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ORIGINAL ARTICLE

HIGH SENSITIVITY C REACTIVE PROTEIN AS A NOVEL MARKER FOR AIRWAY INFLAMMATION AND STEROID RESPONSIVENESS IN ASTHMATIC CHILDREN

By

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Introduction: ***Asthma is a chronic airway inflammatory disease and recently some reports has demonstrated systemic inflammation .High sensitivity C-reactive protein assays can detect minor inflammatory changes that could be missed by other indices of inflammation.***

Aim of study: ***The study was the first to evaluate serum hs-CRP of asthmatic children with different degrees of asthma severity and its relationship to pulmonary function FEV_1% of predicted for age and sex and airway inflammation.***

Methods: ***Serum high sensitivity C-reactive protein levels, sputum cytology study and FEV_1% of predicted for age and sex were estimated in 55 asthmatic children (20 steroid native controlled moderate asthmatics,18 ICS responsive controlled asthmatics and 17 ICS unresponsive uncontrolled asthmatics). Fifteen healthy children as a control group, who matched in age and sex with the other groups.***

Results: ***In the three groups of asthmatics serum hs-CRP concentrations were significantly higher than in controls. serum hs-CRP levels were significantly higher in patients with uncontrolled asthma than in the two groups with controlled disease. Hs-CRP correlated negatively with FEV_1% and positively with sputum eosinophil % in the three groups of asthmatics with very high significant correlation in group C . Hs-CRP correlated positively with neutrophil % in ICS treated asthmatics but it was highly significant in uncontrolled asthmatic group.***

Conclusion: ***Serum hs-CRP levels are significantly correlated to the airway inflammatory markers in asthmatic children. Hs-CRP may point to a novel marker for indirect detection and monitoring of airway inflammation, disease severity, and response to steroid treatment in asthmatic children.***

Key words: ***Childhood asthma, hs- CRP, airway inflammation.***

INTRODUCTION

Asthma is a chronic inflammatory airway disorder characterized by airway hyper responsiveness and inflammation, in which various cells as eosinophils, neutrophils, macrophages, T lymphocytes, cytokines and mediators play a role.(1) It has also been demonstrated that increased levels of amyloid A are present in patients with asymptomatic asthma.(2) C-reactive protein (CRP) is one of the most characteristic markers of the inflammatory process. The monitoring of CRP levels is a good diagnostic tool and is very useful in the assessment of early inflammation, as well as, in treatment monitoring and

efficacy in acute-phase diseases.[3] High sensitivity assays for CRP enabled the identification of CRP at levels that were previously undetectable using routine assays and can reflect even low-grade inflammation in several disorders, such as cardiovascular disease and diabetes mellitus.[4] The CRP is predominantly synthesized in the Liver in response to inflammation and tissue damage. Monocytes, lymphocytes and neutrophiles are also able to produce CRP.[5] CRP is regulated by pro-inflammatory cytokines, with a recognized important role in the pathophysiology of asthma, primarily the tumour necrosis factor-alpha, NF-kappa B , IL-6 and IL-1ß.[6]

Interleukein-6 has growth-regulatory effects on many cells and is involved in T-cell activation, growth, and differentiation. It also activates the gene responsible for CRP synthesis through phosphorylation of the transcription factor.[7] Although its function is still unclear, the CRP may serve as ageneral scavenger protein and play an important role to recognize bacteria and damaged human cells and to mediate their elimination through opsonisation, phagocytosis, and cell-mediated cytotoxicity. The CRP can also activates the classical complement cascade by binding directly to the complement fragment C1q.[8] A correlation of peripheral blood CRP levels with severity, extent, and progression of inflammatory pathologies has been well established. Recent publications suggested that hs-CRP has a contributing role in the pathogenesis of disease in addition to its being merely an inflammatory marker.[9] There are no studies to date that have identified the role of CRP in the pathogenesis of asthma. Increased levels of hs-CRP were found to be significantly associated with respiratory function impairment and bronchial hyper responsiveness (BHR)[10] suggesting that systemic inflammation may be associated with respiratory impairment.[2] Qian et al[11] suggested that hs-CRP might be a sensitive marker for severe asthma.

Aim of the work: The study was the first to evaluate serum hs-CRP of asthmatic children with different degrees of asthma severity with and without inhaled steroids and their relationship to pulmonary function (FEV_1%) and airway inflammation.

PATIENTS AND METHOD

Fifty five children of bronchial asthma were recruited from those attending the outpatient clinic and pediatric department in Zagazig University hospitals. This study was approved by the ethical committee of Faculty of Medicine, Zagazig University and a written consent was obtained from patients and controls. Patients were selected according to the global initiative for asthma.[12] The exclusion criteria were (1) Asthma patient who had complications of other lung diseases or a history suggesting systemic infection, tumors, autoimmune diseases or sinus disease, (2) Patients with recent respiratory tract infection or exacerbation of less than one month prior to study, (3) patients with heart diseases, diabetes mellitus, obesity with body mass index (BMI) > 30 kg / m2 and semi quantitative CRP > 10 mg / L.

Patients were divided into the following groups:

Group A: 20 moderate asthmatic children who never on inhaled steroids (native steroid).

Group B: 18 steroid responsive children with controlled – moderate to severe asthma (treated with inhaled corticosteroids (ICS)).

Group C: 17 steroid unresponsive children with uncontrolled moderate to severe asthma. (treated with inhaled corticosteroids (ICS)).

Group H: 15 healthy children as a negative control group

The steroid-native asthmatics have not been treated ever with ICS and were free from acute exacerbations and respiratory tract infections during the 3 months prior to the study .Patients with controlled moderate to severe asthma had been treated with low- to- medium doses of ICS (fluticasone propionate 250-500 ug / day)at a constant dose for at least 5 months.

According to GINA 2009, controlled asthma was defined as the need for rescue medications twice or less a week (short-acting b2-agonists), no limitation of day activity, no nocturnal symptoms, twice or less a week day time symptoms and FEV_1% > 80% predicted. There was no exacerbations, and no use of systemic steroids in the previous 12 months. The patients with uncontrolled asthma was defined as having three or more features of the following: the need for rescue medications more than twice a week, any limitation of day activity, any nocturnal symptoms , more than twice a week day time symptoms and FEV_1% < 80% predicted or had required one or more hospitalizations for asthma in the last year. They had been taking high-doses of ICS (fluticasone propionate >500-800 ug / day) and long acting b2-agonists for at least 6 month. Fifteen healthy volunteers were used as a negative control group. They were free of respiratory tract infection within 3 months prior to the study, and from either other significant illnesses known to affect sputum cell count or hs-CRP levels (which have been described previously).All of the patients and controls were examined, then underwent spirometry and sputum induction and blood samples were collected to determine serum hs-CRP.

Lung Function Tests: Short-acting bronchodilators were stopped at least 8 hr before the test. Dynamic spirometry was performed by means of a pneumotachograph (Masterlab Jaeger, Wu¨rzberg, Germany), with measurement of forced expiratory volume in 1 sec (FEV_1) ٪ predicted, according to the standards of the European

Respirator Society.[13] The highest values of FEV_1 of three forced expiratory maneuvers were used.

Sputum Induction and Processing: Sputum was collected either spontaneously or induced with hypertonic saline nebulization from all subjects. Prior to sputum induction, children inhaled 200 µg of salbutamol to minimize broncho-constriction during the induction procedure. Sputum was induced by inhalation of 3% hypertonic saline solution for 5 min (DeVilbiss 65 ultrasonic nebulizer; DeVilbiss, Somerset, PA, USA), and the subjects were encouraged to cough and expectorate sputum into sterile containers. FEV_1% was measured after nebulization. Nebulization was stopped if a fall in FEV_1% of >20% compared to baseline values occurred or if troublesome symptoms appeared.[14] The volume of the selected sputum is measured and 0.1% dithiothreitol (Sigma Chemicals, Poole, United Kingdom) added to the sputum in a 4:1 ratio to break up the disulphide bonds and disperse the cells. The cell suspension is aspirated until homogenized and filtered to remove any remaining debris. Phosphate-buffered saline is then added to the cell suspension. The non-squamous cell count and cell viability (with trypan blue) are determined in a haemocytometer. The cell suspension is centrifuged at 400 g for 10 minutes and cytospins made and stained by May-Griunwald Giemsa stain (Bio Optica Milano s.p.a). Four hundred non-squamous cells are counted: an adequate sample is defined as less than 50% squamous cells. The eosinophils and neutrophils count are then expressed as a percentage of the total cell count as it is more accurate than absolute count.[15]

Measurement of serum levels of hs-CRP:

Peripheral blood samples were collected from each patient. Venous blood samples were collected from each patients into plain tubes for serum samples. The samples were left for 30 minutes after collection, and the clotted blood samples were centrifuged at 2,000 to 3,000 x g for 15 minutes the blood samples were then centrifuged at 1700 g for 10 min, immediately aliquoted and frozen until use. Serum hs-CRP was assessed by particle enhanced immunonephelometric assay using BN-PROSPEC analyser (Siemen Medical Solution Diagnostic) with a detection level of 0.175 mg/L. It is a closed system analyzer with its specially manufactured kits from the same company.

Statistics: All obtained data were analyzed statistically by SPSS software version 14.Statistical significance was analysed by using analysis of variance (ANOVA). All values were expressed as range and mean ± SD (standard deviation) for numerical data or number and percent (%) for categorical data. p values of <0.05 were considered significant. The relationship between studied parameters was assayed by correlation. Pearson's linear correlation coefficient was used.

RESULTS

Characteristics of asthmatic patients and healthy controls are presented in Table 1. Serum levels of hs-CRP in all groups of asthmatics were significantly higher than those measured in controls (steroid-native moderate asthma: 2.42± 0.76 mg/l, $p < 0.001$; ICS-responsive controlled asthma: 2.4± 0.7, $p < 0.001$; ICS- unresponsive uncontrolled asthma: 3.73± 0.53, $p < 0.001$; controls: 0.31± 0.14) Table 1.

Serum hs-CRP levels were significantly increased in patients with ICS unresponsive uncontrolled asthma compared with ICS-responsive controlled asthma patients ($p < 0.001$) and steroid-native asthma patients ($p < 0.001$).

There was no significant difference as regard age, bronchial asthma duration and BMI in all studied groups.

There was statistical significant difference as regard mean value of FEV_1% in all asthmatic groups when compared to healthy controls ($p<0.001$) and there was statistical significant difference when group C compared to group A and B ($p<0.001$) Table 1.

Table 1 also shows range and means ± SD of sputum cell counts% among studied groups.

The mean value of sputum eosinophil % was higher with statistical significance among all studied asthmatic groups in comparison to healthy controls ($p<0.001$).

And this difference was highly significant in group A (steroid native) and group C(ICS unresponsive uncontrolled asthmatics) as compared with group B (ICS-responsive controlled asthmatics) ($p<0.001$).

The mean value of neutrophils% was significant ($P < 0.001$) in all asthmatic groups when compared to controls. The mean value of neutrophils was significant ($P < 0.001$) in group C (ICS-unresponsive uncontrolled asthmatics) when compared with group A (steroid native) and B (ICS-responsive controlled asthmatics). The mean value of neutrophils was significant ($P < 0.001$) in Group B when compared with group A.

The sputum percentage of lymphocytes was significantly increased in all asthmatic children in comparison to healthy control group ($P<0.001$). There was no significant difference between asthmatics as regard lymphocytes%.

The mean value of sputum macrophage % was significantly high among healthy control group when compared to all asthmatic groups ($p<0.001$). No significant difference as regard macrophage% in studied asthmatics.

There was statistical significant difference as regard

mean value of FEV_1% in all asthmatic groups when compared to healthy controls ($p<0.001$) and there was statistical significant difference when group C compared to group A and B ($p<0.001$).

There was no significant correlation of age, duration of bronchial asthma, BMI and hs-CRP among all studied patients.

Correlation of hs-CRP to sputum cell counts is shown in Table 2. It was found that there was very high significant positive correlation of hs-CRP to eosinophils % in group A, ($p<0.0001$) and high significant positive correlation to eosinophiles % in group B and C, ($p< 0.001$). There was also high significant positive correlation of hs-CRP to neutrophils % in group B, ($p<0.001$) and and very high significant positive correlation between neutrophils % and hs-CRP in group C ($p<0.0001$). There was very high significant negative correlation of hs-CRP to FEV_1% in all asthmatic groups ($p<0.0001$) Table 2.

Table 1. Demographic and clinical characteristics of controls and asthmatic children in different groups.

	Group A n(20) Mean ±SD Range	Group B n(18) Mean ±SD Rang	Group C n(17) Mean ±SD Range	Group H n(15) Mean ±SD Range	F	P
Age in Years	9.8± 1.4 (8-13)	10.1 ±1.6 (8-13)	10.4 ±1.5 (8-13)	10.2 ± 1.35 (8-13)	0.54	0.65†
Sex F/M	10 / 10	9 / 9	9 / 8	7 / 8		
Bronchial asthma duration (years)	2.43 ±0.75 (1.5-4.5)	2.69 ± 0.84 (1.5-4.5)	2.64 ± 0.8 (1.5-4.5)		0.61	0.54†
BMI Kg/m²	16.1 ± 1.5 (14-19)	16.3 ±1.2 (14-19)	16.5 ±1.4 (14-19)	16.2 ± 1.1 (14-19)	0.25	0.85†
Eosinophiles%	1.76±0.5[a,c] (0.8-3)	1.09± 0.33[a] (0.5-1.9)	1.78± 0.1[a,c] (0.8-2.9)	0.16±0.1 (0.01-0.4)	42.19[a]	<0.001[a]
Neutrophiles%	22.6 ±4[a] (17-30.5)	28.3±3.6[a,b] (22-35)	38.7± 7.7[a,b,c] (24-49.5)	17.7±6.6 (11.9-40)	42.3[a]	<0.001[a]
Lymphocytes%	1.39 ±0.5[a] (0.7-2.4)	1.29± 0.4[a] (0.8-2.1)	1.33± 0.6[a] (0.3-2.4)	0.33± 0.38 (0.0-1.1)	16.8[a]	<0.001[a]
Macrophages%	26.6± 3.8[a] (20-32.2)	26.56± 3.7[a] (20-32.5)	26± 3.02[a] (21.5-30.9)	48.1±4.6 (39-55)	126.16[a]	<0.001[a]
FEV1%	85.2± 5.7[a] (77.5-96.1) 82.7	80.7± 2.6[a] (76-85.5) 80.05	50.7± 3.8[a,b,c] (45.4-57.2) 50.2	97.8± 2 (94-101) 98.5	57.49[a]	<0.001[a]
Hs-CRP mg/l	2.42±0.76[a] (1-4) 2.4	2.4± 0.7[a] (1.1-4) 2.2	3.73±0.53[a,b,c] (2.2-4.5) 3.9	0.31±0.14 (0.2-0.6) 0.3	50.9[a]	<0.001[a]

[a] Values significantly different from control, $p<0.001$.
[b] Values significantly different from patients in group A, $p<0.001$.
c Values significantly different from patients in group B, $p<0.001$.
† Non significant.

Table 2. Correlation between hs-CRP and sputum cells% and FEV_1% in different asthmatics groups.

	Eosinophiles%		Neutrophiles%		Lymphocytes%		Macrophages%		FEV_1%	
	r	p	r	p	r	p	r	p	r	p
A	0.57	<0.001***	0.22	>0.05†	0.21	>0.05†	0.2	>0.05†	-0.84	<0.001***
B	0.53	<0.01**	0.52	<0.01**	0.37	>0.05†	0.01	>0.05†	-0.82	<0.001***
C	0.55	<0.01**	0.62	<0.001***	0.25	>0.05†	0.14	>0.05†	-0.83	<0.001***

*** very Highly Significant.
** Highly Significant.
† Non significant.

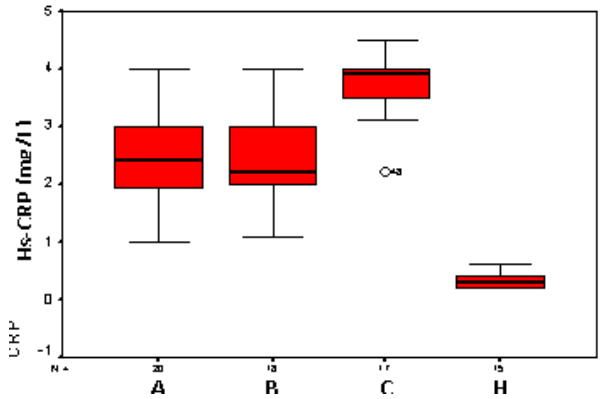

Fig 1. Serum concentration of hs-CRP in different groups.

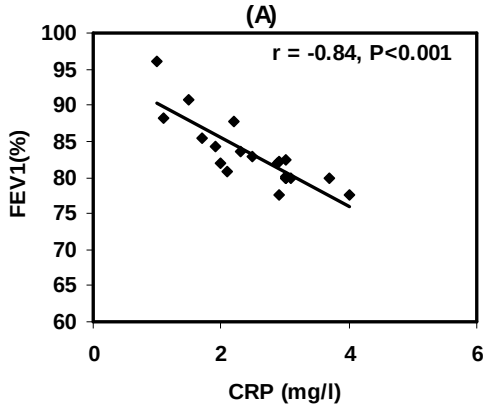

Fig 2. Correlation between FEV_1% and serum hs-CRP levels in group A.

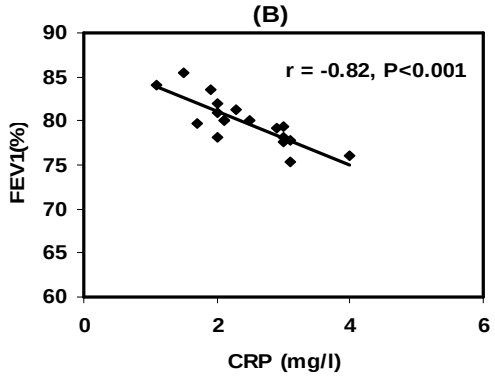

Fig 3. Correlation between FEV_1% and serum hs-CRP levels in group B.

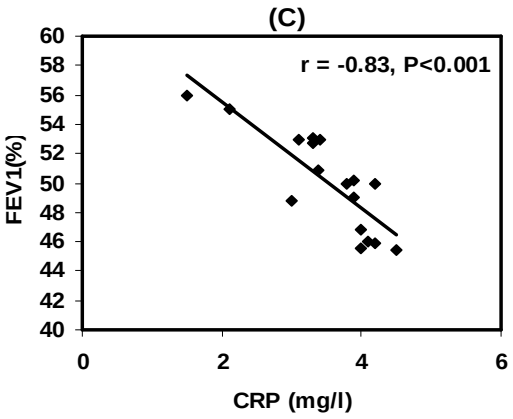

Fig 4. Correlation between FEV_1% and serum hs-CRP levels in group C.

(A)

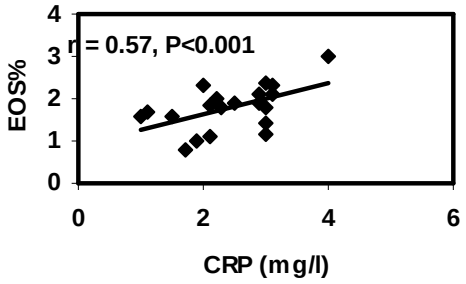

Fig 5. Correlation between eosinophiles% in sputum and hs-CRP in group A.

(B)

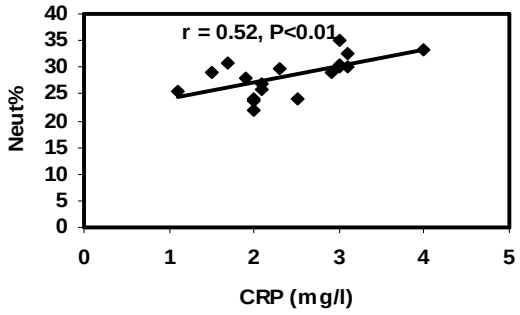

Fig 8. Correlation between neutrophiles% in sputum and hs-CRP in group B.

(B)

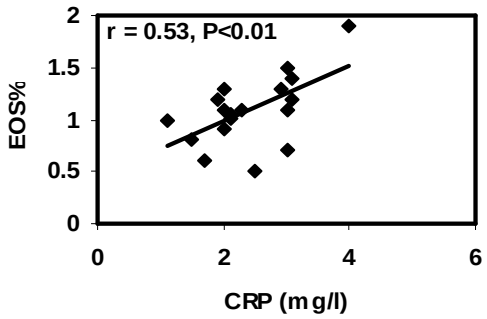

Fig 6. Correlation between eosinophiles% in sputum and hs-CRP in group B.

(C)

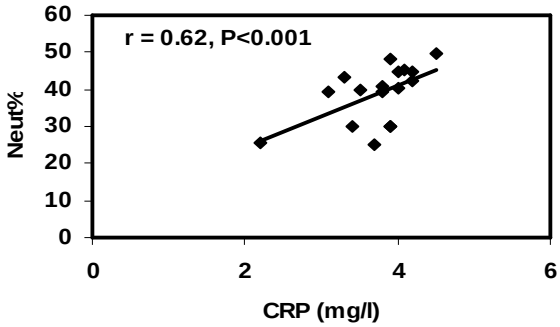

Fig 9. Correlation between neutrophiles% in sputum and hs-CRP in group C.

(C)

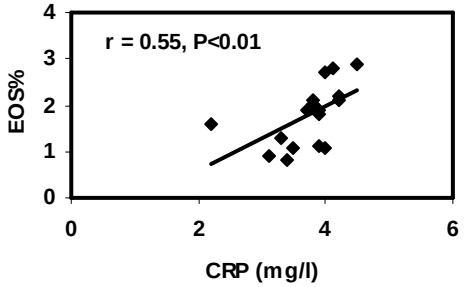

Fig 7. Correlation between eosinophiles% in sputum and hs-CRP in group C.

DISCUSSION

Hs-CRP with its high analytical sensitivity is one of the most characteristic markers of the inflammatory process and can be used to diagnose conditions with low grade inflammation in apparently healthy individuals.[16] Asthma is a chronic inflammatory disorder with not only local but also systemic inflammation exists and may play a role in its pathogenesis.[2] In the present study hs-CRP was significantly higher in all asthmatics compared to healthy controls and in uncontrolled asthmatics hs-CRP was significantly higher than other asthmatics. This was in agreement with a study by Navratil et,al[17] who found that in asthmatic children with uncontrolled asthma serum hs-CRP was increased compared to children with controlled asthma and controls. As asthma is characterized by variable degrees of airways inflammation

and interleukin (IL)-1, IL-6 and nuclear factor k- B, cytokines which regulate hs-CRP[6] had a role in airway inflammation.[18] So it is reasonable to consider that there is a direct association between inflammation severity and hs-CRP level. We used the kit for hs-CRP instead of wide-range CRP because the standard assays for CRP have a lower detection limit of 0.5 mg/L and thus lack the sensitivity required to determine low-grade systemic inflammation levels.

The present study also showed reciprocal correlation between hs-CRP and FEV_1%, where higher FEV_1% significantly correlated with lower hs-CRP levels. These results confirm those of earlier investigations in asthmatic adults who found that high hs-CRP levels were associated with respiratory symptoms[10] and BHR.[2] In a study in asthmatic children soferman et,al[19] has found a strong association between elevated blood hs-CRP levels and impaired respiratory function and he has also found hs-CRP level during exacerbation was significantly higher than the mean level during remission. These findings support the hypothesis that low grade of systemic inflammation present in asthmatic children as found in adults and in our work hs-CRP has been related to severity of asthma and state of asthma control. Several factors might affect serum levels of hs-CRP. The presence of cardiovascular disease, diabetes mellitus or obesity is associated with increased serum hs-CRP level which may be due to adipocyte-derived interleukin-6.[5] In our study, patients and controls were carefully selected to exclude these confounding factors.

The present study showed that there was a significant increase in sputum eosinophils %, neutrophils % and lymphocytes% among all asthmatic children compared to healthy controls, as the contribution of lymphocytes, neutrophils and eosinophils has been well established in the pathophysiology of asthma.[20]

In the present study macrophages% were significantly decreased in asthmatics compared to normal controls. This may be in favor of our patients as Macrophages may have a proasthmatic role by promoting eosinophilic airway inflammation and can be stimulated by allergen/IgEb immune complexes to produce proinflammatory cytokines. However some studies reported that mcrophages may be a negative regulator of established asthma due to the inhibitory effect of macrophages on Th2 by activating Th1. The difficulty in elucidating the role of lung macrophages in asthma may mainly be ascribed to the heterogeneity and plasticity of macrophages.[21]

In the present work, the increase of eosinophiles % in steroid native and ICS-unresponsive uncontrolled asthmatics was highly significant than ICS-responsive controlled asthmatics where steroid with its potent anti-inflammatory effect control eosinophilic inflammation in ICS-responsive controlled asthmatic group. This was in agreement with selected published studies where, up to 80% of corticosteroid-naive subjects and more than 50% of corticosteroid treated subjects[22] with concurrent symptoms had a sputum eosinophil count outside the normal range.

In our study there was significant positive correlation between sputum eosinophils % and hs-CRP in all asthmatic children. Allam et al.[23] found similar result; significant positive correlation between hs-CRP and sputum eosinophil % whether steroid treated patients or not.

The neutrophils % showed higher significant levels in ICS-unresponsive uncontrolled asthmatics (group C) than other groups. Inflammatory phenotypes of severe asthma can be characterised by persistence of eosinophilic or neutrophilic infiltration.[24] Neutrophilia may represent a continuous influx of cells from the bloodstream due to continuous antigenic stimulation of the bronchi in severe persistent asthma[25] or it may be influenced by the high levels of steroid treatment which delay neutrophilic apoptosis.[26] The same results were found by Gibson et al.[27] who used induced sputum to assess 56 patients with persistent asthma taking high doses of inhaled corticosteroids and found that 59% of patients showed evidence of neutrophilic inflammation.

There was also significant positive correlation between hs-CRP and neutrophils % in sputum in ICS treated asthmatics. This significance was very high in ICS-unresponsive uncontrolled group C. In addition to the very high significance correlation of hs-CRP to eosinophils % in the same group C,thus hs-CRP may be a marker of steroid responsiveness in asthmatic children especially those with neutrophilic or mixed inflammatory type with severe persistent symptoms despite high doses of steroid. Takemura et al[28] has found marginally positive correlation of hs-CRP and neutrophils among steroid native asthmatic patients. Our patients were classified according to state of asthma control, while Takemura et al classified his patients according to steroid therapy. Qian et,al[11] suggested that hs-CRP might be a sensitive marker for severe asthma.

Hence the association of eosinophils % and neutrophils % to hs-CRP indicates the relation of hs-CRP to airway inflammation in asthmatic children .Zietkowski et al.[29] has found a significant correlation between hs-CRP levels and exhaled nitric oxide (FENO) in the groups of patients with steroid native mild asthma and unstable severe asthma. (FENO) is a marker of airway inflammation not used in our study. From the previous data, the present study demonstrated that serum hs-CRP levels are associated with airway inflammation[30] and obstruction in pediatric population,[19] as had been demonstrated in

adult populations and may be a predictor of steroid unresponsiveness in a group of uncontrolled asthmatic children.

CONCOLUSION

We concluded that serum hs-CRP levels may be correlated to airway inflammation in children. These data support the possibility of using hs-CRP measurements to assess subclinical, systemic inflammation which make asthma control difficult and provide another useful diagnostic tool for indirectly detecting and monitoring airway inflammation, disease severity, and response to steroid treatment.

Further studies are warranted to confirm clinical significance of hs-CRP measurements, in diagnosis and in treatment of asthmatic patients and paving the way to enhanced individualized therapy.

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