ORIGINAL ARTICLE

Retinol Binding Protein 4 in children with Inflammatory Bowel Disease: a negative correlation with the disease activity

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Abstract

Objectives: Retinol Binding Protein-4 (RBP-4), the action of which was initially thought to be only the transport of vitamin A, is a major circulating adipocytokine involved in the inflammation. We evaluated the serum RBP-4 levels in children with inflammatory bowel disease (IBD) and correlated them with transthyretin (TTR), inflammation markers, disease activity, and body mass index (BMI).

Design: In 41 children of mean age 11.9 ± 3.6 years (range 5-17.7 y) with IBD (19 with Crohn's disease (CD) and 22 with Ulcerative colitis (UC) serum RBP-4, TTR, Amyloid A (SAA), C-Reactive Protein (CRP), Erythrocyte Sedimentation Rate (ESR), disease activity and BMI were prospectively determined and compared with those of 42 matched controls.

Results: No difference in the RBP-4 and TTR serum levels, between patients and controls as well as between active and remission state of the disease was noticed. A negative correlation of serum RBP-4 with the disease activity, SAA and ESR and a positive correlation with TTR was found, but no significant correlation with CRP or BMI was found. Inflammation markers were significantly increased in patients compared to controls and had a positive correlation with the disease activity.

Conclusions: RBP-4 negatively correlated with disease activity of children with IBD probably indicating a protective anti-inflammatory mechanism of action in addition to transport of vitamin A. Hippokratia 2012, 16, 4: 360-365

Key words: retinol binding protein-4, inflammatory bowel disease RBP-4, IBD, children, disease activity index

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Introduction

Inflammatory bowel disease (IBD), Crohn's disease (CD) and ulcerative colitis (UC), constitute a multifactorial disorder characterized by a chronic relapsing inflammation of the digestive tract. As multifactorial disorder, IBD is probably caused by a complex interaction of genetic, microbial, immunological and environmental factors[1-3]. Many studies indicate that cytokines play a key role on inflammation in patients with IBD[4].

Adipose tissue is no longer considered merely an energy-storing depot but rather a metabolically active endocrine organ, that secretes many information-carrying molecules, called adipocytokines. They are fat-derived hormones and cytokines with immune-modulating and metabolic properties. There is evidence that adipocytokines are involved in inflammatory and metabolic pathways in humans. Among the adipocytokines, leptin, adiponectin and resistin appear to play an important role[5].

Anorexia, malnutrition, altered body composition, and development of mesenteric obesity (accumulation of intra-abdominal white adipose tissue -WAT), are well-known features of IBD, mainly of CD, indicating an important role for WAT-secreted proteins[6]. Overexpression of adipocytokines such as leptin, adiponectin, and resistin in mesenteric adipose tissue of patients with CD who have been operated has been reported, suggesting that mesenteric adipocytes in IBD may act as immunoregulatory cells[7-9]. Therefore, it is suggested that adipocytokines may participate in the disease pathogenesis[4].

Based on animal and human studies, it is suggested that the soluble form of Retinol Binding Protein-4 (RBP-4) -the action of which was initially thought to be only the transport of retinol (Vitamin A)- is a major circulating adipokine implicated in systemic insulin resistance. RBP-4 is a small, 21kDa protein that circulates bound to transthyretin (TTR, one of the plasma carriers of thyroid hormone) in the form of an 80kDa protein complex that is not easily filtered through the kidneys. Overall, the available evidence indicates the existence of a high degree complementarity between RBP and TTR, the contact areas of which are highly sensitive to conformational changes and amino acid replacements[10]. TTR is a tryptophan-rich protein, used as one of the nutrition assessment

Table 1: Demographic characteristics and blood chemistry parameters (mean ± SD and ranges in parentheses).

	IBD patients
Age (y)	11.9 ± 3.6 (5-17.7)
Male/ Female	24/17
Crohn/Colitis	19/22
Remission /active	25/16
BMI	19.8 ± 4 (14.2-28.4)
BMI Z-SCORE	-0.05 ± 2.2 (-6.4 – 0.13)
TTR (mg/L)	225.8 ± 63.6 (86.1-351)
RBP-4 (mg/L)	28.5 ± 8.5 (9.8-45.3)
SAA (mg/L)	12.6 ± 20.5 (0.7- 83.4)
CRP (mg/L)	9.9 ± 19.8 (2.2-107.0)
ESR (mmHg)	39.6 ± 28.7 (1.0-115.0)

BMI: body mass index, TTR: transthyretin, RBP-4: Retinol Binding Protein-4, SAA: Serum Amyloid A, CRP: C-Reactive Protein, ESR: Erythrocyte Sedimentation Rate.

proteins, it acts as an anti-acute phase protein, and its plasma concentration decreases during inflammation and bacterial infection. Moreover, TTR plays important roles in various CNS disorders, diabetes mellitus, and lipid metabolism[11]. A specific aspect in the determination of the extent of vitamin A deficiency based on reduced RBP-4 levels is the observation that, beside vitamin A deficiency, the inflammation-associated acute phase response (APR) results in decreased plasma RBP-4 levels despite a sufficient vitamin A status[12].

C-reactive protein (CRP) and serum amyloid A (SAA) increase in the blood of patients with inflammatory conditions and in IBD[13,14]. In IBD patients SAA is useful as a marker for disease activity, with a high sensitivity[14]. The expression of SAA is predominantly in the liver. However, extrahepatic expression of SAA, including adipocytes, has been reported. The association between SAA and leptin suggests an interaction between these two adipokines, which may have implications in inflammatory processes related to obesity and the metabolic syndrome[15].

Studies concerning the relationship of RBP-4 with IBD are limited. Since inflammation is the underlying process in IBD, we aimed to evaluate the serum RBP-4 and its binding TTR in children with IBD and correlate them with inflammation markers such as SAA, CRP and erythrocyte sedimentation rate (ESR), disease activity and BMI in order to find out its possible interference with the disease process.

Patients and methods

Patients

Forty-one children with a confirmed diagnosis of IBD (19 with CD and 22 with UC) according to the ESPGHAN Porto-criteria were included in this study[16,17]. Approval of the local ethical committee and informed parental concern were obtained. The disease was either active or in remission. Its activity was classified according to Pediatric Crohn's Disease Activity Index (PCDAI) for CD patients (score 0 to 10 inactive disease, 11 to 30 mild disease and above 30 moderate to severe disease)[18], while the Pediatric Ulcerative Colitis Activity Index (PUCAI) was used for UC[19]. Evaluation of disease activity

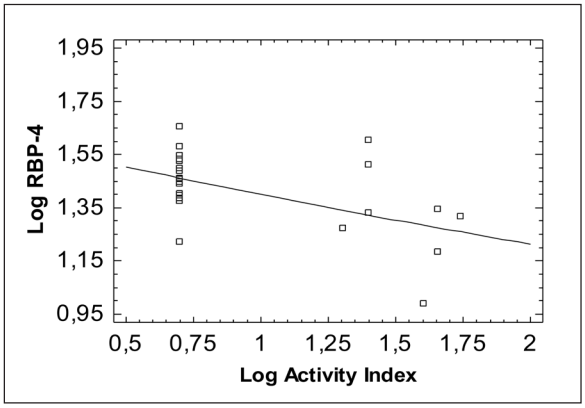

Figure 1: Correlation of Retinol Binding Protein-4 (RBP-4) with activity index.

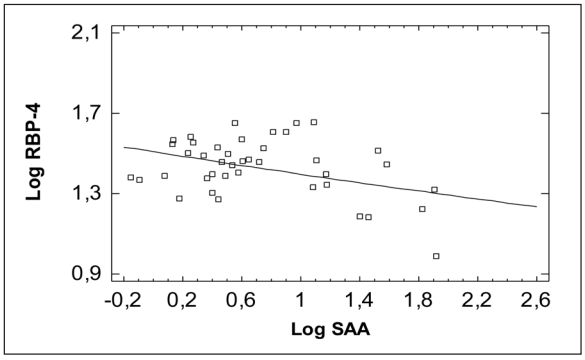

Figure 2: Correlation of Retinol Binding Protein-4 (RBP-4) with Serum Amyloid A (SAA).

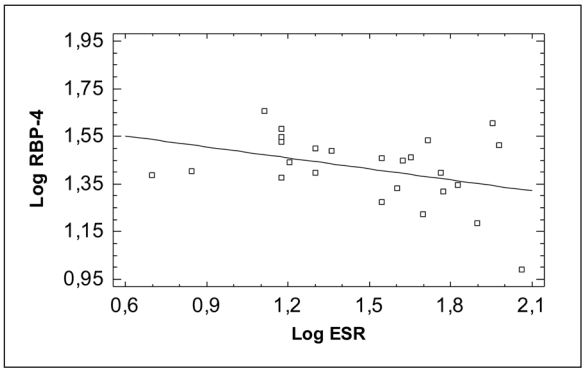

Figure 3: Correlation of Retinol Binding Protein-4 (RBP-4) with Erythrocyte Sedimentation Rate (ESR).

and BMI were performed at the time of serum collection. Serum RBP-4, TTR, SAA and the standard laboratory parameters including red and white blood cell count, hemoglobin, hematocrit, platelet count, total protein, albumin, cholesterol, triglycerides, ESR, CRP, were determined in all patients at a routine clinical and laboratory evaluation. Forty two children were used as controls.

Blood Chemistry

Blood samples were collected in the morning after an overnight fast. Serum concentrations of Transthyretin, SAA and CRP were measured by particle-enhanced immu-

Table 2: Comparison of Retinol Binding Protein-4 (RBP-4) , inflammation markers, transthyretin (TTR) and body mass index (BMI) in children with inflammatory bowel disease (IBD) and controls.

	Patients with IBD n=41		Controls n=42		p-values
	mean	range	mean	range	
BMI	19.8 ± 4	14.2-28.4	19.8 ± 2.3	15.8-24	NS
Z-score	-0.05 ± 2.2	-6.4 – 0.13	-0.07± 0.8	-1.88 – 1.54	
RBP-4 (mg/L)	28.5 ± 8.5	9.75 - 45.33	26.3 ± 5.7	17.3-43.1	0.09
SAA (mg/L)	12.6 ± 20.5	0.7 - 83.4	2.5 ± 0.9	1.7- 4.8	0.00001
CRP (mg/L)	9.9 ± 19.8	2.2 - 107	0.6 ± 0.7	0.1-0.9	0.00001
TTR (mg/L)	225.8 ± 63.6	86.1 - 351	198.3 ± 35.9	149-255	0.07
ESR (mmHg)	39.6 ± 28.7	1 - 115	<20		

SAA: Serum Amyloid A, CRP: C-Reactive Protein, ESR: Erythrocyte Sedimentation Rate.

Table3: Comparison of Retinol Binding Protein-4 (RBP-4) and inflammation markers in active and remission state of children with inflammatory bowel disease (IBD)(mean values).

	Active		Remission		p-values
	mean	range	mean	range	
RBP-4 (mg/L)	28 ± 11.4	9.8- 44.9	28.8 ± 6.3	16.7- 45.3	NS
SAA (mg/L)	20 ± 26.2	1.4- 83.4	7.9 ± 14.6	0.7- 67.1	0.001
CRP (mg/L)	16.9 ± 29.7	3.1-107	5.5 ± 7.3	2.2- 37.5	0.001
TTR (mg/L)	222 ± 85.2	86.1- 348	228.2 ± 46.8	138- 351	NS
ESR (mmHg)	57.5 ± 31.9	17-115	28.1 ± 19.7	1-72	0.001

SAA: Serum Amyloid A, CRP: C-Reactive Protein, TTR: transthyretin, ESR: Erythrocyte Sedimentation Rate.

Table 4: Correlation of Retinol Binding Protein-4 (RBP-4) with different parameters.

	CORRELATION	p -VALUE
ACTIVITY	-0.5117	0.008
BMI	0.1911	NS
SAA	-0.4283	0.03
CRP	-0.0293	NS
ESR	-0.4098	0.04
TTR	0.1502	0.0001

BMI: body mass index, SAA: Serum Amyloid A, CRP: C-Reactive Protein, ESR: Erythrocyte Sedimentation Rate, TTR: transthyretin.

nonephelometric assays (Dade Behring Siemens Healthcare Diagnostics). The intra- and inter-assay coefficients of variation (CVs) were less than 6% and 7% respectively.

Serum RBP-4 concentrations were determined using a sandwich ELISA assay (Immunodiagnostik AG, Behsheim, Germany). Serum samples were diluted so that the absorbance was in the middle of the range of linearity for this assay. The intra-and inter-assay CVs for RBP-4 were 5.0 and 9.7 %, respectively, according to the manufacturer.

Statistical Analysis

Data are expressed as mean ± SD. Differences between groups were analysed using the Student's t-test. The correlation coefficient *r* between the parameters tested was computed using least squares regression analysis. The *p* values reported are two tailed. We used the standardized skewness and standardized kurtosis, to determine whether the sample comes from a normal distribution. Values of these statistics outside the range of -2 to +2 indicate significant departures from normality, which would tend to invalidate many of the statistical procedures normally applied to this data. These values were integrated automatically from the program and indicated that the parameters needed to transform in either logarithmic or reciprocal or square root, where needed it. All the statistical analyses were performed using the STATGRAPHICS 5.1 for Windows program (Graphic Software System, STATPOINT TECHNOLOGIES, INC. Warrenton, Virginia, USA).

Results

A total of 41 children with IBD with a mean age 11.9 ± 3.6 years (range 5-17.5 years) participated in the study. Among them 19 suffered from CD and 22 from UC. According to the activity index 25 were in remission and 16 in moderate to severe disease state. Demographic data and mean values (range) of serum RBP-4, ESR, CRP, SAA and BMI are shown on Table1. Forty two children of mean age 9.9 ± 2.5 years (5.7-14.4 years) were used as controls. No difference of RBP-4, TTR and BMI between patients and controls was found, while the inflammation markers were significantly increased in patients (Table 2).

A statistically significant increase of inflammation

Table 5: Correlation of activity index with inflammatory markers.

	CORRELATION (r)	p-VALUE
SAA	0.5949	0.002
TTR	-0.5	0.01
CRP	0.4429	0.02
ESR	0.6704	0.0002

SAA: Serum Amyloid A, TTR: transthyretin, CRP: C-Reactive Protein, ESR: Erythrocyte Sedimentation Rate.

Table 6: Correlation of BMI with different parameters.

	CORRELATION (r)	p-VALUE
SAA	-0.2059	NS
ACTIVITY	-0.4095	0.04
CRP	0.0218	NS
ESR	-0.1440	NS
RBP-4	0.1911	NS

SAA: Serum Amyloid A, CRP: C-Reactive Protein, ESR: Erythrocyte Sedimentation Rate, RBP-4: Retinol Binding Protein-4.

markers was detected between patients in active phase and those in remission, while no significant difference of RBP-4 and TTR values was found between active and remission state of the disease (Table 3). All the above parameters did not differ between CD and UC patients (data not shown).

A negative correlation of serum RBP-4 with the disease activity, SAA and ESR and a positive correlation with TTR was detected, but no significant correlation with the other parameters was found (Table 4, Figures 1, 2, 3).

All inflammation markers had a positive correlation with the activity index, except TTR which had a negative correlation (Table 5). BMI was negatively correlated with disease activity (Table 6).

Discussion

In the present study we determined the serum levels of RBP-4 and its binding protein TTR in children with IBD, compared them with the values of matched controls and correlated with disease activity, serum inflammation markers and BMI.

A negative correlation of RBP-4 and TTR with the disease activity, SAA and ESR in children with IBD was found. Contrary to our findings, in a previous study on patients with IBD, which aimed to evaluate the possible associations of circulating levels of adipokines and glucose homeostasis with the course and characteristics of the disease, RBP-4 was increased in active and inactive disease compared with controls[20].

Retinol-binding protein is the specific plasma carrier of retinol, encharged of the vitamin transport from the liver to target cells. Ligand binding influences the RBP affinity for TTR, a homotetrameric protein involved in the RBP/TTR circulating complex, and the secretion rate of RBP. In fact, in vitamin A deficiency the RBP release from the hepatocytes dramatically decreases and the protein accumulates in the cells, until retinol is available again[21]. In the present study it is unclear whether the negative correlation of RBP-4 with the disease activity could be due to the reduced vitamin A or it indicates the response of RBP-4 to inflammation during active disease.

Indeed, hypovitaminosis and fat-soluble vitamin deficiency have been reported in adults with IBD. Plasma vitamin A, E and carotenoid concentrations determined in IBD patients were significantly reduced, particularly in those with active disease, with respect to controls[22]. Busvaros et al in a prospective study estimated the serum levels of vitamins A and E in children and young adults with IBD and the prevalence of hypovitaminosis A or E was 16% in the pediatric IBD population[23]. Hypovitaminosis was significantly more prevalent in the Crohn's disease patients who had active disease, but in their study RBP-4 was not estimated in order to compare it with our findings. They concluded that children and young adults with active IBD frequently have low serum levels of vitamin A or vitamin E and that the severity of disease activity was a better predictor of risk for hypovitaminosis than the nutritional status. They also stated that further work is necessary to determine whether the hypovitaminosis seen in children with IBD reflects true deficiency. As an answer to their concern, other studies showed that plasma levels of retinol are of limited value to evaluate the vitamin A status of an individual because of the homeostatic control of RBP-4 in plasma, as well as the strong influence of inflammation on its plasma levels[24,25]. In this respect, the negative correlation of RBP-4 with the disease activity in our study is in accordance with the findings by Busvaros et al since vitamin A reflects RBP-4 levels due to tight regulation of vitamin A by synthesis of RBP-4[23].

RBP-4, as an adipocytokine, has been associated with the pathogenesis of insulin resistance and has gained considerable attention due to its possible link to diabetes, which may be due to the increased inflammatory status in diabetic and obese patients, although several studies have shown controversial results[26-29]. Additionally, negative[30] or positive[31,32] correlations of RBP-4 with BMI in obese children and adolescents have been found.

Studies have demonstrated significantly lower serum retinol levels in patients with inflammation-associated acute phase response (APR) or trauma[25]. The APR leads to transient increase or decrease of plasma concentrations of different proteins due to changed hepatic proteins synthesis, known as positive (e.g. C-reactive protein) or negative (e.g. RBP-4 and transthyretin) acute phase proteins during inflammation. It has been shown that the inflammation-associated APR results in decreased plasma RBP-4 levels despite a sufficient vitamin A status[12]. Based on these difficulties, it has been suggested that the determination of the

molar ratio RBP-4 to TTR index might help to discriminate between deficiency and an inflammatory induced decrease of RBP-4[33]. Thus, in inflammation retinol, RBP-4 and TTR levels decrease, whereas, in nutritional deficiency, only retinol and RBP-4 are decreased[24]. During inflammation, both RBP-4 and TTR levels are decreased due to APR, resulting in an unchanged ratio at lower absolute levels[24]. The negative correlation of both RBP-4 and TTR with the disease activity in the present study is in favour of the interference of inflammation during active disease state. Additionally, an increase of the positive acute phase proteins (SAA and CRP) and ESR was identified in active disease, with a significant negative correlation of RBP-4 with SAA and ESR.

RBP-4 serum levels have been estimated in liver diseases based on its possible interference as an adipocytokine with the inflammation process. An inverse relationship between RBP-4 levels and the degree of liver damage in children with non alcoholic fatty liver disease (NAFLD)[34], as well as with the degree of histological lesion in patients with chronic hepatitis C, who had a significant elevation after treatment with interferon and sustained virological response has been found[35]. Serum RBP-4 has been suggested as a serologic marker for disease severity in patients with chronic liver disease (CLD)[36, 37].

In the present study a statistically significant positive correlation of RBP-4 with the TTR was found and both had a negative correlation with the disease activity. Probably both act as anti- acute phase proteins during the severe inflammation in the active IBD. The negative correlation of RBP-4 with SAA, which is the most sensitive acute phase protein during inflammation, is in accordance with this hypothesis.

In conclusion, in the present study an inverse correlation of RBP-4 and TTR with the disease activity, serum amyloid A and ESR was found. The mechanism of this reduction during active inflammation is not clear but the action of RBP-4 could be through its behavior as an adipocytokine, interfering with the inflammation process and oxidative stress. Therefore, RBP-4 as it happens with TTR- can be used not only as nutrition assessment proteins, but possibly may act as anti-acute phase proteins. It is clear that more studies in children are needed concerning RBP-4 in relation with vitamin A intake as well as with the active and remission stages of IBD.

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Conflict of interest

The authors declare no financial interests and/or conflict of interest related to this study.

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