

The relationship between asthma and essential minerals such as iron, magnesium, phosphorus, and calcium is both intricate and significant. Low iron levels are commonly observed in children with asthma, potentially related to increased oxidative stress and inflammation associated with the condition; however, both deficiencies and excesses of iron can impact asthma in different ways [12]. Magnesium is crucial for muscle relaxation, including the airway muscles, and deficiencies have been linked to increased airway hyperreactivity and inflammation [13]. Similarly, calcium, essential for muscle contraction and immune responses, may exacerbate asthma symptoms when levels are low, likely due to its role in regulating airway smooth muscle function and inflammatory responses [14].

Several studies suggest an imbalance in essential minerals and vitamin D3 levels in children with asthma. This imbalance may be associated with an increased risk and severity of asthma, changes in asthma symptoms, and reduced lung function [15-17]. Vitamin D3 plays a crucial role in the immune system and in modulating inflammation; deficiencies can exacerbate asthma risk by increasing the likelihood of respiratory infections and airway inflammation [18]. However, confounding factors such as age, gender, pollution, and family history of asthma also influence asthma risk. Therefore, while there is evidence suggesting that micronutrient deficiencies and disrupted homeostasis of essential minerals and vitamin D3 might affect asthma, our understanding of these relationships remains incomplete and requires further exploration to determine their independent predictive value and the crude risk associated with asthma.
This study aimed to address the knowledge gap by comparing the status of total and differential leucocyte counts, high-sensitivity C-reactive protein (hs-CRP), immunoglobulin E (IgE), and essential minerals (iron, magnesium, phosphorus, and calcium) and vitamin D3 in children with asthma to those in control groups.

2. Methodology

Subject Enrollment

This case-control study was conducted in the department of biochemistry at Dr Ram Manohar Lohia Institute of Medical Sciences, Lucknow. The study comprised 200 asthmatic cases and 100 age-sex matched normal control from the same geographical area. All children ages had

between 5 to 15 years. Asthma was diagnosed as per the GINA guideline based on the clinical presentation and spirometry analysis [19].

Spirometry Analysis
Questionnaire based detailed information was recorded. Baseline spirometry was measured using fully automated SpiroLab Spirometry to assess the asthma severity. The Forced Expiratory Volume 1 second (FEV1) was estimated. FEV1 ≤60% indicated severe airway obstruction, FEV1 <80% predicted moderate airway obstruction, and FEV1 ≥80% indicated mild airway obstruction.

Sample Collection
Blood sample was collected at child first visit in asthma clinic. 3mL blood was withdrawal via vein puncture; 1 mL in ethylenediamine tetraacetic acid (EDTA) vial for total and differential leucocyte counts and 2mL in plain vial for essential minerals, hs-CRP, IgE, and vitamin D3.

Assessment of Complete Blood Count
For total and differential leucocyte counts, CBC was estimated using 6-part fully automated hematology analyzer with Advanced angle polarized scatter separation (MAPSS) technology (Alinity h-series, Abbott, Japan).

Estimation of Biochemical Parameters (Hs-CRP, Ige, Essential Minerals)
Seum hs-CRP and IgE were measured using commercially available immunoassay system from DiaSys, Diagnostic System GmbH, Germany based on antigen-antibody reaction and fluorescence technology using fully automated analyzer (Beckman Coulter, AU480). However, Iron, magnesium, phosphorus, and calcium were also measured by using system pack commercial reagent on the same instrument.

Vitamin D3 Measurement Using Immunoassay
Serum Vitamin D3 was measured using a fully automated analyzer, Roche Diagnostic (cobas 6000). This is based on a competitive immunoassay that uses an anti-fluorescein monoclonal antibody covalently bound to paramagnetic particles, a vitamin D3 monoclonal antibody labeled with acridinium ester, and a vitamin D3 analog labeled with fluorescein. An inverse relationship exists between the amount of vitamin D3 level in the samples and the amount of relative light units (RLU) detected by the system.

Statistical Data
The frequency data was represented in number and percentage. The variable data was represented in median and quartile ranges. The graph was prepared by using MedCal software. The chi-square test for continuous data and Mann-whitney test for variable data were used to compare the study groups. The data was analyzed using SPSS software 24, Chicago, USA. The p-value less than 0.05 was considered as statistically significant.

3. Result

Table 1 provides an overview of the demographic characteristics of the two groups under study. It shows that there are no significant differences in terms of age, gender, or residential area between the groups. This lack of significant difference indicates that the groups are well-matched with respect to these key demographic factors. The family history of asthma was presented in 49.5% of asthmatic cases. FEV1 status was significantly reduced in asthmatic cases as compared to normal control.

Table 1: Demographical and Clinical data of study population

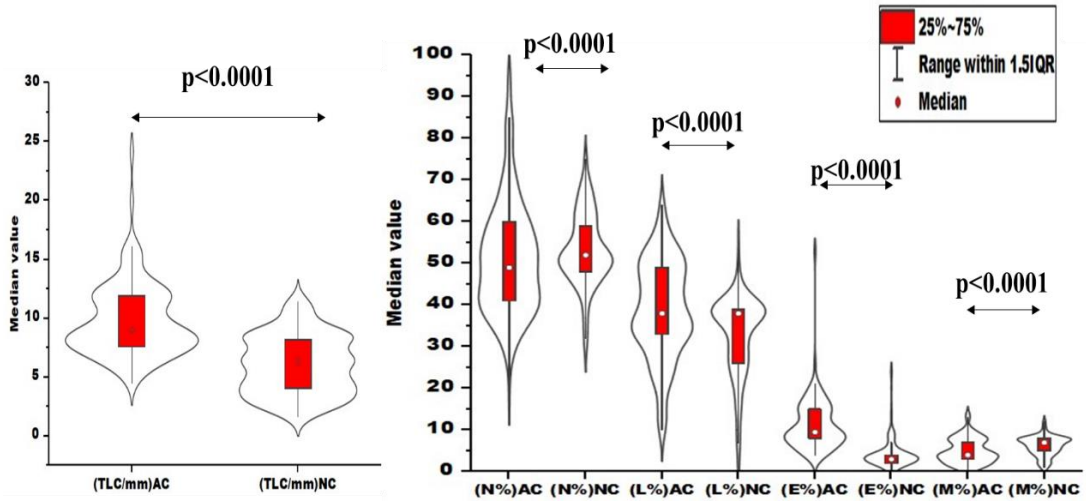
Variables		Asthmatic Cases (n=200); N (%)	Normal Control (n=100); N (%)	p-value
Age (Years)	05-10 Yrs.	92(46.0)	48(48.0)	0.743
	11-15 Yrs.	108(54.0)	52(52.0)	
Gender	Male	101(50.5)	55(55.0)	0.462
	Female	99(49.5)	45(45.0)	
Residence	Urban	115(57.5)	59(59.0)	0.804
	Rural	85(42.5)	41(41.0)	
Socioeconomic Status	Poor	18(9.0)	13(13.0)	0.216
	Lower Middle Class	96(48.0)	38(38.0)	
	Upper Middle Class	86(43.0)	49(49.0)	
Dietary Habit	Vegetarian	96(48.0)	43(43.0)	0.713
	Non-Vegetarian	59(29.5)	32(32.0)	
	Eggetarian	45(22.5)	25(25.0)	
Parental Smoking Habit	Yes	111(55.5)	56(56.0)	0.934
	No	89(44.5)	44(44.0)	
Family h/o of asthma	Yes	99(49.5)	13(13.0)	<0.0001*
	No	101(50.5)	87(87.0)	
FEV1 (%)	Median (Q1-Q3)	75.8 (57.2-87.5)	95(79.2-89.5)	<0.0001*

*Abbreviations: FEV1: Forced expiratory volume in 1 second, h/o: history of. The chi-square test and Mann-whitney test were used to calculate the p-value. *p<0.05 was considered as statistically significant.*

Status of total and differential leucocytes

The study revealed significant differences between asthmatic children and a control group. Asthmatic children had higher levels of leukocyte counts ((Median;9.01 ×103/µL, p<0.0001), neutrophil counts (Median: 54.5%, p<0.0001), lymphocyte counts (Median: 38.50%, p<0.0001), eosinophil counts (Median: 9.50%, p<0.0001).

Figure 1: Violin Plot showed the levels of total and differential Leucocytes counts



Status of biochemical parameters of the study population

The IgE (median: 996.85IU/mL; Q1:331.06-Q3:1676.3 vs median: 57.32IU/mL; Q1:49.09-Q3:71.29) and hs-CRP (median: 3.10 mg/L; Q1:0.90- Q3:6.59 vs median: 0.81 mg/L; Q1:0.75-Q3:0.92) were significantly elevated in asthmatic cases as compared to control ($p<0.05$). However, iron, magnesium, and vitamin D3 were significantly lower in asthmatic cases than the control ($p<0.0001$) (Table 2).

Table 2: Comparison of biochemical parameters in asthmatic cases and normal control

Parameter	Asthmatic Cases (n=200)	Normal Control (n=100)	p-value
IgE (IU/ml)	996.85(331.06-1676.3)	57.32(49.09-71.29)	<0.0001*
Hs-CRP (mg/L)	3.10(0.90-6.59)	0.81(0.75-0.92)	0.001*
Mg (mg/dl)	1.86(1.77-2.09)	2.27(2.11-2.35)	<0.0001*
Iron (µg/dl)	53.0(45.8-66.5)	76.0(51.0-87.5)	<0.0001*
Phosphorus (mg/dl)	4.60(4.10-4.97)	4.90(4.75-4.95)	0.114
Calcium mg/dL)	9.60(8.30-9.90)	9.8(9.5-10.0)	0.432
Vitamin D3 (ng/ml)	9.56(7.83-9.90)	19.29(14.18-20.0)	<0.0001*

*Abbreviations: IgE: Immunoglobulin E, hs-CRP: high sensitivity c reactive protein, Mg: Magnesium. The Mann-whitney test was used to calculate the p-value. *p<0.05 was considered as statistically significant.*

Essential minerals and vitamin D3: associations with lung function (FEV1)

In the univariate regression analysis, iron positively correlated with the FEV1 value (β =0.132, p=0.041). Similarly, magnesium was also positively associated with the FEV1 value (β =0.133, p=0.043). In contrast, vitamin D3 negatively correlated with the FEV1 value (β =-0.165, p=0.012). In the multivariate regression analysis, iron (β=0.112, p=0.049) and magnesium (β

=0.114, p=0.048) were positively associated with FEV1 value, while vitamin D3 was negatively associated with FEV1 value (β =-0.156, p=0.018) (Table 3).

Table 3: Univariate and multivariate Linear regression analysis model as FEV1 ratio-dependent variables

Variables	Univariate		Multivariate	
	β-coefficient	p-value	β-coefficient	p-value
Iron (µg/dL)	0.132	0.041*	0.112	0.049*
Mg (mg/dl)	0.133	0.043*	0.114	0.048*
Phosphorus (mg/dl)	-0.021	0.756	-	-
Calcium mg/dL)	.008	0.899	-	-
Vitamin D3 (ng/mL)	-0.165	0.012*	-0.156	0.018*

*Abbreviation: Mg: Magnesium. Linear regression analysis was used to calculate the beta coefficient. *p<0.05 was considered asstatistically significant.*

4. Discussion

The study was conducted with subjects who were methodically matched based on gender, age and locality, ensuring an unbiased comparison of other variables. Spirometry analysis reveals that the Forced Expiratory Volume in 1 second (FEV1) was significantly lower in asthma, who exhibited symptoms such as wheezing, chest tightness, shortness of breath, and recurrent coughing.

In this context, our findings demonstrated that asthmatic children had significantly lower FEV1 and higher levels of leukocytes, neutrophils, lymphocytes, eosinophils, hs-CRP, and IgE, suggesting a state of inflammation.

Elevated levels of total leucocytes and differential leucocytes, like eosinophils and neutrophils, are common. Eosinophils are often increased because they play a key role in the allergic inflammation that characterizes asthma. When allergens trigger an asthma attack, eosinophils are recruited to the airways, where they release substances that worsen inflammation and damage the airway tissues [20,21]. Neutrophils can also be elevated in asthma, especially in severe or chronic cases. They contribute to ongoing inflammation and can further damage the airways [22,23]. This overall increase in inflammatory cells reflects the chronic inflammation in asthma, leading to symptoms like wheezing, coughing, and shortness of breath.

In asthma, higher serum levels of IgE and hs-CRP levels are linked through interconnected inflammatory pathways. Exposure to allergens triggers a Th2 immune response, leading to increased production of IgE antibodies, which bind to mast cells and cause their degranulation and release of inflammatory mediators [24]. This allergic reaction contributes to airway inflammation and hyperreactivity. Concurrently, the chronic inflammation in the airways activates systemic inflammatory responses, prompting the liver to produce C-reactive protein (CRP), detectable as hs-CRP in the blood. Elevated hs-CRP reflects this systemic inflammation, highlighting the ongoing inflammatory processes associated with asthma. These factors include allergic reactions, airway hyperresponsiveness, and exposure to irritants. These triggers activate inflammatory pathways, such as NF-κB and IL-6, leading to increased production of hs-CRP [25].

Conversely, we also observed that the levels of iron, magnesium, and vitamin D3 were significantly reduced, indicating disrupted homeostasis in asthmatic children. Epidemiological studies have linked iron deficiency to atopic diseases such as asthma, with iron metabolism playing a role in regulating immune responses to allergens [26,27]. Anemia is often associated with atopic diseases in children and adolescents [27]. Iron storage is higher in Th2 immune cells than in Th1 cells, influencing cytokine production [28]. Limited iron availability during lymphocyte stimulation by allergens may favor Th2 responses, leading to allergic sensitization [8]. Asthma patients typically have low levels of cell-free iron, which correlates with reduced lung function but increased iron-loaded cells. Many mammalian allergens, part of the lipocalin family, can bind to bacterial siderophore-iron complexes. These allergens promote Th2 responses when not bound to iron, suggesting that iron deficiency may enhance their stimulatory potential.

Our research also indicated that iron status was lower in asthmatic children due to airway inflammation. Ali et al. [29] suggested that dysregulation of iron status and homeostasis occurs in many diseases, including respiratory conditions. In the respiratory tract, iron-related proteins secreted by epithelial cells, macrophages, and neutrophils regulate free iron levels under physiological conditions. Ghio et al. [30] described the link between asthma and iron-related oxidative stress, with iron being a key catalyst in the Fenton reaction, a major source of free radical formation.

Regression analyses revealed notable associations between essential minerals (iron and magnesium), vitamin D3, and the FEV1/FVC ratio in children with asthma. In univariate analysis, iron and zinc showed a positive correlation with the FEV1/FVC ratio, suggesting that higher concentrations of these minerals might be linked to improved lung function, likely due to their roles in immune function and inflammation. Conversely, vitamin D3 was negatively correlated with the FEV1/FVC ratio, indicating that higher levels of vitamin D3 might be linked to poorer lung function in children with asthma. This finding is somewhat unexpected, given vitamin D3's known roles in immune regulation and potential protective effects against asthma. The relationship between vitamin D3 and lung function in asthma might be influenced by factors such as polymorphisms in the vitamin D receptor or interactions with other nutrients [31]. In multivariate analysis, the positive associations of iron and zinc with the FEV1/FVC ratio persisted, while the negative association of vitamin D3 remained, indicating the robustness of these relationships.

Our study found that iron and magnesium levels were significantly associated with the risk of asthma and wheezing in children, even after adjusting for age, gender, and family history. Deficiencies in these essential minerals and vitamin D3, which are crucial for immune function and inflammation regulation, were linked to an increased risk of asthma. These findings highlight the importance of maintaining adequate levels of these micronutrients in children as part of a comprehensive asthma prevention and management approach.

The study's limitations include its regional focus on India, which may limit the generalizability of the findings. It did not control for confounding factors such as diet, environment, and genetics that could affect essential minerals and vitamin D3 levels. Despite these limitations, the study provides valuable insights into the role of essential minerals and vitamin D3 in asthma.

5. Conclusion

The study found that children with asthma had significantly lower levels of essential minerals (iron and magnesium) and vitamin D3. These elements increase with asthma risk and severity, could contribute to the disease's onset and progression in children.

Ethics statement

The study was approved by the ethics committee of Dr. Ram Manohar Lohia Institute of Medical Sciences, Lucknow, (IEC: 60/21) and conformed to the principles of the Declaration of Helsinki. Written informed consent was obtained from the parents or guardians of all the patients for the research work.

Declaration of competing interest

The authors declare that they have no competing interests.

Author Contribution

SS: Experimental work, preparation of the original manuscript, and data analysis.
MRK: Conceptualization, Manuscript review and editing
AS: Formal analysis, Manuscript review and editing
SS: Clinical details and samples
VT: Conceptualization and fund acquisition

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