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Original Communication

Inflammatory-Driven Vitamin A Transport Dysfunction in Ulcerative Colitis

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Abstract

Background: Retinol-binding protein 4 (RBP4) is a vitamin A transport protein synthesized in the liver and also plays a crucial role in inflammation and immune regulation. Low serum vitamin A levels have been observed in both pediatric and adult patients with ulcerative colitis (UC). The association between serum vitamin A levels and serum RBP4 levels, as well as the underlying mechanism involved inimpaired vitamin A transport during inflammation in UC patients, has yet to been investigated. **Methods**: A validation cohort comprising 103 UC patients and 22 controls was analyzed. Serum RBP4 levels were measured using anenzyme-linked immunosorbent assay (ELISA), and correlations with vitamin A levels and disease severity wereassessed. A dextran sulfate sodium (DSS)-induced colitis mouse model was used to valuatehepatic RBP4 expression and inflammatory changes. Primary hepatocytes from C57BL/6 mice were exposed to inflammatory cytokines to assess the impact of these cytokines on RBP4 expression. **Results**: Serum vitamin A ($p < 0.001$) and RBP4 levels ($p < 0.001$) were significantly lower in UC patients compared to controls, exhibiting a pronounced decreasing trend as disease severity increased (vitamin A: $p < 0.001$; RBP4: $p < 0.001$), while vitamin A levels increased after effective treatment ($p < 0.001$). Hepatic *RBP4* expression was reduced in the DSS-induced colitis model and negatively correlated with *TNF-*α, *IL-6*, and *IL-17*. **Conclusions**: Serum RBP4 levels are decreased in UC patients and negatively correlate with disease severity, possibly due to proinflammatory cytokine-induced suppression which might contribute to inflammation-driven vitamin A transport dysfunction.

Keywords: inflammation; liver-gut axis; retinol binding protein 4; ulcerative colitis; vitamin A

1. Introduction

Vitamin A, a fat-soluble vitamin stored as biologically active derivatives such as retinol and retinoic acid (RA), is essential for visual function, reproductive development, epithelial integrity, and immune system regulation [1–3]. It plays a crucial role in regulating immune homeostasis, especially in the context of mucosal immunity. Upon binding to their nuclear receptors (RARα, β, γ), vitamin A regulates the balance between Th17 and Treg cells, inhibits the expression of inflammatory cytokines interleukin (IL)-6 and IL-23, and enhances transforming growth factor (TGF)-β signaling, thus mitigating disease activity in ulcerative colitis (UC) [4]. Vitamin A deficiency leads to impaired mucosal dendritic cell (DC) function, which is specifically characterized by reduced *ALDH1a2* expression and RA synthesis. TGF-β production is also decreased, impairing immunoregulatory functions. Notably, mucosal DCs lose their capacity to induce Foxp3$^+$ regulatory T cells, ultimately leading to the disruption of intestinal immune homeostasis [5]. It is reported that RA is also synthesized by intestinal epithelial cells and vimentin$^+$CD90$^+$$\alpha$-SMA$^-$ stromal cells (SCs). It aids in the immunoregulatory functions of DCs and helps balance the differentiation of T cell subsets. However, in Crohn's disease (CD), SCs exhibit a significantly reduced capacity to produce RA, leading to dysregulated DC differentiation and excessive activation of pro-inflammatory IFN-γhi/IL-17hi T cells [6].

Vitamin A is absorbed in the gut and stored in the liver. More precisely, dietary vitamin A mainly exists in the form of retinyl esters and provitamin A carotenoids. In the intestine, these compounds are hydrolyzed or cleaved to form retinol. This retinol is then re-esterified within enterocytes and packaged into chylomicrons along with other lipids. These chylomicrons are transported through the lymphatic

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system via the lacteals of the small intestine, and then absorbed and stored by the liver [7]. Vitamin A deficiency, defined as low serum vitamin A levels, can still occur despite adequate intake, which is unrelated to disease location or prior intestinal resection [8]. It indicates that although the digestion of dietary vitamin A, its intestinal absorption, and storage in the liver are functioning properly, the mobilization of vitamin A from the liver to the bloodstream and its subsequent use in the intestines may be impaired. It has been observed that individuals with inflammatory bowel disease (IBD) have a higher prevalence of low serum vitamin A levels, compared to the general population [9], but the underlying regulatory mechanism remains poorly understood.

Retinol-binding protein 4 (RBP4) is predominantly expressed in the liver and serves as the primary transport protein for vitamin A in the circulation [10]. When vitamin A deficiency is detected in the blood and tissues, the body mobilizes hepatic retinol through the bloodstream. The mobilized retinol binds to cellular retinol-binding protein 1 (CRBP1) and is transferred from hepatic stellate cells to hepatocytes. It then associates with RBP4 and forms a stable complex with transthyretin (TTR) to enter the bloodstream, where it binds to target organ receptors for cellular uptake [11]. Once dissociated from TTR and retinol, the circulating apo-RBP4 undergoes renal filtration, with more than 99% being reabsorbed by the proximal renal tubules [12]. Mice with global *RBP4* deficiency showed elevated levels of hepatic retinol and retinyl ester, while circulating retinol levels were decreased by nearly 90% [13]. Conversely, acute *RBP4* overexpression in the liver resulted in increased serum retinol levels and reduced liver retinyl esters, highlighting the crucial role of hepatocyte *RBP4* in mobilizing retinol into the circulation [14].

Given the critical role of vitamin A in regulating immune responses and the frequent occurrence of low serum vitamin A levels in IBD patients, it is crucial to elucidate the mechanisms underlying vitamin A metabolism and transport in inflammatory settings. Exploring how inflammation impacts vitamin A mobilization could offer novel insights into the gut-liver axis and its dysregulation in UC.

2. Materials and Methods

2.1 Reagents

Murine TNF-α (0–100 ng/mL, AF-315-01A) and IL-1β (100 IU/mL, AF-211-11B) were obtained from Peprotech Inc. (Cranbury, NJ, USA), and murine IFN-γ (10 ng/mL, IF005) from Sigma-Aldrich (St. Louis, MO, USA). All reagents were stored and handled according to the manufacturers' instructions in order to maintain their biological activity.

2.2 Patient Selection

The inclusion criteria for the experimental group in this study were as follows: patients diagnosed with UC who visited the Department of Gastroenterology at Tianjin Medical University General Hospital between January 2023 and June 2024, meeting the diagnostic criteria outlined in the "Chinese Guidelines for the Diagnosis and Treatment of Ulcerative Colitis (2018) [15]", with complete clinical data. The control group consisted of patients with colonic polyps who visited the same department during the same period, with complete clinical data. The exclusion criteria for both groups included: patients receiving vitamin A supplementation; those with other infectious or autoimmune diseases; those with cardiovascular, hepatic, pulmonary, renal, or hematological diseases; patients with malignant tumors; those who had used or increased the use of medications affecting coagulation function within the past three months; and patients with incomplete clinical data. Clinical data and peripheral blood samples were collected from both the UC and control groups. Written informed consent was acquired from all participants. This study was conducted in strict accordance with the guidelines of the Declaration of Helsinki (as revised in Brazil, 2013) and was approved by the Ethics Committee of Tianjin Medical University General Hospital (Approval No. IRB2024-YX-523-01).

2.3 Treatment

All UC patients included in this study were in the active phase of the disease, and their treatment plans were formulated based on the "Consensus on the Diagnosis and Treatment of Inflammatory Bowel Disease (2018, Beijing) [15]". For patients with mild-to-moderate UC, mesalazine sustained-release granules were administered at a dose of 4 g/day; for distal colitis, localized mesalazine treatment was primarily used (suppositories for proctitis, once every night; enemas for rectosigmoiditis, administered every day). For patients with severe UC, mesalazine was combined with corticosteroids or immunosuppressants for treatment. The 5-ASA regimen should be initiated immediately upon diagnosis of UC as first-line therapy, and is appropriate for patients with mild to moderate disease activity. If symptoms were not adequately controlled after 2–4 weeks of 5-ASA monotherapy, or in cases with extensive disease or moderate-to-severe activity at onset, corticosteroids should be added to constitute a 5-ASA plus corticosteroid regimen. For patients who are refractory to corticosteroids or develop steroid dependence, immunosuppressant may be introduced in addition to 5-ASA, comprising the 5-ASA plus immunosuppressant regimen.

2.4 Animal Studies

Twelve healthy SPF-grade C57BL/6 male mice (20–22 g, 6–8 weeks old) were obtained from Sipeifu Biotechnology Co., Ltd. They were housed under standard conditions at room temperature, 40%–60% humidity, and with free access to water and food. After one week of acclimatization, mice were randomly assigned to normal control (NC) and model (dextran sulfate sodium, DSS) groups,

each with 6 animals. The DSS group received 2.5% DSS (molecular weight 35,000–50,000 Da, MP Biomedicals, Irvine, CA, USA) in drinking water for 7 days to induce colitis. All animal procedures followed the "Guide for the Care and Use of Laboratory Animals" and were approved by the Animal Ethics Committee of the Institute of Radiation Medicine, Chinese Academy of Medical Sciences (Approval No. IRM-DWLL-2023266). A euthanasia method by cervical dislocation was established as the humane endpoint.

2.5 Isolation of Primary Mouse Hepatocytes

Mice were anesthetized by inhalation using isoflurane at a concentration of 2%–3% with an oxygen flow rate of 1–2 L/min. They were placed in an anesthesia chamber for 2–3 minutes until anesthetized, then transferred to a surgical platform where anesthesia was maintained via a face mask. After disinfection with 75% ethanol and securing on an operating table, an indwelling needle was inserted into the inferior vena cava to allow perfusion with 15 mL of calcium-free perfusion solution at 20 mL/min. This was followed by perfusion with 0.5% collagenase IV in calcium-containing solution II. Digestion ceased when the liver became soft and indented under pressure. The liver was then isolated, washed with PBS, and placed in a culture dish. After adding 15 mL of fresh HDMEM complete medium (Gibco, 11965092, Carlsbad, CA, USA), the liver capsule was gently torn with tweezers to release the hepatocytes into suspension. This was filtered through a 200-mesh strainer and the suspension centrifuged at 4 °C and 50 rpm for 3 minutes. After discarding the supernatant, the procedure was repeated three times. Density gradient centrifugation with 40% Percoll (Cytiva, 17-0891-01, Marlborough, MA, USA) was then performed at 4 °C and 550 rpm for 10 minutes to enhance cell viability. After discarding the supernatant, fresh medium was added, and trypan blue staining (Gibco, 15250061, Carlsbad, CA, USA) was used to evaluate cell viability. Experiments were only conducted if the cell viability exceeded 80%[16].

2.6 Quantitative RT-PCR

Total RNA was isolated from the colon tissues of patients and mice using the Total RNA Extraction Reagent (Vazyme, R401-01, Nanjing, Jiangsu, China). Reverse transcription was performed to synthesize cDNA. Quantitative real-time PCR was performed using Universal qPCR SYBR Master Mix (Vazyme, Q711, Nanjing, Jiangsu, China), with the PCR reactions performed in a real-time PCR detection system (QuantStudio 3/5 Real-Time PCR Software, ThermoFisher, Waltham, MA, USA). The experiment was conducted in triplicate to ensure reproducibility. The relative expression level of target genes was calculated using the $2^{-\Delta\Delta Ct}$ method, with GAPDH serving as the internal control. The primers used for this experiment are listed in Table 1.

2.7 Western Blotting

Total protein was extracted from colon tissues and cells using efficient RIPA lysis buffer (Solarbio, R0010, Beijing, China). The protein concentration of samples was quantified with a microplate reader and BCA protein assay kit (Solarbio, PC0020, Beijing, China). After measuring the sample volume, protein loading buffer (Solarbio, P1015, Beijing, China) was added at one-third of the sample volume. The mixture was boiled at 100 °C for 10 minutes and then cooled to room temperature. Proteins were resolved using polyacrylamide gel electrophoresis, and subsequently transferred onto a PVDF membrane. The membrane was incubated at 4 °C overnight with a rabbit anti-RBP4 primary antibody (Proteintech, 11774-1-AP, Rosemont, IL, USA) and a mouse anti-β-actin primary antibody (Abcam, ab8226, Cambridge, UK). Following washes with TBST, a goat anti-rabbit secondary antibody (Sabbiotech, L3073, College Park, MD, USA) and a goat anti-mouse secondary antibody (Sabbiotech, L35661, College Park, MD, USA) were applied and the membrane incubated for 2 h at room temperature. Target proteins were detected using the ECL Plus luminescent solution (Solarbio, PE0010, Beijing, China) and a Western blotting system, with β-actin serving as the internal control.

2.8 Histopathological Analysis

Paraffin-embedded colon tissue sections (4 µm) were first dewaxed using an alcohol gradient and then stained with hematoxylin, differentiated, and counterstained with eosin (Solarbio, G1120, Beijing, China). They were subsequently dehydrated, cleared, and mounted with neutral resin. Pathological changes were observed after scanning with a digital scanner (PRECICE 500B, Unicmed Medical Technology Co., Ltd., Beijing, China).

2.9 Enzyme-linked Immunosorbent Assay (ELISA)

Blood samples were stored in EDTA tubes. Prior to evaluation by ELISA, the samples were centrifuged for 10 minutes at 2–8 °C and 2000 g. The supernatant was analyzed for RBP4 using an ELISA kit as per the manufacturer's protocol (Abcam, ab108897, Cambridge, UK). A 96-well plate was used for the experiment, with each well receiving 50 µL of RBP4 standard or sample, followed by gentle tapping and incubation for 2 h. Manual washing was performed five times with 200 µL of 1× wash buffer, or six times with 300 µL if using a machine. Each well was then treated with 50 µL of 1× biotinylated RBP4 antibody and incubated for 1 h. After washing, 50 µL of 1× SP conjugate was added, followed by a 30-minute incubation. The microplate reader was prepared in advance. After washing, 50 µL of chromogen substrate was added, and the plate incubated for 12 minutes. Stop Solution (50 µL) was then added, changing the color from blue to yellow, and the absorbance at 450 nm was read immediately.

Table 1. Quantitative RT-PCR primers.

Gene	Specie	Forward primer (5′-3′)	Reverse primer (5′-3′)
GAPDH	mouse	GGAGAAACCTGCCAAGTATG	TGGGAGTTGCTGTTGAAGTC
TNF-α	mouse	GGTGCCTATGTCTCAGCCTCTT	GCCATAGAACTGATGAGAGGGAG
IL-1β	mouse	TGGACCTTCCAGGATGAGGACA	GTTCATCTCGGAGCCTGTAGTG
IFN-γ	mouse	GCCACGGCACAGTCATTGA	TGCTGATGGCCTGATTGTCTT
RBP4	mouse	TGTAGCCTCCTTTCTCCAGCGA	ACAGGTGCCATCCAGATTCTGC
IL-6	mouse	TTGCCTTCTTGGGACTGAT	TTGCCATTGCACAACTCTT
IL-17	mouse	GGCCCTCAGACTACCTCAAC	TCTCGACCCTGAAAGTGAAGG
iNOS	mouse	ACATCGACCCGTCCACAGTAT	CAGAGGGGTAGGCTTGTCTC
COX-2	mouse	TGCTGTACAAGCAGTGGCAA	GCAGCCATTTCCTTCTCTCC

RT-PCR, reverse transcription polymerase chain reaction; *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase; *TNF-α*, tumor necrosis factor alpha; *IL-1β*, interleukin-1 beta; *IFN-γ*, interferon gamma; *RBP4*, retinol binding protein 4; *IL-6*, interleukin-6; *IL-17*, interleukin-17; *iNOS*, inducible nitric oxide synthase; *COX-2*, cyclooxygenase-2.

2.10 Measurement of Plasma Vitamin A

Whole blood samples were collected from all subjects in a non-fasting state, and the plasma isolated within 24 h and stored at –20 °C until further analysis. Plasma vitamin A levels were measured using high-performance liquid chromatography (HPLC) (ABLE Jasco, Hachioji-shi, Tokyo, Japan) and a commercially available *in vitro* diagnostic kit for vitamin A (Bio-Rad Laboratories, #195-5869, Hercules, CA, USA). All procedures were performed according to the manufacturer's instructions. Quantification was carried out using internal standards and calibrators provided by the kit manufacturer.

2.11 Statistical Analysis

Data analysis was performed using GraphPad Prism 10 (GraphPad Software Inc., GraphPad Software, San Diego, CA, USA) and R software version 4.3.1 (R Foundation for Statistical Computing, Vienna, Austria). For categorical variables, the chi-square test was used to compare proportions between groups. When the expected frequency in any cell was less than 5, the continuity-corrected chi-square test or Fisher's exact test was applied. Quantitative data with a normal distribution were expressed as mean $\pm$ standard deviation, while non-normally distributed data were presented as median (interquartile range). The normality of data can be tested using the Shapiro-Wilk test, and equality of variance was assessed using Levene's test. Differences between two groups were analyzed using Student's unpaired *t*-test, or the Mann-Whitney U test. For comparisons among four groups, we first performed an omnibus test (one-way ANOVA for normally distributed data or Kruskal-Wallis test for non-normally distributed data) to assess overall group differences. If the omnibus test indicated statistically significant differences, post-hoc pairwise comparisons were conducted using Tukey's HSD test (for ANOVA) or Dunn's test with Bonferroni correction (for Kruskal–Wallis), to control for Type I error. For paired comparisons of vitamin A levels before and after treatment, a paired *t*-test was applied for normally distributed data; otherwise, the Wilcoxon signed-rank test was used. For the binary outcome variable, Firth's penalized likelihood logistic regression was used to reduce bias arising from sample imbalance and complete separation. The model was implemented using the logistf package in R. Correlations between groups were assessed using Spearman's and Pearson's correlation coefficients. p-values < 0.05 were considered to be statistically significant.

3. Results

3.1 Clinical Characteristics and Laboratory Parameters of UC Patients and Control Subjects

A total of 103 UC cases sand 22 control subjects were included in the study. The baseline characteristics of these participants are presented in Table 2. The UC patients were younger than the control group, with a mean age of 38.1 $\pm$ 13.9 years compared to 57.5 $\pm$ 12.4 years ($p < 0.001$). There were no significant differences in sex, smoking, or alcohol consumption between the UC and control groups. All 103 UC cases (100%) experienced diarrhea, compared to only 3/22 (13.6%) individuals in the control group ($p < 0.001$). The UC group had more frequent visible blood in stool compared to the control group (92/103 *vs*. 1/22, $p < 0.001$). However, no significant difference in abdominal pain was noted between the UC and control groups. Patients in the UC group exhibited lower albumin levels (29 [26–32] *vs*. 40.5 [36.25–43.75], $p < 0.001$), higher platelet counts (336 [281.5–437] *vs*. 233 [187.25–249], $p < 0.001$) and increased monocyte levels (0.68 [0.53–0.95] *vs*. 0.43 [0.39–0.52], $p < 0.001$) compared to the controls. There was no significant difference in lymphocyte count and neutrophil levels between the UC and control groups.

3.2 Serum Vitamin A and RBP4 Concentrations in Patients With UC and Control Subjects

The severity of UC patients was classified according to the modified Truelove and Witts criteria [17]. A total

Table 2. Clinical characteristics and laboratory data of UC patients and control subjects.

Variables	UC (n = 103)	Control (n = 22)	*p*-value
Age (years)	38.1 ± 13.9	57.5 ± 12.4	<0.001
Gender (male/female)	35/68	10/12	0.4395
Smoking	9/103	3/22	0.4415
Alcohol Consumption	10/103	3/22	0.6991
Clinical sign			
Diarrhea	103/103	3/22	<0.001
Visible blood in stool	92/103	1/22	<0.001
Abdominal pain	72/103	10/22	0.052
Albumin (g/L)	29 (26–32)	40.5 (36.25–43.75)	<0.001
PLT (10^9/L)	336 (281.5–437)	233 (187.25–249)	<0.001
LYM# (10^9/L)	1.85 (1.43–2.17)	1.80 (1.42–2.42)	0.7311
NEUT# (10^9/L)	5.68 (4.08–7.63)	4.49 (4.12–6.32)	0.4899
Mon# (10^9/L)	0.68 (0.53–0.95)	0.43 (0.39–0.52)	<0.001

Abbreviations: UC, Ulcerative Colitis; n, number; PLT, Platelet; LYM#, Lymphocyte count; NEUT#, Neutrophil count; Mon#, monocyte count. $p < 0.05$ was considered statistically significant.

of 22 patients were classified as having mild disease, 45 as moderate disease, and 36 as severe disease. Serum vitamin A levels were found to be significantly lower in UC patients compared to the control group ($p < 0.001$) (Fig. 1A). A significant difference in serum vitamin A levels was observed among the four groups (Kruskal-Wallis test, $p < 0.001$). Further analysis demonstrated that, relative to the control group, vitamin A levels were significantly reduced in all patient groups (all $p < 0.05$ after Bonferroni correction), with a clear downward trend corresponding to increasing disease severity (Fig. 1B and **Supplementary Table 1**). Further analysis revealed that serum RBP4 levels in the UC group were lower than those in the control group ($p < 0.001$) (Fig. 1C). Similarly, serum RBP4 levels differed significantly among the four groups (Kruskal-Wallis test, $p < 0.001$). Post hoc analysis showed that all patient groups had significantly lower RBP4 levels compared with the control group (all $p < 0.05$ after Bonferroni correction), with RBP4 levels decreasing as disease severity increased (Fig. 1D and **Supplementary Table 1**). Subsequently, we have conducted separate analyses for males and females to investigate gender-specific differences in the relationship between vitamin A, RBP4, and disease severity. There were no significant differences between males and females in vitamin A levels ($p = 0.12$) or RBP4 levels ($p = 0.2926$) in the "Mild" disease severity group, and similarly in the "Moderate" disease severity group, vitamin A levels ($p = 0.1835$) and RBP4 levels ($p = 0.8733$), as well as in the "Severe" disease severity group vitamin A levels ($p = 0.765$) or RBP4 levels ($p = 0.3396$) (**Supplementary Table 2**).

3.3 Lower Serum RBP4 Correlates With Higher UC Disease Activity Index and Lower Vitamin A Levels

Special inflammatory scoring indices can be used to quantify the degree of inflammation, thereby facilitating standardization and comparison between groups. These indices include the systemic immune-inflammation index (SII), neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), neutrophil-to-albumin ratio (NAR), monocyte-to-lymphocyte ratio (MLR), lymphocyte-to-C-reactive protein ratio (LCR) and C-reactive protein-to-albumin ratio (CAR) [18,19]. Results for the inflammatory scoring indices of the UC and control groups are presented in Table 3. The CRP and ESR levels were significantly higher in the UC group compared to the control group (CRP: $p < 0.001$; ESR: $p < 0.001$). Similarly, the SII, PLR, NAR, MLR and CAR indices were markedly increased in the UC group (SII: $p < 0.001$; PLR: $p < 0.001$; NAR: $p = 0.0012$; MLR: $p < 0.001$; CAR: $p < 0.001$). Furthermore, the LCR was significantly lower in the UC group compared to the control group ($p < 0.001$). However, no statistically significant difference in the NLR was observed between the two groups.

Since RBP4 is a transport protein for vitamin A in the bloodstream, we also analyzed the correlation between serum RBP4 and vitamin A levels in UC patients. These showed a significant positive correlation (r = 0.762, $p < 0.001$) (Fig. 2A). We next analyzed for correlations between serum RBP4 levels and disease activity indexes in the 103 UC cases. The results showed that serum RBP4 levels were negatively correlated with ESR (r = –0.4871, $p < 0.001$), CRP (r = –0.3347, $p < 0.001$), MLR (r = –0.1951, $p = 0.0483$), and CAR (r = –0.328, $p < 0.001$). Similarly, serum vitamin A levels also exhibited significant negative correlations with ESR (r = –0.427, $p < 0.001$), CRP (r = –0.5345, $p < 0.001$), MLR (r = –0.3201, $p = 0.001$), and CAR (r = –0.5594, $p < 0.001$) (Fig. 2B–I). These results indicate that lower serum RBP4 levels were associated with higher disease activity in UC patients.

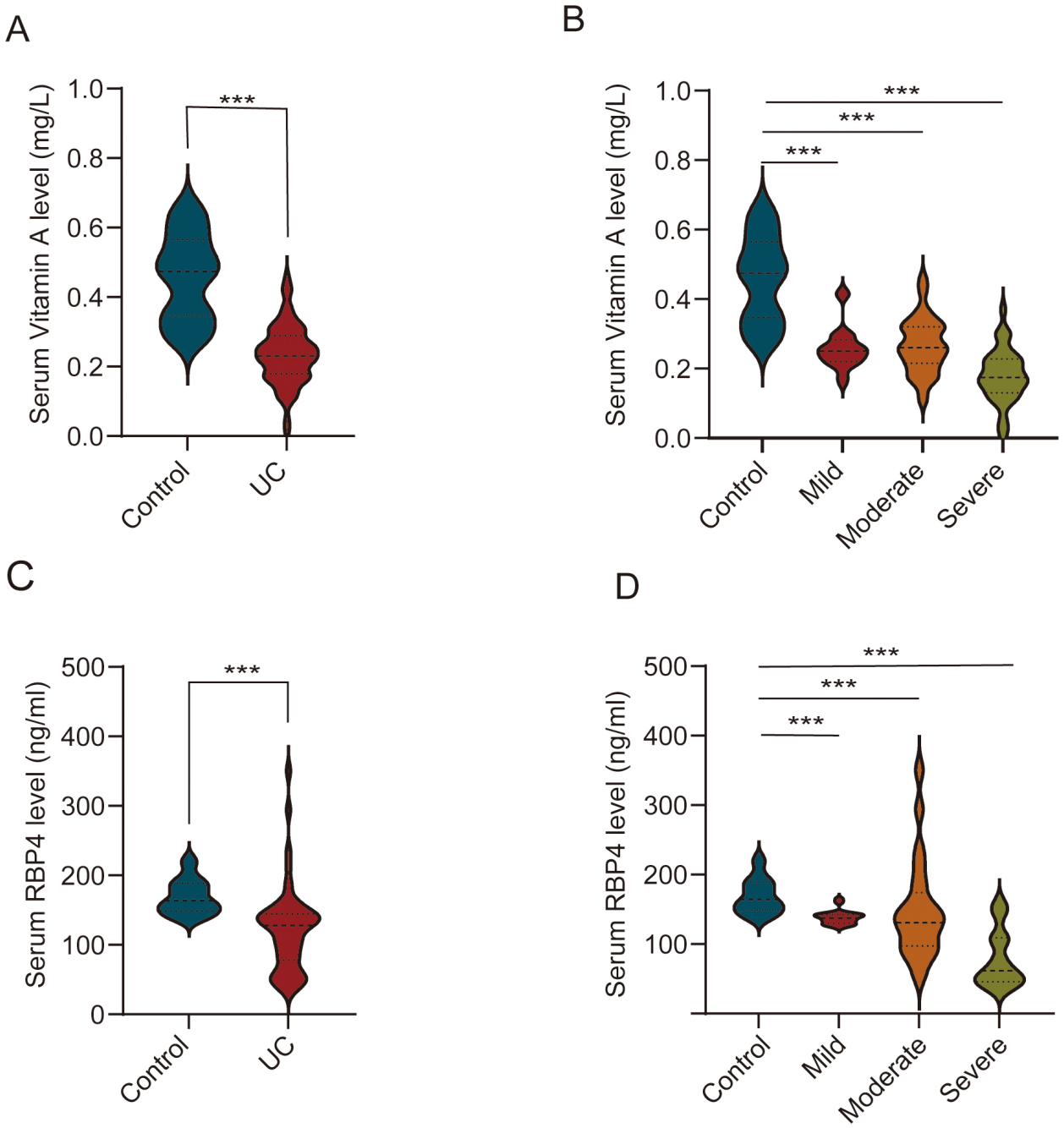

Fig. 1. Serum concentrations of vitamin A and RBP4 in control subjects and UC patients. (A) Comparison of vitamin A levels between control subjects and UC patients. (B) Comparison of vitamin A levels among UC patients stratified by disease severity. (C) Comparison of RBP4 levels between control subjects and UC patients. (D) Comparison of RBP4 levels among UC patients stratified by disease severity. ***$p < 0.001$. RBP4, Retinol-binding protein 4; UC, ulcerative colitis.

To control for the influence of potential confounding variables, we implemented a multivariable binary logistic regression model to analyze the determinants of disease severity. Disease severity was dichotomized, with mild cases coded as 0 and moderate/severe cases as 1. "Severity" was set as the dependent variable, while "Vitamin A" and "RBP4" were included as the main independent variables, with age, gender, BMI, and other disease activity indexs as covariates. The results indicated that none of the individual predictors reached statistical significance (all p-values > 0.05) (**Supplementary Table 3**). The associations between vitamin A, RBP4, and disease severity were not statistically significant, potentially due to residual confounding factors that were not fully accounted for, such as other metabolic markers or inflammatory factors.

Table 3. Disease activity index in UC patients and control subjects.

Variable	UC group (n = 103)	Control (n = 22)	*p*-value
ESR (mm/h)	33 (20.5–40)	21.5 (17–26)	<0.0010
CRP (μg/mL)	1.88 (0.445–3.445)	0.245 (0.22–0.3225)	<0.0010
Disease activity index			
SII	1376.97 (752.7951–2635.114)	526.33 (287.40–754.84)	<0.0010
NLR	3.21 (1.84–4.58)	2.90 (1.99–3.66)	0.4981
PLR	180.56 (150.86–261.17)	125.82 (93.38–139.07)	<0.0010
NAR	0.20 (0.128–0.252)	0.117 (0.103–0.176)	0.0012
MLR	0.376 (0.279–0.515)	0.235 (0.225–0.283)	<0.0010
LCR	1.074 (0.552–3.353)	8.091 (5.456–10.281)	<0.0010
CAR	0.0647 (0.014–0.1106)	0.0059 (0.0046–0.0086)	<0.0010

Abbreviations: UC, Ulcerative Colitis; n, number; CRP, C-reactive protein; ESR, Erythrocyte sedimentation rate; SII, Systemic Immune-inflammation Index; NLR, Neutrophil-to-Lymphocyte Ratio; PLR, Platelet-to-Lymphocyte Ratio; NAR, Neutrophil-to-Albumin Ratio; CAR, C-Reactive Protein-to-Albumin Ratio; MLR, Monocyte-to-Lymphocyte Ratio; LCR, Lymphocyte-to-C-Reactive Protein Ratio. $p < 0.05$ was considered statistically significant.

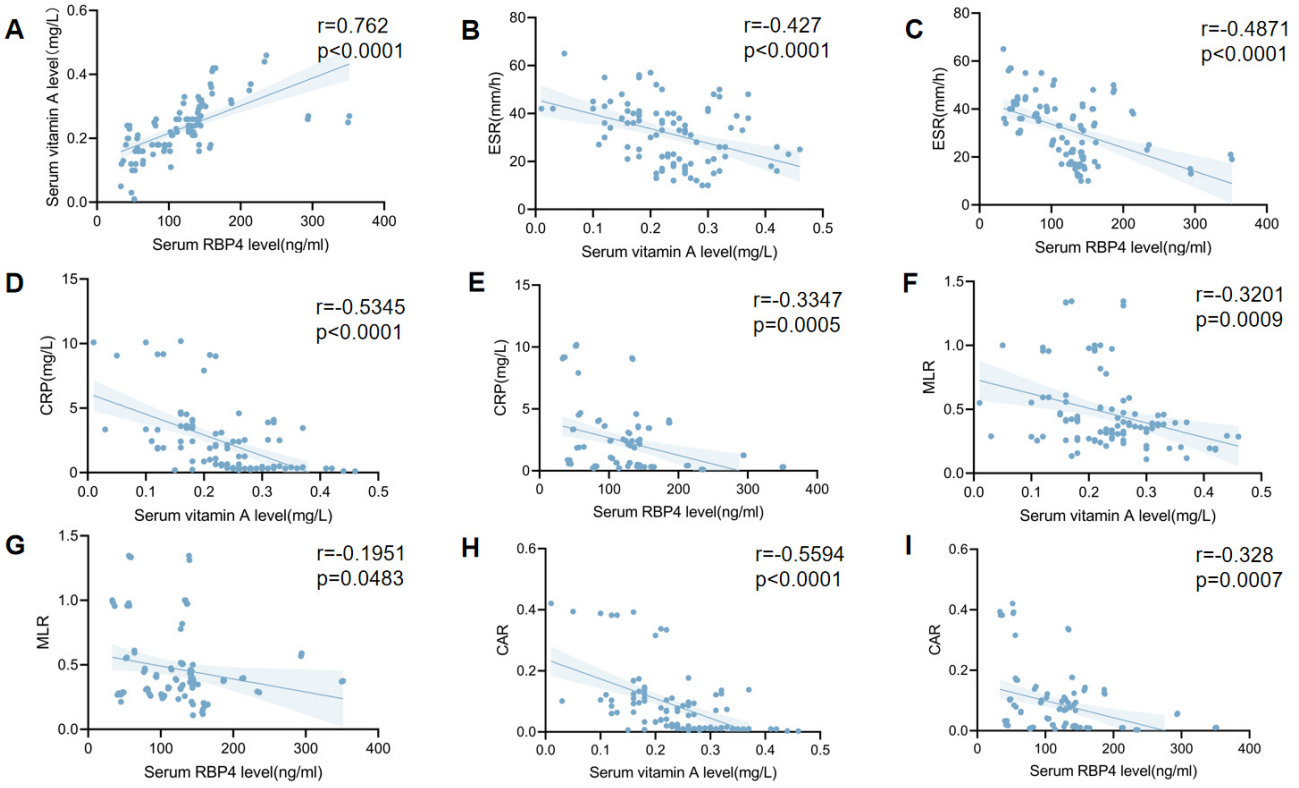

Fig. 2. Correlation analysis of RBP4 with vitamin A and disease activity markers. The correlation coefficients were calculated using the Pearson method. (A) Represents RBP4 and vitamin A. (B) Represents vitamin A and ESR. (C) Represents and ESR. (D) Represents vitamin A and CRP. (E) Represents RBP4 and CRP. (F) Represents vitamin A and MLR. (G) Represents RBP4 and MLR. (H) Represents vitamin A and CAR. (I) Represents RBP4 and CAR. ESR, Erythrocyte sedimentation rate; CRP, C-reactive protein; MLR, monocyte-to-lymphocyte ratio; CAR, C-reactive protein-to-albumin ratio.

3.4 Inflammatory Cytokines Reduce Serum RBP4 Expression by Hepatocytes

Serum RBP4 is primarily synthesized and secreted by the liver [20]. To investigate the potential mechanisms underlying the reduction of serum RBP4, we first isolated primary hepatocytes and stimulated them *in vitro* with inflammatory cytokines (TNF-α, IFN-γ, and IL-1β) to assess the expression of RBP4. Under the stimulation of inflammatory factors, the protein level of RBP4 and the mRNA level of *RBP4* are both decreased (Fig. 3A,B). Subsequently, we

established a DSS mouse model to evaluate the expression levels of *TNF-α*, *IFN-γ*, *IL-1β* and *RBP4* in liver tissue. Histopathological images indicated the presence of inflammation in the intestine of DSS model mice (Fig. 3C). The expression levels of *TNF-α*, *IFN-γ*, *IL-6*, *IL-17*, *iNOS*, *COX-2* and *IL-1β* in both the liver and intestinal tissues of the DSS group were observed to be higher than those of the control group, whereas the *RBP4* levels in the liver tissue were lower (Fig. 3D,E). Furthermore, the levels of *RBP4* show a significant negative correlation with those of *TNF-α*, *IL-6*, and *IL-17* in liver tissue (Fig. 3F).

3.5 Serum Vitamin A Levels Increase in UC Patients After Treatment

When stratified by treatment regimens, significant differences in vitamin A levels were observed among different treatment groups ($p = 0.0045$; **Supplementary Table 1**). To evaluate the serum vitamin A level in UC patients following treatment, whole blood samples were collected from 62 UC patients, including 18 with mild UC, 29 with moderate UC, and 15 with severe UC. Following treatment, the serum vitamin A levels in UC patients were found to increase significantly compared to pre-treatment levels ($p < 0.001$) (Fig. 4A). Stratified analysis based on UC severity revealed the levels in patients with mild UC were significantly higher after treatment compared to pre-treatment levels ($p = 0.0189$) (Fig. 4B), while those in patients with moderate UC were also significantly higher after treatment ($p = 0.0011$) (Fig. 4C). Similarly, patients with severe UC also exhibited a significant increase in serum vitamin A level after treatment compared to pre-treatment levels ($p = 0.0023$) (Fig. 4D). After stratifying patients according to medication regimen, we compared vitamin A levels before and after treatment in each group. The results showed that there were significant differences in vitamin A levels before and after treatment in the 5-ASA group ($p < 0.001$), the 5-ASA plus corticosteroids group ($p < 0.001$), and the 5-ASA plus immunosuppressants group ($p < 0.001$) (Fig. 4E–G).

4. Discussion

Vitamin A possesses antioxidant properties that enable it to neutralize free radicals, thereby reducing cellular damage induced by oxidative stress. This action suppresses the secretion of pro-inflammatory cytokines and reduces the inflammatory response [21]. Therefore, the presence of vitamin A deficiency in UC and its impact on disease progression is a crucial area of research. The prevalence of vitamin A deficiency in the pediatric IBD population has been reported as 16%. Serum vitamin A levels are generally lower in children and adolescents with active IBD [22]. Pang *et al.* [23] reported that vitamin A supplementation significantly ameliorates UC by altering the gut microbiota composition and increasing short-chain fatty acid production, thus reducing inflammation and improving intestinal barrier function. This study found that serum vitamin A levels in UC patients were lower than those in the control group, and an increase in vitamin A levels was observed following treatment. Our study's observations on vitamin A levels aligned with those of previous research. The level of vitamin A in the blood circulation is primarily maintained by the liver. When the level of vitamin A in the blood decreases, the liver releases stored vitamin A to maintain homeostasis.

RBP4 is primarily synthesized in the liver and serves as a carrier for transporting vitamin A in the bloodstream. However, the role of RBP4 in UC is still unclear and warrants further investigation. In a study on pediatric patients with IBD, Roma *et al.* [20] found the RBP4 level was negatively correlated with disease activity. Similarly, the current study found that UC patients have lower serum RBP4 levels compared to healthy controls, and a positive correlation was observed between serum RBP4 and vitamin A levels in UC patients. Since vitamin A undergoes significant changes under inflammatory conditions, RBP4 likely plays a crucial role in the inflammation-mediated disruption of vitamin A metabolism. Notably, lower serum RBP4 levels in UC patients were associated with higher disease activity.

In our study, both vitamin A and RBP4 levels were significantly associated with UC disease severity in univariate analysis, but these associations were not statistically significant in the multivariate analysis after adjusting for additional variables. To investigate this, we performed separate logistic regressions by individually adding each covariate to the base model (vitamin A + RBP4). Key observations from **Supplementary Table 4** include: (1) vitamin A: OR range: 0.0027–0.044 (consistently <1, protective effect); p-value fluctuations: significant in univariate models ($p < 0.05$ for some covariates, e.g., ESR: OR = 66.35, $p = 0.048$); Non-significant when adjusting for CRP, BMI, or NLR ($p > 0.05$). (2) RBP4: OR range: 0.996–1.019 (minimal effect size); Consistent non-significance: p-values remained >0.05 across all models, suggesting weak independent association with severity. The non-significant findings for vitamin A and RBP4 in multivariable analysis may stem from several factors. First, we calculated the variance inflation factors (VIF) for all covariates, with values ranging from 1.00 to 2.72, all below the commonly used threshold for significant multicollinearity (VIF = 5) (**Supplementary Table 5**). Additionally, a causal diagram (DAG) has been added to clarify our adjustment strategy and to visually present the relationships among the exposure variables (vitamin A, RBP4), outcome (UC status), and confounders (age, BMI, CRP, CAR, PLR, MLR, ESR, gender, LCR, NAR, NLR, SII) (**Supplementary Fig. 1**). This indicates that the model did not exhibit notable multicollinearity. Therefore, although certain covariates (such as CRP and ESR) are correlated with the main predictors (Vitamin A and RBP4), the VIF results suggest that multicollinearity did not substantially affect the robustness of our model. Second, residual confounding from unmeasured

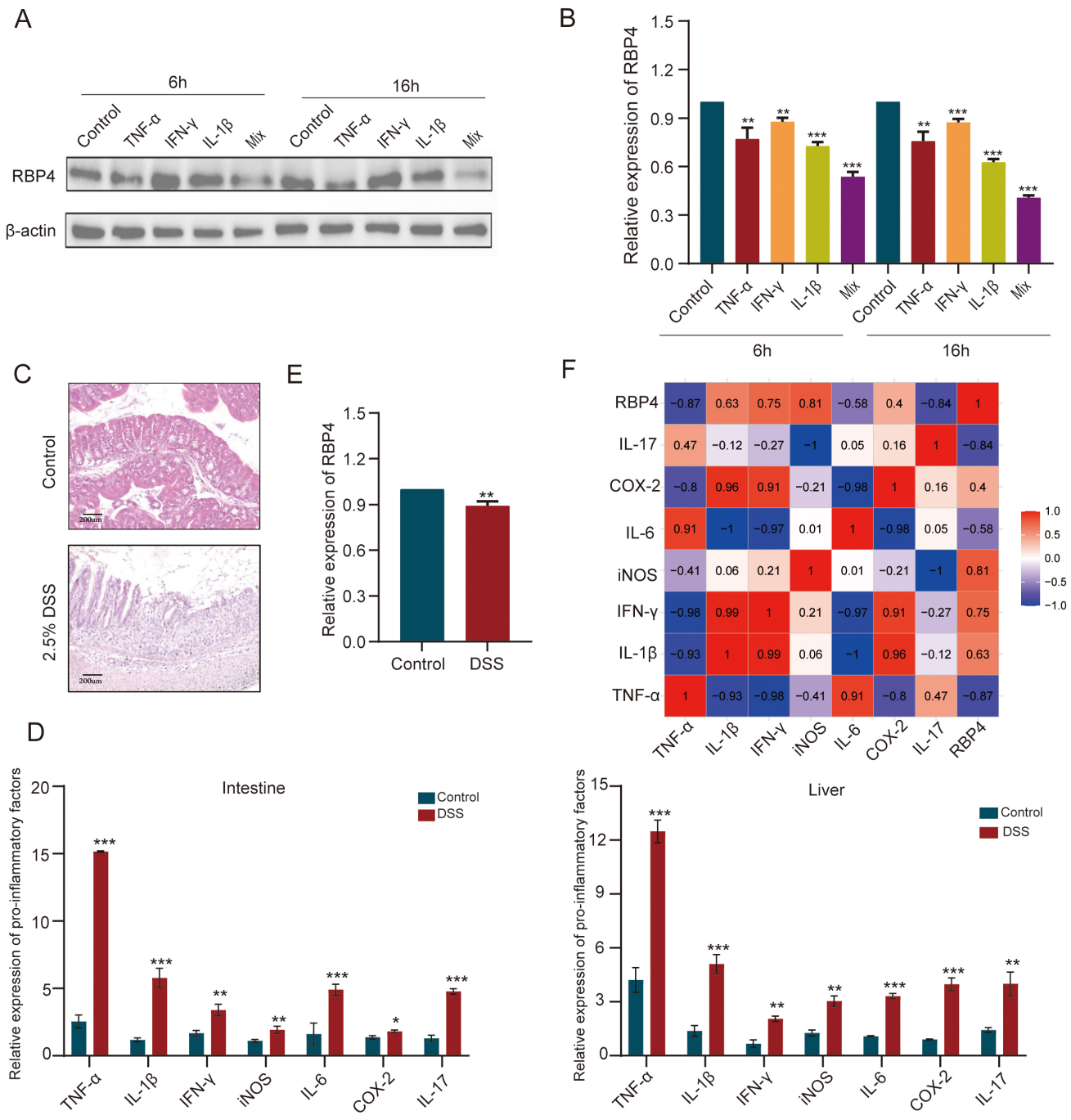

Fig. 3. Expression level of *RBP4* and its correlation with pro-inflammatory cytokines under inflammatory stimulation. (A) The protein level of RBP4 in primary mouse hepatocytes under stimulation with inflammatory cytokines. (B) The mRNA expression level of *RBP4* in primary mouse hepatocytes stimulated by inflammatory cytokines. (C) Histopathological images of colonic tissue from DSS model mice. (D) The mRNA levels of *TNF-α*, *IL-1β*, *IL-6*, *IL-17*, *iNOS*, *COX-2* and *IFN-γ* in tissue of the DSS and control mice. (E) The mRNA levels of *RBP4* in the liver tissue of DSS mice. (F) The correlation between *pro-inflammatory cytokines* and *RBP4* in the liver tissue of DSS mice. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. DSS, dextran sulfate sodium; *TNF-α*, tumor necrosis factor alpha; *IL-1β*, interleukin-1 beta; *IL-6*, interleukin-6; *IL-17*, interleukin-17; *iNOS*, inducible nitric oxide synthase; *COX-2*, cyclooxygenase-2; *IFN-γ*, interferon gamma.

variables (e.g., dietary habits or genetic factors) might bias estimates, supported by widened confidence intervals for vitamin A in BMI-adjusted models (95% CI: [2.79 $\times$ 10^{-6}, 21.19]). Third, limited statistical power due to the modest sample size (n = 103) hindered detection of small effect sizes, exemplified by vitamin A's consistent protective

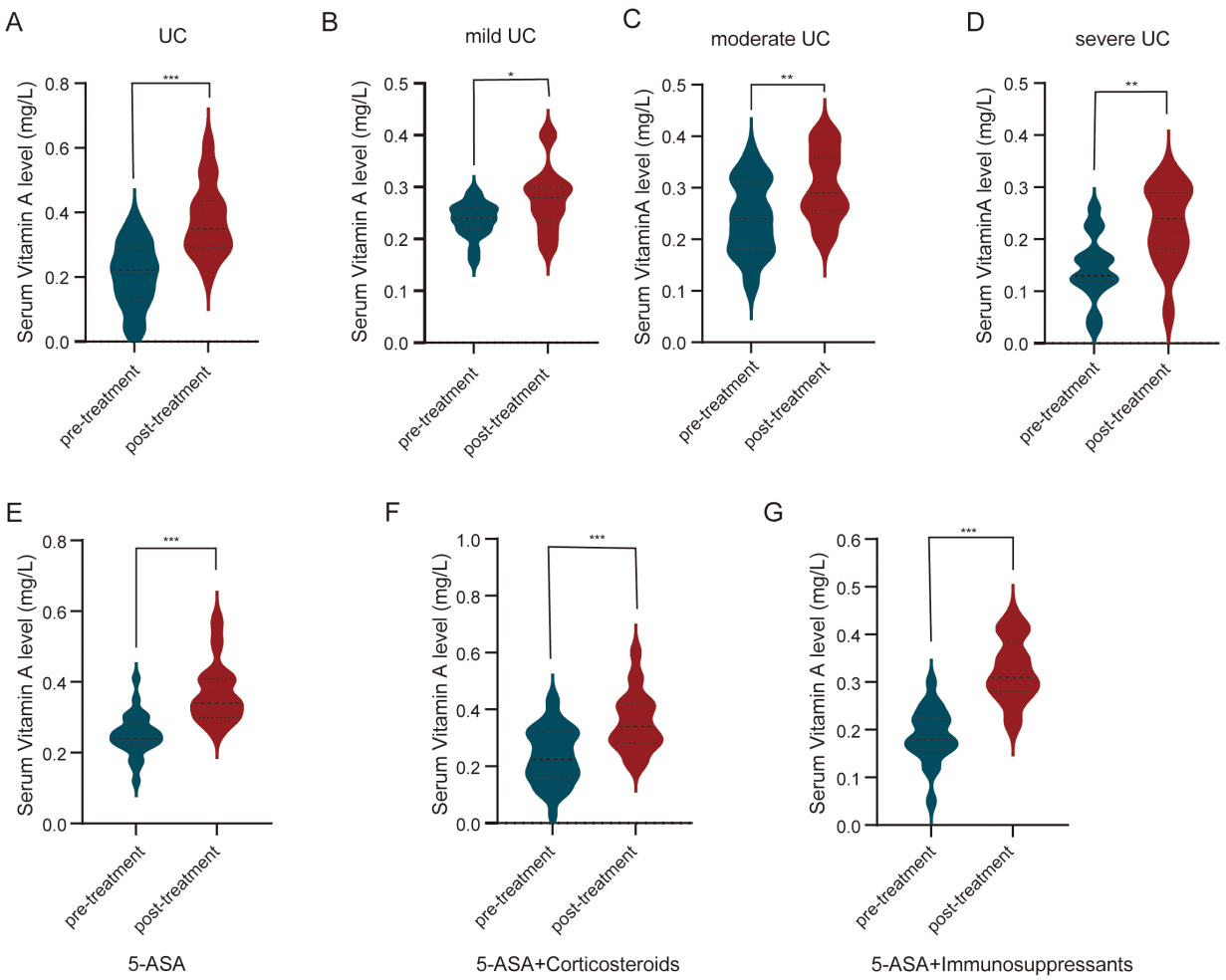

Fig. 4. Vitamin A levels in UC patients before and after treatment. (A) Comparison of Vitamin A levels in UC patients before and after treatment. (B) Comparison of Vitamin A levels before and after treatment in patients with mild UC. (C) Comparison of Vitamin A levels before and after treatment in patients with moderate UC. (D) Comparison of Vitamin A levels before and after treatment in patients with severe UC. (E) Comparison of vitamin A levels before and after 5-ASA treatment in UC patients. (F) Comparison of vitamin A levels before and after 5-ASA plus corticosteroids treatment in UC patients. (G) Comparison of vitamin A levels before and after 5-ASA plus immunosuppressants treatment in UC Patients. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. 5-ASA, 5-Aminosalicylic Acid.

trend (OR <1) but marginal significance (e.g., OR $= 0.035$). Additionally, overadjustment may have occurred if covariates like CRP or ESR acted as mediators on the causal pathway, introducing bias.

Furthermore, the progression and severity of UC are closely associated with elevated levels of pro-inflammatory cytokines. Analysis of the GEO dataset GSE107499, which includes 119 UC colon tissue samples, revealed significantly higher levels of IL-8, IL-1β, TNF-α, and IL-6 in inflamed tissues compared to non-inflamed tissues ($p < 0.05$) (**Supplementary Fig. 2**). Supporting this, Colombel *et al.* [24] investigated UC patients using the Mayo Clinical Score (MCS) to assess disease activity and measured inflammatory cytokine levels in endoscopic biopsy samples. Their findings showed that patients with higher endoscopy scores (MCS $\geq$2) exhibited significantly elevated levels of IL-6, IL-8, IL-1β, and TNF-α compared to those without endoscopic activity. Additionally, another study reported markedly increased IL-1 levels in UC mucosal biopsy samples compared to controls [25].

Several studies have highlighted the potential value of circulating RBP4 in monitoring treatment response and disease progression. For instance, RBP4 has been proposed as a predictive and monitoring biomarker for adalimumab treatment efficacy in patients with ankylosing spondylitis [26]. A meta-analysis found that circulating RBP4 levels were consistently elevated in patients with gestational diabetes mellitus (GDM) compared to non-GDM controls, suggesting that RBP4 may also be a biomarker for GDM [27]. Similarly, in obstructive sleep apnea syndrome (OSAS), circulating RBP4, along with adiponectin and irisin, was identified as a potential novel biomarker. These markers, alongside clinical data, could facilitate the diagnosis of OSAS when polysomnography is unavailable

[28]. Furthermore, in pediatric non-alcoholic fatty liver disease (NAFLD), RBP4 levels were inversely associated with the degree of liver damage, supporting its role as a noninvasive biomarker [29].

Consistent with these findings, the present study observed a negative correlation between serum RBP4 levels and UC severity. To further explore underlying metabolic factors and their potential contribution to UC, we conducted a multivariable linear regression analysis with vitamin A levels as the dependent variable, BMI as the independent variable, and age and gender as covariates. However, as shown in **Supplementary Table 6**, none of the independent variables (BMI, age, gender) demonstrated a statistically significant effect on vitamin A levels. Similarly, as shown in **Supplementary Table 7**, BMI did not exhibit a significant impact on RBP4 levels. These results suggest that serum RBP4 in UC is not strongly influenced by BMI, age, or gender, reinforcing the need for further studies to better understand its regulation and role in UC pathology.

We also explored the biological factors that may decrease RBP4 expression in patients with UC. Initially, our *in vitro* study revealed that the protein and mRNA levels of *RBP4* were both decreased in primary hepatocytes when exposed to inflammatory cytokines such as TNF-α, IFN-γ, and IL-1β. In the DSS model, we observed a negative correlation between *RBP4* levels and pro-inflammatory factors levels in liver tissue. Our results using the DSS mouse model extended this observation, with liver inflammatory cytokine expression found to be inversely correlated with *RBP4* levels. It is suggested that a systemic inflammatory response suppresses RBP4 synthesis.

However, some reports in the literature have suggested that elevated RBP4 levels may contribute to the promotion of inflammation. Zhang *et al.* [30] reported that elevated expression of the adipokine RBP4 can lead to a more severe periodontal inflammatory state in obese rats. RBP4 induces insulin resistance by promoting inflammation in adipose tissue through the activation of pro-inflammatory cytokines in macrophages. This mechanism is dependent on JNK and TLR4, but independent of retinol and STRA6 [31]. Additionally, RBP4 triggers insulin resistance and glucose intolerance by activating antigen-presenting cells in adipose tissue, which subsequently induces Th1 polarization of CD4 T cell and inflammation [32]. Elevated plasma RBP4 levels and inflammation were observed in patients with chronic kidney disease (CKD) without obesity and diabetes, both of which were ameliorated by transplantation. The increase in plasma RBP4 did not involve adipose tissue expression, suggesting it was primarily due to passive accumulation, or that it was linked to CKD-related inflammation [33]. The anti- or pro-inflammatory roles of RBP4 may depend on an interplay of factors, including the disease context, tissue-specific expression, genetic factors, cytokine crosstalk, and metabolic environment.

In summary, to our knowledge this study is the first to propose that vitamin A levels in UC patients undergo a transient decrease due to inflammation-induced suppression of RBP4 expression, which subsequently disrupts vitamin A transport. Moreover, serum RBP4 levels exhibited a negative correlation with the severity of UC. Future research should aim to develop an evaluation system based on serum vitamin A and RBP4 levels that can be used to assess disease severity and predict outcome in UC patients. Our current study's findings offer strong support for this concept. Moreover, our study revealed that the hepatic capacity for RBP4 synthesis and secretion, as well as vitamin A metabolism, was correlated with the severity of intestinal inflammation, indicating a significant role for liver-gut axis. Then these findings also provide important evidence to inform the development of therapeutic strategies that modulate chronic inflammatory diseases by targeting the gut-liver axis.

Nevertheless, this study has several limitations. First, there was some imbalance in sample distribution. To minimize potential bias in regression parameter estimation arising from this issue, we used Firth regression to ensure the robustness of our conclusions. This method introduces a penalization term to the conventional likelihood function, effectively reducing parameter bias caused by imbalanced group sizes or extreme variable distributions, and thereby improving the robustness of parameter estimation and the reliability of statistical inference. The results showed that the model had a good overall fit (LRT p-value < 0.001), and most variable estimates had finite, accurate confidence intervals and adjusted p-values (**Supplementary Table 8**). For example, in this multivariable analysis, vitamin A (OR = exp(–11.73), 95% CI: [exp(–48.02), exp(102.15)], p = 0.1525) and RBP4 (OR = exp(–0.0048), 95% CI: [exp(–0.2885), exp(0.2778)], p = 0.6965) were not significantly associated with UC status. In contrast, age showed a significant association (OR = exp(–0.0975), 95% CI: [exp(–0.5654), exp(–0.0009)], p = 0.0477). This result may be mainly due to the relatively small sample size, which could lead to random variation or confounding effects. These results, along with the effect sizes and confidence intervals for other covariates detailed in **Supplementary Table 8**, allow for a more nuanced interpretation of the magnitude and precision of associations in our study and support the robustness of our findings despite the sample size imbalance. Another limitation is that our control group consisted of patients with colonic polyps, rather than healthy individuals. This choice was primarily due to ethical and practical constraints in obtaining blood samples from healthy subjects undergoing colonoscopy. Although polyp patients might have subclinical inflammation or metabolic alterations, potentially introducing confounding effects and limiting the generalizability of our results, prior studies have shown negligible differences in serum retinol levels between individuals with colonic polyps and truly healthy controls.

For example, one study [34] reported nearly identical median plasma retinol levels and interquartile ranges for both healthy subjects and polyp patients, suggesting no significant difference in vitamin A status between these groups. Therefore, while our findings should be interpreted with caution due to this control selection, available evidence indicates that the impact on vitamin A and RBP4 comparisons is likely minimal. Future studies should, where feasible, include healthy controls to further strengthen baseline comparisons and validation. A larger clinical cohort is also needed to confirm the association between lower serum vitamin A levels and disease activity. Further multi-center studies should clarify the associations between serum vitamin A, RBP4, and UC severity and prognosis. Long-term follow-up of UC patients should also be performed to dynamically monitor changes in serum vitamin A and RBP4 levels, and to evaluate their relationship with disease progression and treatment outcomes. Second, the mechanisms of the gut-liver axis in this process require further study to elucidate how inflammatory responses influence RBP4 expression. AI-based data analysis may also be useful for developing a clinical assessment system based on serum vitamin A and RBP4, improving evaluation of disease activity, severity, and prognosis in UC. Additionally, potential therapies targeting the vitamin A–RBP4 pathway should be explored, which may lead to novel diagnostic, monitoring, and treatment strategies for UC.

5. Conclusions

Serum vitamin A levels are reduced in UC patients, and these reductions are correlated with increased disease severity. Similarly, serum RBP4 levels are decreased and show a negative correlation with disease severity, possibly due to suppression by proinflammatory cytokines, which may contribute to inflammation-driven dysfunction of vitamin A transport. These findings offer new insights into the mechanism of inflammation-induced dysfunction of vitamin A transport in UC, while advancing our understanding of liver-gut axis.

Availability of Data and Materials

The data used to support the findings of this study are available from the corresponding author upon request.

Author Contributions

ZZ: Contributed to study design, data analysis and interpretation, and drafting of the manuscript; JMM: Contributed to data acquisition and drafting of the manuscript; YJ: Contributed to study design and data acquisition; XJS: Contributed to data acquisition; YFL: Contributed to data analysis; XYW: Contributed to data analysis; ZLF: Contributed to data analysis; HZL: Contributed to data interpretation; QYT: Contributed to data interpretation; HTZ: Contributed to data acquisition; QHY: Critically reviewed the manuscript for important intellectual content; MY: Critically reviewed the manuscript for important intellectual content; XL: Contributed to study design, data analysis and interpretation, and critically reviewed the manuscript for important intellectual content; XCC: Contributed to study design, critically reviewed the manuscript for intellectual content, and gave final approval for the version to be published. All authors have contributed to the editorial changes made to the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The research protocol was approved by the Animal Ethics Committee of the Institute of Radiation Medicine, Chinese Academy of Medical Sciences (Ethic Approval Number: IRM-DWLL-2023266) and the Ethical Committee of Tianjin Medical University General Hospital (Ethic Approval Number: IRB2024-YX-523-01), and all of the participants provided signed informed consent.

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Conflict of Interest

XDJ, HL, HL, and FZ are the employee of Tianjin Kingmed Diagnostics Laboratory Company. There is no conflict of interest between Tianjin Kingmed Diagnostics Laboratory Company and the research described in this article.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/IJVNR37353.

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