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Original Articles

CHRONIC LOW-GRADE SYSTEMIC INFLAMMATION IN CHILDREN WITH ASTHMA AND OBESITY

Niya A. Krasteva,
Boiko R. Shentov,
Adelaida L. Ruseva[1],
Chaika K. Petrova,
Simeon P. Petkov[1]

Department of Children `s Diseases, Medical University – Pleven, Bulgaria
[1]Department of Clinical Immunology, Allergology and Clinical Laboratory, Medical University – Pleven, Bulgaria

Summary

The rising incidence of bronchial asthma and obesity in children raises the question of whether there is a link between them. Chronic low-grade systemic inflammation could be one of the linking mechanisms. We aimed to determine the serum concentrations of high-sensitive C-reactive protein (hs-CRP), interleukin 6 (IL-6) and tumour necrosis factor α (TNF-α) in children with asthma and obesity and to seek a relationship between these inflammatory markers and asthma control. We investigated 88 children aged 6 to 17 years – 25 asthmatic obese children (AsOb), 25 asthmatic non-obese children (AsNOb), 19 obese non-asthmatic children (ObNAs), and 19 non-obese non-asthmatic children as controls. Serum levels of IL-6 and hs-CRP were significantly increased in asthmatic obese and ObNAs compared to AsNOb and the control group. Serum TNF-α concentration was similar in the four studied groups. There were no statistically significant differences in serum levels of these inflammatory markers between controlled and partially controlled/uncontrolled asthmatics (obese and non-obese). Knowing the possible mechanisms of interaction between bronchial asthma and obesity would contribute to a more effective therapeutic approach in these patients.

Key words: asthma, obesity, inflammation, markers, children

Introduction

Bronchial asthma is one of the most prevalent chronic conditions in children. In recent years, the prevalence of obesity among children has also increased. A link is suspected between asthma and obesity, which has not been fully elucidated yet and remains debatable. Various theories have been suggested, and many possible factors have been discussed: mechanical, genetic, immunological and inflammatory [1].

It is considered that there is a chronic low-grade systemic inflammation in obese people, which is characterized by increased circulating leukocytes and increased serum concentration of cytokines, cytokine receptors, chemokines and acute phase proteins. It is known that adipose tissue is not inert and has an endocrine function. Fatty tissue is composed of adipocytes, stromal preadipocytes, immune cells and endothelium, and responds rapidly and dynamically to excess food intake by hypertrophy and hyperplasia.

Corresponding Author:
Niya A. Krasteva
Department of Children`s Diseases,
Medical University – Pleven
1, St. Kl. Ohridski Str.
Pleven, 5800
Bulgaria
e-mail: niya_krasteva@abv.bg

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The hypertrophied adipocytes have insufficient vascularisation to provide adequate perfusion of the growing fat tissue, thus leading to tissue hypoxia, cellular apoptosis and activation of the macrophages infiltrated into the fatty tissue. Activated macrophages produce cytokines, which fall into the peripheral circulation and lead to chronic low-grade systemic inflammation [2]. This inflammation affects the cellular and molecular pathways of the immune system signalling. It is suspected that systemic inflammation could modulate airway inflammation, thus leading to more severe asthma and difficult control in obese individuals [3].

Interleukin 6 (IL-6) is a soluble mediator with a pleiotropic effect. It is a small-sized glycoprotein composed of 212 amino acids, including a 28 amino acid signal peptide. IL-6 is produced by macrophages, dendritic cells, mast cells, neutrophils, B cells and by some CD4 effector Th cells, non-leukocyte cells – adipocytes, endothelial cells, fibroblasts. It is involved in inflammation, immune response and hematopoiesis. Its role in the pathogenesis of bronchial asthma has been discussed [4].

Tumour necrosis factor α (TNF-α) is involved in the pathophysiology of many inflammatory lung diseases, including chronic bronchitis, chronic obstructive pulmonary disease, respiratory distress syndrome and bronchial asthma. TNF-α plays a role in the activation of NF-κB transcriptional nuclear factor. This, in turn, mediates the inflammatory response through activating metalloproteinases and neutrophil migration and induces the production of proinflammatory cytokines IL-1 and IL-6, which are involved in the differentiation and proliferation of T and B lymphocytes [5].

C-reactive protein (CRP) is a part of pentraxin family. It is a cyclic pentameric protein with a molecular weight of 120 kD, consisting of five identical non-covalently bound subunits. CRP is secreted in response to inflammatory agents and binds to the pathogen, activates complement and assists phagocytosis. CPR is synthesized by hepatocytes under the influence of IL-6 and TNF-α, but it could be also be produced by other cells - monocytes, lymphocytes, atherosclerotic plaques and respiratory tract epithelial cells [6]. There is data in the literature that high-sensitive C-reactive protein (hs-CRP) could be used as a biomarker for grading of childhood asthma concerning clinical classification, induced sputum cellularity, and spirometry as well as monitoring of response to inhaled corticosteroids [7].

This study aimed to determine the serum concentrations of hs-CRP, IL-6 and TNF-α in children with asthma and obesity and to seek a relation between these inflammatory markers and asthma control.

Materials and Methods

A total of 88 children aged 6 to 17 years admitted to the Pediatric Department of Georgi Stranski University Hospital, Pleven from January – September 2017 were studied. The participants were divided into 4 groups: 25 asthmatic obese children (AsOb), 25 asthmatic non-obese children (AsNOb), 19 obese non-asthmatic children (ObNAs) and 19 non-obese non-asthmatic children – controls (CTR). The university ethics committee approved the study. Informed consents and assents were obtained from the parents and the children over 14 years of age, respectively. Anthropometric measurements - height and weight were performed with standard equipment. Body mass index was calculated by the formula: BMI=weight (kg)/height (m^2). Obesity was defined as BMI>95th percentile according to international standards for children based on age and gender [8]. Abdominal obesity was defined by the Bulgarian age- and sex-specific waist circumference reference values [9]. Bronchial asthma was assessed by the degree of control according to the Global Initiative for Asthma (GINA) – controlled, partially controlled and uncontrolled [10]. The treatment of bronchial asthma with inhaled corticosteroids was equated to milligram equivalent fluticasone propionate. Children with acute or other chronic diseases were not included in the study.

Venous blood was taken from each participant and the serum concentrations of IL-6, TNF-α and hs-CRP were determined. IL-6 and TNF-α were tested with human ELISA assays. Hs-CRP was tested with a latex particle immunoturbidimetric assay for highly sensitive quantification of CRP in human serum or plasma.

The statistical processing of the data was carried out with statistical software packages Statgraphics and Excel for Windows. For the

comparison of mean values in normal or close to normal distribution of cases, single-factor variance analysis ANOVA was used with Tukey, Scheffe, Bonferroni post hoc tests. For the comparison of medians in a non-normal distribution of cases, Kruskal-Wallis H-test was used. The results are presented in tables and figures. The significance of the results and conclusions was considered at $p<0.05$.

Results

The mean age of all the participants in the study was 10.66±3.27 years. The girls were 43 (48.90%), and the boys were 45 (51.10%). The number of boys was significantly higher in the groups of asthmatic obese and non-obese children, as compared to the groups of ObNAs and CTR where the number of girls was more significant ($p<0.05$). The data about age, gender and body mass index for each group is presented in Table 1.

We did not, however, establish a correlation between gender and serum concentration of the studied markers. All the asthmatic children (obese and non-obese) were using low doses inhaled corticosteroids only or in combination with leukotrienes. The mean dose of inhaled corticosteroid was 160 micrograms fluticasone propionate in asthmatic obese children and 140 micrograms in AsNOb, respectively $p=0.304$.

After controlling for age and gender, we found a positive correlation between hs-CRP and waist circumference in the groups of asthmatic children (obese and non-obese) – $r=0.44$, $p=0.001$. A stronger positive correlation was also found between hs-CRP and waist circumference in the groups of non-asthmatic children (obese and non-obese) – $r=0.6$, $p=0.001$.

The highest levels of IL-6 were observed in the group of ObNAs – median 3.4 (interquartile range IQR 0.6-5.8) and asthmatic obese children – median 2.95 (IQR 1.3-5.9) but without significant difference between the two groups. Compared to them, the levels of IL-6 in the group of AsNOb – median 0.8 (IQR 0.3-1.2) and the control group – median 0.7 (IQR 0.2-3.6) were significantly lower (Kruskal Wallis Test 19.01, $p<0.001$). There was no difference in serum IL-6 levels between the last two groups (Figure 1).

Serum levels of TNF-α in the asthmatic obese children were median 2.2 (IQR 1.6-2.5), as well as in the AsNOb – median 2.2 (IQR 1.8-3.7). Serum levels of TNF-α were higher in the group of ObNAs – median 2.5 (IQR 1.7-3.2) and the control group – median 2.35 (IQR 1.8-4.1). Data analysis revealed no statistically significant differences ($p=0.716$) in serum TNF-α levels found in the four groups (Figure 2).

The highest levels of hs-CRP were observed

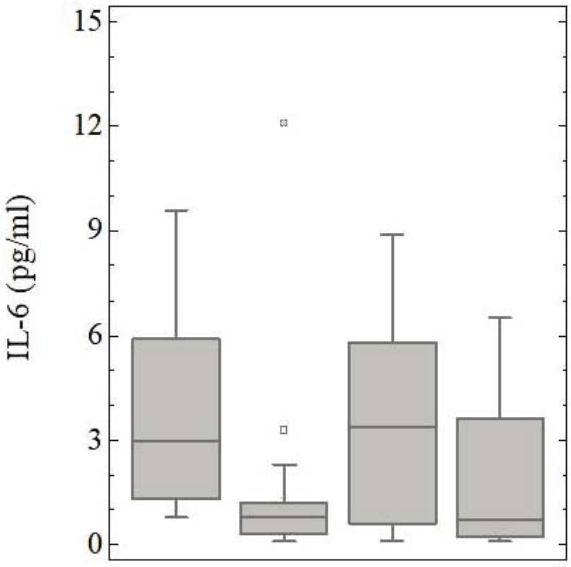

Figure 1. Serum IL-6 concentrations in the four groups

Table 1. Age, gender and BMI of the studied groups

Parameters	AsOb (n=25)	AsNOb (n=25)	ObNAs (n=19)	CTR (n=19)
Age in years (mean±SD)	9.91±2.74	9.32±2.66	12.07±3.36	11.99±3.70
BMI kg/m^2 (median, IQR)	25.24 (23.43-26.70)	16.48 (15.58-17.78)	29.71 (25.30-37.16)	17.30 (15.52-19.30)
Gender Girls/Boys (ratio)	8/17	9/16	14/5	12/7

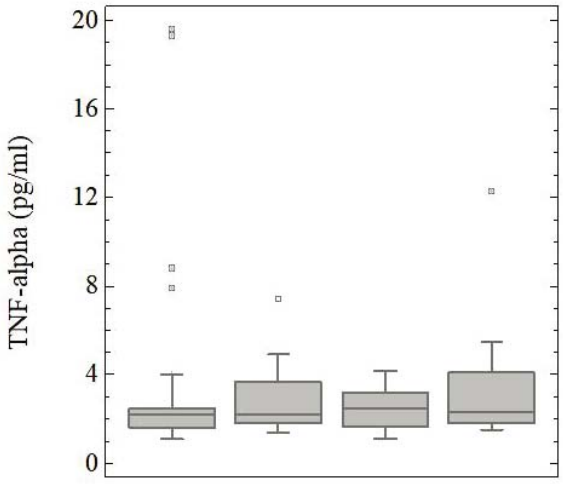

Figure 2. Serum concentrations of TNF-α in the four groups

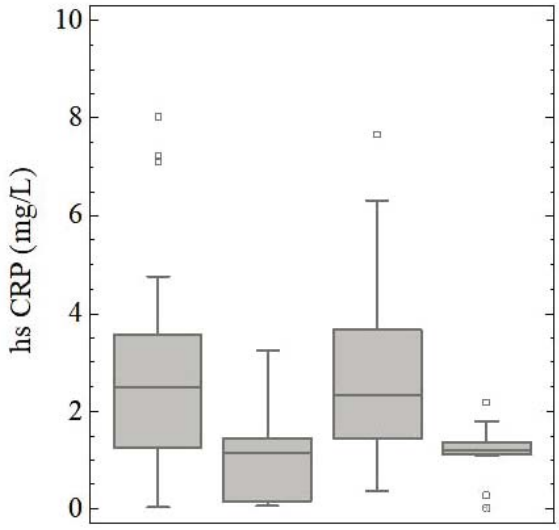

Figure 3. Serum concentrations of hs-CRP in the four groups

in the group of asthmatic obese children - median 2.48 (IQR 1.24-3.57). The levels of hs-CRP in the group of ObNAs – median 2.33 (IQR 1.44-3.66) were lower but not significantly different. Compared to them, the levels of hs-CRP in AsNOb (median 1.14, IQR 0.16-1.46) and the control group (median 1.20, IQR 1.10-1.38) were significantly lower (Kruskal Wallis Test 19.92, p<0.001). Comparison of the levels of hs-CRP between the last two groups did not reveal a difference (Figure 3).

We searched for a relationship between serum levels of IL-6, TNF-α, hs-CRP and asthma control. Serum levels of IL-6 in asthmatic obese children with controlled asthma did not differ significantly from those partially controlled/uncontrolled (p=0.783). The comparison of serum TNF-α levels also revealed no difference (p=0.948). Serum levels of hs-CRP in obese children with partially controlled/uncontrolled asthma were higher, as compared to children with good asthma control but the difference did not reach significance – p= 0.080 (Table 2).

When we compared the serum levels of IL-6, TNF-α and hs-CRP between the controlled and partially controlled/uncontrolled AsNOb, we did not find any significance (Table 3). Analysis of the results showed that there was no correlation between the control of asthma and systemic inflammatory markers.

Table 2. Serum levels of IL-6, TNF-α and hs-CRP in asthmatic obese children according to asthma control

Markers	Controlled (n=8)	Partially controlled /Uncontrolled (n=17)	P
IL-6 (pg/ml)	2.95 (1.25-4.70)	2.75 (1.30-7.20)	0.783
TNF-α (pg/ml)	2.20 (1.80-2.40)	2.00 (1.50-7.90)	0.948
Hs-CRP (mg/L)	1.28 (0.38-2.04)	2.60 (1.85-3.90)	0.080

Table 3. Serum levels of IL-6, TNF-α and hs-CRP in AsNOb according to asthma control

Markers	Controlled (n=10)	Partially controlled /Uncontrolled (n=15)	P
IL-6 (pg/ml)	0.90 (0.50-1.40)	0.80 (0.30-1.10)	0.358
TNF-α (pg/ml)	2.85 (2.00-4.10)	2.00 (1.70-3.30)	0.182
Hs-CRP (mg/L)	0.80 (0.09-1.46)	1.14 (0.26-1.49)	0.617

Discussion

Data about chronic systemic inflammation in asthma is insufficient and still controversial, especially for childhood asthma. According to some authors, there is a connection between chronic systemic inflammation and inflammation in the respiratory tract, and this is one of the connecting mechanisms between asthma and obesity.

In our study, the serum levels of IL-6 and hs-CRP were significantly increased in obese children (with and without asthma) compared to the groups of children with healthy body weight. There was no significant difference in serum IL-6 and hs-CRP levels in the asthmatic obese children, as compared to the AsNOb, as well as when the asthmatic obese ones were compared to AsNOb and the control group. Serum TNF-α concentrations were similar in the four studied groups. A significant positive correlation between hs-CRP and waist circumference was seen.

Increased serum levels of hs-CRP in obese children have been confirmed in other Bulgarian studies, as well as increased serum levels of IL-6 and TNF-α [11-13]. Galcheva et al. (2011) have also observed a positive correlation between waist circumference and hs-CRP but in prepubertal children [13]. Our results agree with those reported by Magrone et al. (2014) who also demonstrated elevated serum levels of IL-6 in obese children (with and without asthma), compared to the other two groups of normal-weight children (with and without asthma) and the serum levels of TNF-α alpha were not significantly different [14].

Confirmation of our results can also be found in the study by Zedan et al. (2015) [15]. These authors found a significant increase in IL-6 and TNF-α in obese children (asthmatics and non-asthmatics), but unlike us, they reported significantly increased hs-CRP in asthmatic obese children, compared to the other three groups. Similar results were reported from another study [16]. Contrary to our results, Jensen et al. (2013) [17] did not establish significant differences in serum levels of IL-6 and hs-CRP between the four groups. Results that differ from the ones we obtained are those obtained by Deraz et al. (2012) [7] who found significantly higher serum levels of hs-CRP in children with bronchial asthma versus a control group of healthy children. Besides, serum levels were significantly higher in the steroid naïve group (asthmatic children who have not been treated with inhaled corticosteroids), as compared to those who have used them. These authors believe that hs-CRP can be used as an indirect marker for monitoring airway inflammation. In another study, higher serum levels of hs-CRP in children with asthma than the control group were also found [18].

The differences between these results could be attributed to the criteria used in selecting the participants with asthma - according to the severity of the disease, the presence of exacerbation, the phenotype, the degree of control, and the therapy applied. According to some authors, chronic systemic inflammation in asthma could be affected by inhaled corticosteroids (ICS), and serum levels of hs-CRP in asthmatics who have received ICS are similar to those in healthy subjects [19, 20]. Also, serum hs-CRP concentration in asthmatics was found to be significantly higher among steroid naïve patients than in controls, but there was no difference when we compared asthmatics receiving ICS with the control group [21].

The asthmatic children we studied had no asthma attacks and were using ICS. This fact may be one of the reasons why we did not detect chronic inflammation in normal weight asthmatics. However, this inflammation was present in asthmatic obese children, although they had no attacks and were using ICS. This fact makes us conclude that chronic systemic inflammation in obese asthmatic children is mainly due to obesity, and not so much to the presence of asthma.

According to the study of Olafsdottir et al. (2005) [22], in patients with non-allergic asthma, serum levels of hs-CRP were significantly higher than in the control group of healthy subjects, but there was no difference between patients with allergic asthma and the control group. In another study, it is also stated that neutrophil asthma patients had higher levels of hs-CRP and IL-6 than those with non-neutrophil asthma and healthy subjects, as the last two groups had similar levels of these markers [23]. It is known that allergic asthma is the most common

phenotype in childhood. This could explain the lack of systemic inflammation in non-obese asthmatic children in our study.

We did not find an association between markers of chronic low-grade systemic inflammation and asthma control. These results are the same as those obtained by Sigari et al. (2013) [24], who did not establish a difference in serum concentrations of hs-CRP in groups of patients with different asthma control. However, some authors have found a correlation between markers of systemic inflammation and asthma control – children with poor asthma control had higher levels of hs-CRP, IL-6 and TNF-α, as compared to well-controlled asthmatics [25-27].

Conclusions

The widespread prevalence of bronchial asthma and obesity, as well as their rising incidence, determine the greater possibility of a combination of these conditions. Knowing some clinical features of bronchial asthma in obese children and possible mechanisms of interaction could contribute to more effective therapeutic approach in these patients. Chronic low-grade systemic inflammation is present in children with obesity, which may be a part of the linking mechanisms with asthma, but further investigations are needed to elucidate them.

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