Retinol-binding protein 4 and plasminogen activator inhibitor-1 as potential prognostic biomarkers of non-allergic asthma caused by obesity in adolescents

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Abstract

Background: Non-allergic asthma caused by obesity is a complication of the low-grade chronic inflammation inherent in obesity. Consequently, the serum concentrations of adipokines such as retinol-binding protein 4 (RBP4) and plasminogen activator inhibitor-1 (PAI-1) increase. No gold standard molecule for the prediction of non-allergic asthma among obese patients has been identified.

Objective: To evaluate RBP4 and PAI-1 as prognostic biomarkers of non-allergic asthma caused by obesity.

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Methods: A cross-sectional study between four groups of adolescents: (1) healthy (n = 35), (2) allergic asthma without obesity (n = 28), (3) obesity without asthma (n = 33), and (4) non-allergic asthma with obesity (n = 18).

Results: RBP4 was higher in the non-allergic asthma with obesity group than in the obesity without asthma group (39.2 ng/mL [95% confidence interval (CI): 23.8-76.0] vs. 23.5 ng/mL [95% CI: 3.2-33.5], p < 0.01), and PAI-1 was higher in the non-allergic asthma with obesity group than in the obesity without asthma group (21.9 ng/mL [95% CI: 15.7-26.5] vs. 15.9 ng/mL [95% CI: 9.4-18.2], p < 0.05). Receiver operating characteristic (ROC) curve analysis demonstrated that the serum RBP4 cut-off value was >42.78 ng/mL, with an area under the ROC curve (AUC) of 0.741 (95% CI: 0.599-0.853, p = 0.001), considered acceptable. The PAI-1 cut-off value was >12.0 ng/mL, with an AUC of 0.699 (95% CI: 0.554-0.819, p = 0.008), considered fair.

Conclusions: RBP4 may be useful to predict non-allergic asthma among obese adolescents in clinical practice.

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Introduction

Obesity and overweight are defined by the World Health Organization (WHO) as “abnormal or excessive fat accumulation that may impair health.” Every year, at least 2.8 million people die of overweight and obesity. Asthma is defined according to the Global Initiative for Asthma (GINA) as “a heterogeneous disease, usually characterized by chronic airway inflammation. It is defined by the history of respiratory symptoms such as wheeze, shortness of breath, chest tightness, and cough that vary over time and in intensity, together with variable airflow limitation. Airflow limitation may later become persistent.” According to the WHO, 235 million people in the world suffer from asthma.

Obesity causes a low-grade chronic inflammatory state, and this inflammation has been associated with non-allergic asthma, metabolic diseases, autoimmune diseases, and certain types of cancer. Systematic reviews and meta-analyses have shown that obesity precedes asthma and that an increase in body mass index (BMI) increases the relative risk (RR) of suffering from asthma throughout the life in both children and adults.5,7

Holguin et al.8 described a special phenotype of non-allergic asthma caused by obesity. With late onset, this asthma starts after age 12, is more common in women, is more severe than regular asthma, and is resistant to inhaled steroids. It is characterized by a Th1 and Th17 immune response with increased levels of TNF-α, IL-8, IL-6, and IL-17A.9-11

The major cell type in visceral adipose tissue in obesity is type 1 macrophages (M1), which are the main source of inflammatory interleukins, such as tumor necrosis factor (TNF-α), interleukin (IL)-18, and IL-6, which cause low-grade chronic inflammation.12-13 This low-grade chronic inflammation is mainly orchestrated by TNF-α, IL-18, IL-6, and IL-17A.14 These interleukins play important roles because they cause structural and irreversible damage to the respiratory epithelium in this group of patients.15 As a consequence of this chronic inflammation, serum concentrations of certain adipokines are increased, such as retinol-binding protein 4 (RBP4) and plasminogen activator inhibitor-1 (PAI-1).16

Serum RBP4 is the only hepatocyte-secreted factor responsible for the transport of retinol (vitamin A) in the circulation and is secreted by M1 macrophages and adipocytes.17 In the pediatric population, Huang et al.18 reported that the concentration of RBP4 is higher in obese adolescents than in healthy adolescents. Few clinical studies of the association between RBP4 and asthma have been reported, and their findings are controversial.19,20

Retinol-binding protein 4 is produced mainly by adipocytes and by M1 macrophages located in visceral adipose tissue,21 and according to Periyalil et al.,22 patients with asthma and obesity have a greater number of M1 macrophages in visceral adipose tissue than obese patients without asthma. Consequently, patients with non-allergic asthma caused by obesity can be assumed to have higher serum concentrations of RBP4 than patients with obesity without asthma.

Plasminogen activator inhibitor-1 is an adipokine that is upregulated in obesity mainly in visceral adipose tissue, and its main function is inhibiting fibrinolysis.23,24 The deposition of fibrin and collagen is a characteristic of remodeling in the respiratory epithelium in asthmatic patients.25 PAI-1 induces the deposition of fibrin in the airways, inhibiting the activation of the active enzyme plasmin by tissue-type plasminogen activator (t-PA) or urokinase-type plasminogen activator (u-PA), which both degrade fibrin. Therefore, there is no fibrinolysis, and as a result, there is an increase in the deposition of fibrin in the extracellular matrix of the bronchial epithelium, so the respiratory epithelium will have irreversible damage and bronchial remodeling.26,27 Studies in murine models such as those by Eitzman et al.,28 Oh et al.,29 and Swaisgood et al.30 concluded that PAI-1 plays an important role in the deposition of fibrin in the respiratory tract in the murine asthma model. Few clinical studies have explored the association between PAI-1 and asthma, and the phenotypes of asthma in such studies are heterogeneous; consequently, the results are controversial.31,32

Plasminogen activator inhibitor-1 is increased in obesity33; therefore, in patients with superimposed inflammatory diseases, such as obesity and asthma, serum concentrations of PAI-1 can be logically assumed to be...
higher than those in healthy individuals, individuals with allergic asthma without obesity, and individuals with obesity without asthma.

The multifactorial etiology of non-allergic asthma caused by obesity imposes an important limitation on the treatment of this disease.\textsuperscript{14,16} The most important intervention is to prevent the disease in obese patients. Unfortunately, the general practitioner, pediatrician, and allergist do not currently have a biomarker that can predict non-allergic asthma in obese patients. The aim of the present study was to evaluate RBP4 and PAI-1 as potential prognostic biomarkers of non-allergic asthma caused by obesity.

Based on a previous theory, our hypothesis is based on the fact that adolescents with non-allergic asthma caused by obesity present lower chronic inflammation than healthy adolescents, adolescents with allergic asthma without obesity, and obese adolescents without asthma.

Materials and methods

Study design

A cross-sectional study of four groups of adolescents was carried out from June 2018 to October 2019. All patients were sampled by convenience in the Department of Allergy and Paediatric Clinical Immunology and in the Obesity Clinic of the Children’s Hospital of Mexico Federico Gómez, Mexico City.

Ethical approval

The study was approved by the research, ethics in research, and biosecurity committee of the Children’s Hospital of Mexico Federico Gómez with an approval number HIM/2013/015. This study adhered to the Declaration of Helsinki (2013)\textsuperscript{17} and was conducted in accordance with the Mexican Regulation for Research in the Field of Health. Before any procedures, the parents of all participating adolescents signed informed consent, and the patients themselves also signed informed assent. No patient was identified during the study, and all data were kept confidential.

Subjects

The following four groups of adolescents were established: (1) healthy, (2) allergic asthma without obesity, (3) obesity without asthma, and (4) non-allergic asthma with obesity. The age range of all subjects was 11-18 years. The inclusion criteria for the allergic asthma without obesity group were a diagnosis of asthma determined by a physician according to the clinical criteria of GINA 2018\textsuperscript{3}; an asthma evolution ≥6 months; a positive skin prick test (SPT) for at least one air allergen; asthma confirmation by spirometry with an inhaled \( \beta_2 \)-agonist with reversibility ≥12% or 200 mL of the forced expiratory volume in 1 second (FEV1) with respect to the baseline value; well- or partially controlled asthma; and a normal BMI. For the obesity group, the inclusion criterion was a BMI ≥95th percentile as defined by the National Center for Health Statistics, Centers for Disease Control and Prevention [CDC].\textsuperscript{38} Finally, the criteria for the non-allergic asthma with obesity group were a BMI ≥95th percentile according to the CDC\textsuperscript{38}; obesity before the onset of asthma; a diagnosis of asthma determined by a doctor according to the clinical criteria of GINA 2018\textsuperscript{3}; asthma evolution ≥6 months; a negative SPT; asthma confirmation by spirometry with an inhaled \( \beta_2 \)-agonist with reversibility ≥12% or 200 mL of the FEV1 compared to the baseline value; and well- or partially controlled asthma. The exclusion criteria for the four groups were systemic and chronic degenerative diseases and treatment with systemic steroids in a cut-off or continuous cycle within 3 months before measurement of the serum concentrations of the adipokines. Elimination criteria: the voluntary decision of parents/guardians or patients to withdraw from the study at any time (their medical care was continued).

Protocol and laboratory measurements

The first visit included a medical history, a physical exam by a pediatric allergist, anthropometric measurements collected by a trained nutritionist, and, if the eligibility criteria were met, signing of the informed consent by the parents/guardians and patients. A spirometry test was performed on all patients with asthma using a spirometer (Vmax Spectra 209, Yerba Linda, CA, USA) by a technician trained in respiratory physiology. FEV1 was adjusted for differences in age, sex, and height using Polgar values to obtain the percentage of predicted values of FEV1.\textsuperscript{39} The spirometric procedure was performed according to standardization criteria last recommended by the American Thoracic Society and European Respiratory Society.\textsuperscript{40} All patients with asthma underwent SPT for aeroallergens (Dermatophagoides pteronyssinus, Dermatophagoides farinae, Blattella germanica, Periplaneta americana, standardized cat hair, dog epithelium, and pollens of grasses, weeds, and trees) (ALK-Abelló®, Copenhagen, Denmark), with positive control of histamine and negative control of glycerin. Asthma was considered allergic if the size of the hive was ≥3 mm.

Height was measured to the nearest 0.5 cm using a stadiometer, and weight was measured to the nearest 0.1 kg using a medical balance (Health Metre 402 KL, USA). When weighed, the participants wore light clothing and took off their shoes. BMI was calculated by the standard formula, (weight in kilograms)/(height in metres\(^2\)). Tanner stage was assessed according to the standardized method.\textsuperscript{41}

On the second visit, a blood sample of 10 mL from the left cubital vein (at 8 to 9 am, after 12 h of fasting) was taken. The concentration of RBP4 was determined by enzyme-linked immunosorbent assay (ELISA) (Millipore, USA). To assess PAI-1, serum and plasma were frozen and kept at -70°C until analysis, and the concentration of PAI-1 in citrated plasma was determined by ELISA (Oncogene-Science, Cambridge, MA, USA). Finally, on the third visit, the results of adipokine concentrations were given to the parents/guardians and patients.
**Statistical analysis**

The data are presented as the medians and their respective 95% confidence intervals (CIs), and the ordinal and qualitative variables are presented as frequencies and percentages. The Kruskal-Wallis test was carried out to identify significant differences in medians, and the Wilcoxon signed-rank test was performed as a post hoc analysis to determine which groups were significantly different. Comparisons of proportions were analyzed by Pearson’s Chi-squared test. To evaluate the accuracy of adipokines as prognostic indicators, receiver operating characteristic (ROC) curve analysis was performed, and the area under the curve (AUC) and the best cut-off was defined as the value maximizing the Youden’s index with their respective sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). A multivariate linear regression model was used to adjust confounding variables. MedCalc® software, version 19.5.2 (Belgium) and GraphPad® Prism software, version 8 (La Jolla, CA) were used for ROC curve analysis. In all analyses, a value of p < 0.05 with a 95% CI was considered statistically significant. All data were evaluated with IBM® SPSS® Statistics v. 22.0 (SPSS, Inc., IBM, Chicago, IL, USA).

**Results**

With regard to demographic and baseline characteristics, patients with obesity without asthma were younger than the healthy subjects and those in the allergic asthma without obesity group and shorter than those in the non-allergic asthma with obesity group. There were no differences in the proportion of the sexes and Tanner stage between the four groups (Table 1).

As expected, both groups of adolescents with obesity had higher weight, BMI, BMI percentile, and BMI z-score than the two groups without obesity. There were no differences in the weight, BMI, BMI percentile, and BMI z-score between the non-allergic asthma with obesity group and the obesity without asthma group (Table 1).

The non-allergic asthma with obesity group had the highest serum concentration of RBP4. Notably, the non-allergic asthma with obesity group had higher serum RBP4 levels than the obesity without asthma group. No statistically significant differences were found between the healthy, allergic asthma without obesity and obesity without asthma groups (Table 1 and Figure 1).

The non-allergic asthma with obesity group also had higher serum PAI-1 levels than the healthy and obesity without asthma groups. Important to our hypothesis, the non-allergic asthma with obesity group had higher serum PAI-1 levels than the obesity without asthma group. We did not observe any statistically significant differences between the healthy, allergic asthma without obesity and obesity without asthma groups (Table 1 and Figure 1).

To evaluate the accuracy of RBP4 and PAI-1 as diagnostic tests for prognosis, ROC curve analysis was performed only in the obesity without asthma and non-allergic asthma with obesity groups. As shown in Figure 2, when the cut-off value for RBP4 was set to >42.78 ng/mL, the ROC curve had an AUC value of 0.741 (95% CI: 0.599-0.853) (p = 0.001), indicating that RBP4 had a 74.1% probability of accurately discriminating whether an obese adolescent had the outcome of non-allergic asthma.

Figure 3 shows the test characteristics of RBP4; the sensitivity was low, so there was a 50% probability of a false negative; however, the accuracy of the diagnostic test was compensated by the high specificity of 90.9%.

Interestingly, the cut-off for RBP4 is close to the median of the non-allergic asthma with obesity group; therefore, RBP4 has poor sensitivity (50%). Nevertheless, the cut-off point was substantially higher than the median of the obesity group without asthma; thus, it is specific enough (90.9%) to identify non-allergic asthma in an adolescent with obesity in clinical practice (Figures 1 and 3).

### Table 1

Demographic and baseline characteristics.

<table>
<thead>
<tr>
<th></th>
<th>Healthy (n = 35)</th>
<th>Allergic asthma without obesity (n = 28)</th>
<th>Obesity without asthma (n = 33)</th>
<th>Non-allergic asthma with obesity (n = 18)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>13.4 (12.7, 14.5)</td>
<td>14.0 (13.1, 14.5)</td>
<td>11 (11.0, 14.5) &amp; &amp; &amp; &amp;</td>
<td>13.0 (11.4, 15.6)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><strong>Male, n (%)</strong></td>
<td>19 (54.3%)</td>
<td>16 (57.1%)</td>
<td>19 (57.6%)</td>
<td>13 (72.2%)</td>
<td>0.6*</td>
</tr>
<tr>
<td><strong>Tanner stage II, III, IV, V (n)</strong></td>
<td>13, 7, 15, 0</td>
<td>4, 10, 11, 3</td>
<td>17, 8, 7, 1</td>
<td>4, 6, 7, 1</td>
<td>0.07*</td>
</tr>
<tr>
<td><strong>Weight, kg</strong></td>
<td>48.0 (42.8, 51.6)</td>
<td>49.1 (47.0, 52.2)</td>
<td>65.7 (57.3, 71.8) &amp; &amp; &amp; &amp;</td>
<td>69.2 (63.4, 79.9) &amp; &amp; &amp; &amp;</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Height, cm</strong></td>
<td>156.0 (153.0, 160.0)</td>
<td>156.0 (153.0, 163.0)</td>
<td>152.0 (145.0, 158.0)</td>
<td>159.0 (152.0, 169.0) ψ</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td><strong>BMI, kg/m²</strong></td>
<td>19.4 (17.2, 20.5)</td>
<td>20.0 (19.2, 20.8)</td>
<td>27.3 (25.5, 29.2) &amp; &amp; &amp; &amp;</td>
<td>27.8 (26.8, 30.7) &amp; &amp; &amp; &amp;</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>BMI percentile, %</strong></td>
<td>50.0 (25.0, 50.0)</td>
<td>50.0 (25.0, 50.0)</td>
<td>95.1(95.1, 96.4) &amp; &amp; &amp; &amp;</td>
<td>96.1 (95.1, 97.7) &amp; &amp; &amp; &amp;</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>BMI z-score</strong></td>
<td>0.08 (-0.5, 0.5)</td>
<td>0.36 (-0.6, 0.6)</td>
<td>1.9 (1.8, 2.0) &amp; &amp; &amp; &amp;</td>
<td>1.9 (1.8, 2.1) &amp; &amp; &amp; &amp;</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>RBP4, ng/mL</strong></td>
<td>12.7 (3.2, 19.9)</td>
<td>27.6 (3.8, 43.9)</td>
<td>23.5 (3.2, 33.5)</td>
<td>39.2 (23.8, 76.0) &amp; &amp; &amp;</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td><strong>PAI-1, ng/mL</strong></td>
<td>12.6 (10.0, 17.0)</td>
<td>16.7 (8.4, 22.3)</td>
<td>15.9 (9.4, 18.2)</td>
<td>21.9 (15.7, 26.5) &amp; &amp; &amp;</td>
<td>0.05</td>
</tr>
</tbody>
</table>

*Pearson’s chi-squared test.
Values are expressed as the median and 95% confidence intervals.
BMI: body mass index; RBP4: retinol-binding protein 4; PAI-1: plasminogen activator inhibitor-1.
& & p < 0.01 vs. healthy; & & & p < 0.001 vs. healthy; ### p < 0.001 vs. allergic asthma without obesity; ψ p < 0.05 vs. obesity without asthma; ψψ p < 0.01 vs. obesity without asthma.
RBP4 and PAI-1 as prognostic biomarkers of non-allergic asthma caused by obesity

Figure 1 Serum levels of RBP4 and PAI-1 in the four groups of adolescents. The data are presented as the median and interquartile range (IQR). The Kruskal-Wallis test with Wilcoxon signed-rank test post hoc analysis was performed to test for significant differences between the medians; the p-values are shown in the figure; blue dashed lines represent the cut-off value calculated for each adipokine according to Youden's index.

Figure 2 Comparison of the receiver operating characteristic (ROC) curves for RBP4 and PAI-1 between adolescents with obesity without asthma and adolescents with non-allergic asthma with obesity.

<table>
<thead>
<tr>
<th>Adipokine serum</th>
<th>Area under the ROC curve (95% CI)</th>
<th>Cut-off value (ng/mL)</th>
<th>Standard error</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBP4</td>
<td>0.741 (0.599–0.853)</td>
<td>&gt;42.78</td>
<td>0.072</td>
<td>0.001</td>
</tr>
<tr>
<td>PAI-1</td>
<td>0.699 (0.554–0.819)</td>
<td>&gt;12.0</td>
<td>0.074</td>
<td>0.008</td>
</tr>
</tbody>
</table>

RBP4: retinol-binding protein 4; PAI-1: plasminogen activator inhibitor-1.

When the cut-off value for PAI-1 was set to >12.0 ng/mL, the ROC curve had an AUC of 0.699 (95% CI: 0.554–0.819) (p = 0.008), indicating that PAI-1 had a nearly 70.0% probability of accurately discriminating whether an adolescent with obesity had the outcome of non-allergic asthma (Figure 2).

Figure 4 shows the test characteristics of PAI-1; the specificity of 45.5% was low, so there was a 54.5% probability of a false positive; however, the accuracy of the diagnostic test was compensated by a high sensitivity of 94.4%. In addition, the cut-off value was notably lower than the medians of both the non-allergic asthma with obesity group and the obesity without asthma group. Consequently, PAI-1 is very sensitive (94.4%) but has poor specificity (45.5%) (Figures 1 and 4).

The serum concentrations of RBP4 and PAI-1 were associated with non-allergic asthma with obesity. An obese patient with a serum RBP4 concentration >42.78 ng/mL was 10 times more likely to suffer from non-allergic asthma than a patient with a concentration ≤42.78 ng/mL [OR: 10.0 (95% CI: 2.2–45.0), p < 0.01]. In addition, an obese adolescent with a serum PAI-1 concentration >12 ng/mL had a 14-fold greater likelihood of having non-allergic asthma than an obese patient with a serum PAI-1 concentration ≤12.0 ng/mL [OR: 14.2 (95% CI: 1.7–119.2), p < 0.01].

There were no differences in RBP4 between males versus females (20.1 ng/mL [95% CI: 12.7–33.9] vs. 22.6 ng/mL...
Leija-Martínez JJ et al. [95% CI: 10.9–29.0], p = 0.6) or in PAI-1 between males versus females (16.7 ng/mL [95% CI: 12.8–18.4] vs. 15.1 ng/mL [95% CI: 11.9–18.2], p = 0.5). The sex, age, and Tanner stage showed no independent effects on the serum concentrations of RBP4 and PAI-1 in the four groups (Table 2).

Discussion

As shown in Table 1 and Figure 1, the findings of this study partially support our hypothesis. The non-allergic asthma with obesity group had higher serum RBP4 concentrations than the healthy and obesity without asthma groups, similarly Huang et al.,20 who found no significant difference in serum RBP4 between the non-allergic asthma with obesity and asthma without obesity groups.

Regarding serum PAI-1 concentrations, our hypothesis was partially supported: The non-allergic asthma with obesity group had higher PAI-1 concentrations than the healthy and obesity without asthma groups. Interestingly, the findings in the allergic asthma without obesity group do not support our hypothesis. These findings are different from those reported by Cho et al.,32 where patients with asthma and obesity had higher PAI-1 concentrations than patients with asthma without obesity. Notably, the characteristics of the patients in the study by Cho et al.32 are different from those in our study: they studied adult patients, and the asthma phenotype was different; their patients with asthma and obesity did not exclusively have the non-allergic form; and not all patients with asthma without obesity were exclusively allergic.

The phenotype of allergic asthma without obesity is not within the causal chain of non-allergic asthma caused

![Test characteristics of PAI-1](image)

**Test characteristics of PAI-1**

<table>
<thead>
<tr>
<th>Cut-off point &gt; 12.0 ng/mL</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>94.4%</td>
</tr>
<tr>
<td>Specitivity</td>
<td>45.5%</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>48.6%</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>93.8%</td>
</tr>
<tr>
<td>Positive likelihood ratio</td>
<td>1.7</td>
</tr>
<tr>
<td>Negative likelihood ratio</td>
<td>0.12</td>
</tr>
</tbody>
</table>

PAI-1: plasminogen activator inhibitor-1.

**Figure 4** Interactive dot diagram comparing the levels of PAI-1 between adolescents with obesity without asthma and adolescents with non-allergic asthma with obesity.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Multivariate linear regression model adjusted for the variables sex, age, and Tanner stage in the four groups of adolescents.</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBP4</td>
<td></td>
</tr>
<tr>
<td>n = 114</td>
<td></td>
</tr>
<tr>
<td>Adjusted R² = 0.16</td>
<td></td>
</tr>
<tr>
<td>β (95% CI)</td>
<td>p-Value</td>
</tr>
<tr>
<td>---------</td>
<td>---------</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>Reference</td>
</tr>
<tr>
<td>Male</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>-2.5 (-9.7, 4.6)</td>
</tr>
<tr>
<td>Tanner stage</td>
<td></td>
</tr>
<tr>
<td>Tanner II</td>
<td>Reference</td>
</tr>
<tr>
<td>Tanner III</td>
<td>-5.3 (-33.1, 22.5)</td>
</tr>
<tr>
<td>Tanner IV</td>
<td>-12.2 (-64.0, 39.7)</td>
</tr>
<tr>
<td>Tanner V</td>
<td></td>
</tr>
<tr>
<td>Groups</td>
<td></td>
</tr>
<tr>
<td>Non-allergic asthma with obesity</td>
<td>Reference</td>
</tr>
<tr>
<td>Healthy</td>
<td></td>
</tr>
<tr>
<td>Allergic asthma without obesity</td>
<td>-36.9 (-63.4, -10.3)</td>
</tr>
<tr>
<td>Obesity without asthma</td>
<td>-56.8 (-83.0, -30.6)</td>
</tr>
</tbody>
</table>

RBP4: retinol-binding protein 4; PAI-1: plasminogen activator inhibitor-1.
by obesity, but it was included to study how inflammatory adipokines behave in this group of patients. That is, the origin of allergic asthma is mainly a Th2 immune response with an elevation of IL-4, IL-5, and IL-13, resulting in elevated levels of specific immunoglobulin E; in contrast, in non-allergic asthma caused by obesity, the cause of asthma is chronic low-grade inflammation induced mainly by TNF-α, IL-18, IL-6, and IL-17A.9–11

A potential explanation for the lack of significant difference in PAI-1 between the classical allergic asthma group and the non-allergic asthma with obesity group is that PAI-1 biology may be important in both phenotypes. Cho et al.26,27,32 and Oh et al.29 reported that PAI-1 plays an important role in lung damage in patients with allergic asthma. PAI-1 may be essential in both asthma phenotypes, which could explain the lack of statistically significant differences.

The most important findings in our study are that patients with non-allergic asthma with obesity had higher serum RBP4 and PAI-1 levels than patients with obesity without asthma. This study demonstrated that patients with obesity who have higher concentrations of RBP4 and PAI-1 will have a greater risk of developing non-allergic asthma; thus, RBP4 and PAI-1 may be useful as prognostic biomarkers for the outcome of non-allergic asthma among adolescents with obesity. A possible explanation for this phenomenon is that individuals with non-allergic asthma caused by obesity have more M1 macrophages12 than obese subjects without asthma and therefore have greater RBP4, PAI-1, and cytokine production, which causes low-grade inflammation.16

This study demonstrated an association between the serum concentration of RBP4 and non-allergic asthma with obesity, where a cut-off value >42.78 ng/mL was taken as a risk factor for non-allergic asthma. Above this cut-off, an obese patient was 10 times more likely to develop non-allergic asthma compared to an obese patient with a concentration below the cut-off [OR: 10.0 (95% CI: 2.2–45.0), p ≤ 0.01]. Similarly, we also observed an association between the serum concentration of PAI-1 and non-allergic asthma with obesity, where a PAI-1 concentration >12.0 ng/mL was a risk factor for non-allergic asthma in the group of adolescents with obesity. According to our data, an obese patient with a concentration above this cut-off was 14 times more likely to suffer from non-allergic asthma than an obese patient with a concentration below the cut-off [OR: 14.2 (95% CI: 1.7–119.2), p ≤ 0.01]. The CIs for the ORs of both RBP4 and PAI-1 are very wide, possibly because the sample size was small and the numbers of patients with non-allergic asthma and obesity (n = 18) and patients with obesity without asthma (n = 33) were not balanced, which is an important limitation of our study.

Regarding the accuracy of a serum RBP4 >42.78 ng/mL as a diagnostic test to predict non-allergic asthma among adolescents with obesity, we can report that the AUC of 0.74 is considered acceptable, indicating that RBP4 can discriminate whether an adolescent with obesity will develop non-allergic asthma with 74% accuracy. As indicated in Figure 3, we had a 50% probability of a false negative; however, the accuracy of the test was compensated by the 9.1% probability of a false positive. The LR+ of 5.5 (95% CI: 1.7–17.8) is considered good and relevant for clinical practice. If the patient had a positive test (RBP4 > 42.78 ng/mL), they have 5.5 times the likelihood of obtaining a true-positive result compared to a false negative; however, the LR- of 0.55 (95% CI: 0.3–0.9) is considered bad and not relevant for clinical practice. Thus, with a negative test (RBP4 ≤ 42.78 ng/mL), there is a 0.55-fold greater likelihood of obtaining a false-negative result compared to a true negative, possibly because the sample number was small and the numbers of patients with the disease and without the disease were not balanced.

For the accuracy of a serum PAI-1 >12.0 ng/mL as a diagnostic test to predict non-allergic asthma among adolescents with obesity, the AUC was 0.69, which is considered fair, indicating that PAI-1 had nearly 70% accuracy in discriminating whether an adolescent with obesity would develop non-allergic asthma. The sensitivity of 94.4% is considered good, but the accuracy is diminished by the 55.5% probability of a false positive. The serum concentration of PAI-1 does not truly have clinical relevance, and the LR- of 1.7 (95% CI: 1.2–2.4) is bad. Additionally, the PPV is not sufficient to accurately predict non-allergic asthma among obese adolescents. These values indicate that we are not certain whether a positive test (PAI-1 > 12.0 ng/mL) is a true positive or a false positive. The low specificity may be explained by the small and unbalanced sample size in each group. The LR+ of 0.12 (95% CI: 0.01–0.8) is considered good. With a negative test (PAI-1 ≤ 12.0 ng/mL), there is a 0.1-fold greater likelihood of a false-negative result compared to a true negative.

Therefore, PAI-1 is unlikely to predict non-allergic asthma among obese adolescents in clinical practice. However, PAI-1 is an interesting molecule for this disease.27,29,32 Additionally, there is an association between PAI-1 and the phenotype of non-allergic asthma caused by obesity, so further research in basic and clinical immunology should be conducted. It is also important to highlight that the confounding variables such as age, sex, and Tanner stage did not influence the serum levels of RBP4 and PAI-1.

The study has some limitations. The sample size is small, and the groups of non-allergic asthma with obesity and obesity without asthma were not balanced. As a cross-sectional study, it cannot establish a causal association. Knowledge of the biological mechanisms of these adipokines specifically in non-allergic asthma caused by obesity is limited, and more studies in basic and clinical sciences are needed to better explain this phenomenon. In conclusion, associations exist between RBP4 and PAI-1 levels and non-allergic asthma caused by obesity, but it was included to study how inflammatory adipokines behave in this group of patients. That is, the origin of allergic asthma is mainly a Th2 immune response with an elevation of IL-4, IL-5, and IL-13, resulting in elevated levels of specific immunoglobulin E; in contrast, in non-allergic asthma caused by obesity, the cause of asthma is chronic low-grade inflammation induced mainly by TNF-α, IL-18, IL-6, and IL-17A.9–11

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**Authorship contributions**

All the authors conducted the literature search and worked together to construct the design and analysis. The authors accept responsibility for the content of this manuscript.

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**Conflicts of interest**

The authors report no conflicts of interest.

**References**


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