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ABO blood group-dependent association of serum retinol-binding protein 4 with eosinophilic inflammation and spirometric parameters in adults with asthma

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Abstract

Growing evidence links retinol binding protein-4 (RBP4) to inflammation; however, its association with inflammatory cell indices and spirometric parameters in asthma remains unclear, and the potential modifying role of ABO blood group has yet to be explored. The main aim was to assess the associations of serum RBP4 with inflammatory cell indices and spirometric parameters in adults with asthma stratified by blood group O and non-O.

Adults with physician-diagnosed asthma attending a respiratory clinic between January and June 2026 were consecutively enrolled in this cross-sectional study. Spearman's correlation assessed associations involving inflammatory cell indices, whereas Pearson's correlation evaluated associations between normally distributed serum RBP4 and spirometric parameters. Of the 88 adults with asthma enrolled, 64 had available ABO blood group data (38 O and 26 Non-O) and were included in the blood group-stratified analyses. In the O group, RBP4 was positively correlated with eosinophil count ($\rho=0.35$, $p=0.03$), eosinophil-to-neutrophil ratio ($\rho=0.42$, $p<0.01$), and eosinophil-to-lymphocyte ratio ($\rho=0.41$, $p=0.01$), whereas no significant associations were observed in the non-O group. Serum RBP4 was not correlated with spirometric parameters in either group. The forced expiratory volume in one second/forced vital capacity ratio was inversely correlated with eosinophil and lymphocyte counts only in the O group, while lymphocyte count showed an inverse correlation with forced expiratory flow between 25% and 75% of forced vital capacity in the non-O group. In conclusion, serum RBP4 was positively associated with eosinophilic inflammatory markers only in adults with asthma belonging to blood group O, whereas no such associations were observed in the non-O group.

Key words: ABO blood-group system, asthma, retinol binding proteins, eosinophils, spirometry.

Introduction

Asthma is a heterogeneous chronic inflammatory respiratory disorder with distinct inflammatory phenotypes that influence disease severity, outcome, and therapeutic response [1-3]. Eosinophilic inflammation is the hallmark of type 2 asthma; however, increasing evidence indicates that neutrophilic and mixed inflammatory phenotypes also contribute to disease heterogeneity [3,4]. Retinol-binding protein-4 (RBP4), a 21-kDa adipokine primarily synthesized by the liver and adipose tissue, has emerged as a mediator of metabolic and immune regulation beyond its classical role in retinol transport [5-9]. Experimental and clinical studies have demonstrated that RBP4 promotes inflammatory signaling through activation of antigen-presenting cells, macrophages, and Toll-like receptor-dependent pathways, leading to increased production of pro-inflammatory cytokines and systemic inflammation [8,9]. Studies investigating the relationship between RBP4 and asthma have reported inconsistent findings [10-14]. Elevated serum RBP4 levels have been observed in asthma, particularly in obesity-related non-allergic asthma, and in allergic sensitization, whereas other studies found no differences in RBP4 levels or consistent associations with pulmonary function, highlighting the need for further investigation. The ABO blood group system has been associated with cardiovascular, infectious, neoplastic, and immune-mediated diseases. ABO antigens are expressed on respiratory epithelial cells and mucosal secretions, where they may influence host–environment interactions [2,15,16]. Biological differences between blood group O and non-O phenotypes provide a plausible mechanism through which ABO blood groups may modify inflammatory responses [15,16]. Evidence regarding the association between ABO blood groups and asthma is inconsistent [2,17,18]. While some identified an increased prevalence of asthma or atopy among individuals with blood group O, others found associations with non-O phenotypes or no significant relationship. Similarly, evidence regarding differences in lung function and asthma severity across ABO blood groups remains inconclusive.

Despite increasing evidence linking RBP4 to inflammation and metabolic dysfunction, its association with inflammatory cell indices in asthma remains unclear. Moreover, the potential role of ABO blood group in modifying the relationship between serum RBP4 and asthma-related inflammatory phenotypes has not been explored. Therefore, this study assessed the associations of serum RBP4 with inflammatory cell indices and spirometric parameters in adults with asthma stratified by blood group O and non-O.

Materials and Methods

This cross-sectional study included adult patients with physician-diagnosed asthma who attended the respiratory clinic at Al-Sadr Medical City, Najaf, Iraq, between January and June 2026. The study was reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement (*Supplementary Table 1*). The study protocol was approved by the Ethics Committee of the University of Kufa, College of Medicine (Approval No. MEC-10; January 7, 2026) and was conducted in accordance with the principles of the Declaration of Helsinki. Verbal informed consent was obtained from all participants prior to enrollment. A formal sample size calculation was not performed. Instead, all consecutive eligible patients attending the study center during the study period were enrolled. Eligible participants were adults aged ≥ 18 years with physician-diagnosed asthma who were able to perform acceptable spirometry. Patients with a recent asthma exacerbation (within four weeks), chronic obstructive pulmonary disease, active respiratory infection, autoimmune or chronic inflammatory disorders, malignancy, pregnancy, severe hepatic or renal dysfunction, or other systemic conditions affecting inflammatory biomarkers were excluded. Demographic and clinical data, including ABO blood group, age, sex, body mass index (BMI), smoking status, asthma duration, and comorbidities, were obtained from patient interviews and review of medical records. The primary outcome was the association between serum RBP4 and inflammatory cell indices according to ABO blood group (O versus Non-O). Secondary outcomes included the associations between serum RBP4, inflammatory cell indices, and spirometric parameters. To minimize measurement bias, all laboratory and spirometric measurements were performed using standardized procedures in accordance with international guidelines and manufacturers' recommendations.

Spirometry was performed with a calibrated spirometer following the American Thoracic Society/European Respiratory Society recommendations. The forced expiratory volume in one second/forced vital capacity ratio (FEV_1/FVC), peak expiratory flow (PEF), and forced expiratory flow between 25% and 75% of forced vital capacity (FEF_{25-75}) were recorded as percentages of predicted values. At least three acceptable maneuvers were obtained, and the best values were included in the analysis.

Blood sample collection

After an overnight fast, approximately 5 mL of peripheral venous blood was collected under aseptic conditions. Blood samples for complete blood count (CBC) analysis were processed

immediately. For serum RBP4 measurement, samples were allowed to clot at room temperature for 10–20 minutes, centrifuged for 20 minutes, and the resulting serum was aliquoted and stored at –80°C until laboratory analysis.

Inflammatory cell indices derived from CBC

CBC analysis was performed using an automated hematology analyzer in accordance with the manufacturer's instructions. Absolute neutrophil, lymphocyte, and eosinophil counts were recorded, and inflammatory cell indices, including the neutrophil-to-lymphocyte ratio (NLR), eosinophil-to-lymphocyte ratio (ELR), and eosinophil-to-neutrophil ratio (ENR), were subsequently calculated.

RBP4 measurement

Serum RBP4 concentrations were quantified using a commercially available sandwich enzyme-linked immunosorbent assay (ELISA) kit (Human Retinol-Binding Protein ELISA Kit, Cat. No. PRS-01350hu; Nanjing Pars Biochem Co., Ltd., Nanjing, China) in accordance with the manufacturer's protocol. All standards and serum samples were assayed in duplicate. Measured RBP4 concentrations were converted to mg/L prior to statistical analysis.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics version 23.0 (IBM Corp., Armonk, NY, USA). Continuous variables were assessed for normality using the Shapiro–Wilk test and visual inspection of histograms and Q–Q plots. Normally distributed variables are presented as mean $\pm$ standard deviation (SD), whereas non-normally distributed variables are expressed as median and interquartile range (IQR). Categorical variables are presented as frequencies and percentages. Participants without available ABO blood group information were excluded from blood group-stratified analyses. No imputation of missing data was performed. Comparisons between the O and Non-O blood groups were performed using the independent-samples t test or the Mann–Whitney U test for continuous variables, as appropriate, and the chi-square test for categorical variables. Correlation analyses were conducted separately for the O and Non-O groups. Spearman's rank correlation coefficient (ρ) was used to assess correlations between inflammatory cell indices and serum RBP4 or spirometric parameters because the inflammatory cell indices were not normally distributed. The associations between serum RBP4 and spirometric parameters were evaluated using Pearson's correlation coefficient (r), as both serum RBP4 and the

analyzed spirometric parameters were normally distributed. Statistical significance was defined as a two-tailed P value <0.05.

Results

A total of 88 adults with asthma were enrolled. ABO blood group information was available for 64 participants (38 blood group O and 26 Non-O), who were included in the blood group-specific analyses. The remaining 24 participants were excluded from these analyses because their ABO blood group was unavailable. Among the 64 participants with available ABO blood group information, 38 (59%) belonged to the O group and 26 (41%) to the Non-O group. The two groups were comparable with respect to age, sex distribution, body mass index (BMI), asthma duration, smoking status, serum RBP4 concentrations, spirometric parameters, and inflammatory cell indices (all $P > 0.05$). Hypertension was significantly more prevalent in the O group than in the Non-O group (50% vs. 15%, $P < 0.01$). (Table 1)

Association between serum RBP4 and inflammatory cell indices

Spearman correlation analysis demonstrated distinct association patterns according to ABO blood group. In the O group, serum RBP4 showed significant positive correlations with eosinophil count ($\rho = 0.35$, $P = 0.03$), eosinophil-to-neutrophil ratio (ENR; $\rho = 0.42$, $P < 0.01$), and eosinophil-to-lymphocyte ratio (ELR; $\rho = 0.41$, $P = 0.01$). No significant correlations were observed with lymphocyte count, neutrophil count, or neutrophil-to-lymphocyte ratio (NLR). In contrast, no significant associations between serum RBP4 and any inflammatory cell index were identified in the Non-O group (all $P > 0.05$). (Table 2)

Associations of RBP4 and inflammatory cell indices with spirometric parameters

No significant correlations were observed between serum RBP4 and spirometric parameters, including FEV₁/FVC, peak expiratory flow (PEF), or FEV_{25–75}, in either the O or Non-O blood groups (all $P > 0.05$), as in Table 3. In the O group, FEV₁/FVC showed significant inverse correlations with eosinophil count ($\rho = -0.32$, $P = 0.04$) and lymphocyte count ($\rho = -0.46$, $P < 0.01$), whereas no significant associations were observed with neutrophil count, ENR, ELR, or NLR. In the Non-O group, none of the inflammatory cell indices correlated significantly with FEV₁/FVC. No significant correlations were identified between inflammatory cell indices and PEF in either blood group. For FEV_{25–75}, lymphocyte count demonstrated a modest inverse correlation

in the Non-O group ($\rho = -0.29$, $P = 0.04$), whereas no other inflammatory cell indices were significantly associated with FEF_{25–75} in either blood group. (Table 4)

Discussion

The principal finding of the present study was that serum RBP4 correlated positively with eosinophilic inflammatory markers, including eosinophil count, ENR, and ELR, in patients with blood group O only, whereas these associations were absent in the Non-O group. In contrast, serum RBP4 was not associated with spirometric parameters in either blood group. Furthermore, inflammatory cell indices showed only limited associations with spirometric parameters, suggesting that the observed relationships between RBP4 and eosinophilic inflammation were largely independent of lung function impairment. A notable observation was the comparable demographic, clinical, and laboratory characteristics between the O and Non-O groups, including serum RBP4 concentrations, inflammatory cell indices, and spirometric parameters. These findings indicate that the blood groups differed primarily in the pattern of biomarker associations rather than in baseline inflammatory burden or disease severity. The higher prevalence of hypertension among participants with blood group O should be interpreted cautiously because of the relatively small sample size and the absence of corresponding differences in other cardiovascular risk factors.

Increased blood eosinophil counts have consistently been associated with asthma severity, poor disease control, and exacerbation risk. Likewise, eosinophil-derived indices, particularly ELR, have emerged as accessible inflammatory biomarkers reflecting eosinophilic activity in allergic asthma and may predict therapeutic response to biologic treatment [1,3,19]. The blood group-specific associations observed in this study suggest that serum RBP4 may preferentially reflect eosinophilic inflammatory activity in patients with blood group O, supporting a potential modifying role of ABO blood group in the relationship between RBP4 and eosinophilic inflammation. The biological mechanisms underlying these findings remain speculative but are biologically plausible. RBP4 is increasingly recognized as an adipokine with immunomodulatory properties rather than solely a vitamin A transport protein [5,6,20]. Circulating RBP4 correlates with inflammatory activity in several immune-mediated disorders, including psoriasis, Crohn's disease, hepatitis C, and metabolic diseases, supporting its role as a systemic inflammatory mediator rather than a passive metabolic biomarker [6,7,20–22]. These findings provide biological

plausibility for a potential link between elevated serum RBP4 and eosinophilic inflammatory pathways in asthma, although the underlying mechanisms remain to be elucidated. Emerging evidence further supports the role of metabolic dysfunction in asthma pathogenesis. Obesity, insulin resistance, and type 2 diabetes are associated with greater asthma severity and poorer disease control, particularly in obesity-related asthma phenotypes [23]. As an adipokine closely linked to insulin resistance and metabolic inflammation, RBP4 may represent a biological link between metabolic dysregulation and airway inflammation through systemic inflammatory activation and altered retinoid signaling [5,12,23]. Although the present study did not evaluate metabolic status, these mechanisms provide additional biological plausibility for the observed association between serum RBP4 and eosinophilic inflammatory markers and warrant further investigation.

The blood group-specific nature of these correlations is noteworthy. ABO antigens are expressed on respiratory epithelial cells and mucosal surfaces, where they influence glycosylation patterns, epithelial barrier function, and immune regulation [2]. In addition, ABO glycosyltransferase activity has been associated with differences in circulating von Willebrand factor, adhesion molecules, and inflammatory mediators, providing a plausible biological basis for variation in inflammatory responses among blood groups [2]. Accordingly, individuals with blood group O may possess a distinct immunological microenvironment that modifies the association between circulating RBP4 and eosinophilic inflammatory pathways. Although the precise mechanism cannot be established from this cross-sectional study, these biological differences may explain why significant correlations between serum RBP4 and eosinophilic inflammatory markers were observed only in the O group. Previous studies investigating the association between ABO blood groups and asthma have yielded inconsistent findings, with some reporting a predominance of blood group O in asthma or allergic diseases and others finding no significant association with asthma susceptibility or pulmonary function [2,15,17]. Our findings extend this evidence by suggesting that ABO blood group may modify the association between serum RBP4 and eosinophilic inflammation rather than susceptibility to asthma.

Serum RBP4 was not associated with FEV₁/FVC, PEF, or FEF_{25–75} in either blood group, consistent with previous studies reporting no clear relationship between circulating RBP4 and pulmonary function despite altered RBP4 concentrations in specific asthma phenotypes [10,13]. These

findings suggest that circulating RBP4 primarily reflects inflammatory or metabolic processes rather than airflow limitation.

The inverse correlations of eosinophil and lymphocyte counts with FEV₁/FVC in the O group are consistent with the contribution of airway inflammation to airflow obstruction and the established association of blood eosinophilia with asthma severity and poor disease control [1,3,24]. In contrast, the weak inverse association between lymphocyte count and FEF_{25–75} in the Non-O group should be interpreted cautiously given its modest strength and the potential influence of multiple comparisons.

This study has several limitations. Its cross-sectional design precludes causal inference, and ABO blood group information was available for only 64 of the 88 enrolled participants, limiting the statistical power of subgroup analyses, particularly in the Non-O group. Inflammatory phenotyping was based solely on peripheral blood biomarkers without assessment of sputum eosinophils or cytokine profiles, and the single-center design may limit the generalizability of the findings. Despite these limitations, the present findings provide preliminary evidence that ABO blood group may modify the association between serum RBP4 and eosinophilic inflammation in asthma. Validation in larger multicenter prospective studies may support the potential role of ABO blood group as a stratification factor for interpreting inflammatory biomarkers and improving biomarker-based inflammatory phenotyping in asthma.

In conclusion, serum RBP4 was positively associated with eosinophilic inflammatory markers only in adults with asthma with blood group O, but not in those with Non-O blood groups, while showing no association with pulmonary function. These findings suggest that ABO blood group may modify the association between serum RBP4 and eosinophilic inflammation. Further multicenter prospective studies are warranted to validate these findings and clarify the underlying mechanisms.

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Online supplementary file

Supplementary Table 1. STROBE checklist for cross-sectional studies.

Table 1. Baseline characteristics of the study population according to ABO blood group.

Variables	O group (n=38)	Non-O group (n=26)	p-value
Age, mean±SD	45±14	46±10	0.72
BMI, mean±SD	29±4	29±7	0.99
Asthma duration (years), mean±SD	14±13	19±12	0.43
Male, n (%)	17 (45)	11 (42)	0.52
Hypertension, n (%)	19 (50)	4 (15)	<0.01
Diabetes mellitus, n (%)	9 (24)	5 (19)	0.76
Coronary artery disease, n (%)	3 (8)	2 (7)	0.67
Smoking, n (%)	2 (5)	1 (4)	0.59
Serum RBP4 (mg/L), mean±SD	64±18	71±20	0.15
Spirometric parameters			
FEV1/FVC	85±10	85±9	0.74
PEF	48±18	46±12	0.67
FEF ₂₅₋₇₅	72±34	74±30	0.79
Inflammatory cell indices			
Eosinophil count (×10 ³ /μL), median (IQR)	0.3 (0.1-0.6)	0.2 (0.0-0.5)	0.32
Neutrophil count (×10 ³ /μL), median (IQR)	4.9 (3.6-5.6)	5.1 (4.0-8.4)	0.11
Lymphocyte count (×10 ³ /μL), median (IQR)	2.3 (1.9-3.2)	2.6 (2.1-2.9)	0.66
Eosinophil/lymphocyte ratio, median (IQR)	0.1 (0.0-0.2)	0.1 (0.0-0.2)	0.50
Eosinophil/neutrophil ratio, median (IQR)	0.0 (0.0-0.1)	0.0 (0.0-0.0)	0.17
Neutrophil/lymphocyte ratio, median (IQR)	2.0 (1.4-2.3)	2.1 (1.6-2.8)	0.15

BMI, body mass index; RBP4, retinol-binding protein 4; FEV₁/FVC, forced expiratory volume in one second/forced vital capacity ratio; PEF, peak expiratory flow; FEF₂₅₋₇₅, forced expiratory flow between 25% and 75% of forced vital capacity; IQR, interquartile range; SD, standard deviation.

Table 2. Spearman correlations between serum RBP4 and inflammatory cell indices according to ABO blood group.

Cell indices	RBP4			
	O group		Non-O group	
	Spearman's ρ	p-value	Spearman's ρ	p-value
Eosinophil count	0.35	0.03	0.06	0.63
Lymphocyte count	0.10	0.52	-0.04	0.76
Neutrophil count	-0.19	0.24	0.20	0.15
ENR	0.42	<0.01	0.04	0.77
ELR	0.41	0.01	0.04	0.77
NLR	-0.11	0.48	0.20	0.15

RBP4, retinol-binding protein 4; ENR, eosinophil-to-neutrophil ratio; ELR, eosinophil-to-lymphocyte ratio; NLR, neutrophil-to-lymphocyte ratio; ρ, Spearman's rank correlation coefficient.

Table 3. Correlation between serum RBP4 and spirometric parameters according to ABO blood group.

Spirometric parameters	RBP4			
	O group		Non-O group	
	r	P value	r	P value
FEV1/FVC	-0.12	0.47	0.05	0.70
PEF	0.11	0.49	-0.05	0.69
FEF ₂₅₋₇₅	-0.06	0.68	0.06	0.65

RBP4, retinol-binding protein 4; FEV₁/FVC, forced expiratory volume in one second/forced vital capacity ratio; PEF, peak expiratory flow; FEF₂₅₋₇₅, forced expiratory flow between 25% and 75% of forced vital capacity; r, Pearson's correlation coefficient.

Table 4. Spearman correlations between inflammatory cell indices and spirometric parameters according to ABO blood group.

FEV₁/FVC				
	O group		Non-O group	
	Spearman's ρ	P value	Spearman's ρ	P value
Eosinophil count	-0.32	0.04	-0.14	0.32
Lymphocyte count	-0.46	<0.01	0.01	0.94
Neutrophil count	-0.14	0.37	-0.01	0.30
ENR	-0.26	0.11	-0.11	0.41
ELR	-0.22	0.18	-0.14	0.33
NLR	0.20	0.23	-0.03	0.80
PEF				
	O group		Non-O group	
	Spearman's ρ	P value	Spearman's ρ	P value
Eosinophil count	-0.19	0.25	-0.10	0.45
Lymphocyte count	-0.03	0.83	-0.20	0.15
Neutrophil count	-0.03	0.85	-0.22	0.11
ENR	-0.15	0.35	0.00	0.97
ELR	-0.23	0.16	-0.07	0.58
NLR	-0.06	0.68	-0.07	0.96
FEF₂₅₋₇₅				
	O group		Non-O group	
	Spearman's ρ	P value	Spearman's ρ	P value
Eosinophil count	-0.24	0.13	-0.25	0.07
Lymphocyte count	-0.06	0.69	-0.29	0.04
Neutrophil count	0.01	0.94	-0.16	0.25
ENR	-0.21	0.20	-0.17	0.21
ELR	-0.27	0.08	-0.17	0.21
NLR	-0.06	0.69	0.10	0.49

FEV₁/FVC, forced expiratory volume in one second/forced vital capacity ratio; PEF, peak expiratory flow; FEF₂₅₋₇₅, forced expiratory flow between 25% and 75% of forced vital capacity; ENR, eosinophil-to-neutrophil ratio; ELR, eosinophil-to-lymphocyte ratio; NLR, neutrophil-to-lymphocyte ratio; ρ , Spearman's rank correlation coefficient.