

EVALUATION OF HIGH SENSITIVITY C REACTIVE PROTEIN LEVELS IN BRONCHIAL ASTHMA

Samarth Arya¹, Roopakala MS², Wilma Delphine Silvia CR³, Mohan Rao⁴, Chandrashekhar S⁵,
Prasanna Kumar KM⁶

Abstract

Background: High sensitivity C reactive protein (hsCRP) is an inflammatory marker known to be related to smoking, obesity and cardiovascular disease. **Aim:** To estimate and compare serum hsCRP levels in mild, moderate, and severe asthmatics and in normal subjects and to obtain a mathematical model describing the relationship between serum hsCRP levels and severity of asthma. **Materials and methods:** A stratified sample of 54 asthmatic patients within age group of 18-60 years and 15 healthy controls within 18-60 years were included in this study and classified according to GINA classification. Serum hsCRP levels were estimated by turbidometry method. **Results:** Mean hsCRP levels ranged from 1.5mg/L in normal subjects to 13.90 mg/L in severe asthmatics. **Conclusion:** Serum hsCRP levels were high in asthmatics as compared to normal subjects. On an average, the levels increased as the severity of asthma increased. However, the mean values of hsCRP showed no significant difference among mild, moderate & severe groups of asthma.

Author Affiliations: ¹Department of Orthopaedics, Manipal Hospital, #98, HAL Airport Road, Bangalore, ²Department of Physiology, MS Ramaiah Medical College, Bangalore, ³Department of Biochemistry, Akash Institute of Medical Sciences & Research Centre, Bangalore, ⁴Department of Chest Medicine, M.S. Ramaiah Medical College and Teaching Hospitals, Bangalore, ⁵Department of Immunology, ChanRe Rheumatology and Immunology Center, Basaweshwara Nagar, Bangalore, Karnataka, India, ⁶Consultant endocrinologist and Diabetologist, Bangalore Diabetes Hospital and CDEC, Bangalore-52, Karnataka, India.

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***Corresponding Author:** Dr. Roopakala MS, Professor & HOD Department of Physiology, MS Ramaiah Medical College, Bangalore, India-560054. Contact Number: 09845832910, E mail: widel2008@gmail.com

INTRODUCTION

Bronchial Asthma is a chronic, inflammatory lung disease characterized by breathing difficulties such as wheezing, coughing and shortness of breath. Between 100 and 150 million people around the globe suffer from asthma and this number is rising. India has an estimated 15-20 million asthmatics.^[1] In some patients, exposure to allergens such as animal dander, dust and mould spores can trigger an allergic reaction that can cause asthma signs and symptoms.^[2]

Although profound insights have been made into the pathophysiology of asthma, the exact mechanisms inducing and regulating the disease are still not fully understood since, there may be multiple factors causing the complexity. Researchers are still trying to understand the precise underlying mechanism that causes the disease in order to develop more effective treatment. Further, a new school of thought is that both allergens and viruses are capable of making a person with the right genetic predisposition to asthma. As a result, some people with allergies may not develop asthma, while others who develop asthma will not have allergies, because their disease is rooted in a virus, respiratory syncytial virus. Airway inflammation is a characteristic feature of bronchial asthma.^[3]

There is an increased local inflammatory activity in the airway mucosa of asthma patients. Acute phase reactants have been implicated for their involvement as proinflammatory molecules in various inflammatory diseases. However, little is known regarding their role in the allergic airway disease.^[4]

C reactive protein (CRP) is a highly sensitive marker of inflammation, infection, and tissue damage which contributes to host defense against infection by activating the complement pathways.^[5] The rise in CRP is driven by its rate of synthesis and falls rapidly when the pathological stimulus ceases with plasma half life

of approximately 19 hours.^{[6][4]} Highly sensitive assays for CRP (hsCRP) are available and reflect low-grade inflammation. CRP is produced by hepatocytes under transcriptional control by the proinflammatory cytokine interleukin (IL)-6, but adipose tissue has an active role in its metabolism by producing about 30% of IL-6.^[7]

MATERIALS & METHODS:

A cross sectional sample of urban adult population in Bangalore was considered. The study was conducted at MS Ramaiah Medical College & Teaching Hospital, Bangalore. A detailed explanation of the purpose of the study, the procedure adopted and the safety measures undertaken while sampling blood were clearly given to the asthma patients attending the out patient department of chest medicine of the hospital. 54 patients in the age group of 18-60 years, with an average age of 40 completed years and with male: female ratio of 31:23. with an acute attack of bronchial asthma, volunteered to participate in the project; 15 healthy volunteers within 18-60 years with a mean age of 38 completed years and with a male: Female ratio of 5:10, as control group were enrolled after taking an informed consent from them. Asthmatics who had taken bronchodilators within 24 hours prior to assessment, other allergic disorders, immunocompromised patients, chronic respiratory diseases other than asthma were excluded from the study. The study was approved by the Institution's Ethical Committee, and informed consent was obtained from every subject.

For each of the patient the FEV1 value and the hsCRP value were measured. The procedure for obtaining FEV1 value as follows: The pulmonary function test was done using computerized spirometry-spirobank G. the patients were first asked to sit comfortably and breath through the mouth with a pinchcork applied to the nose. A disposable mouth piece

was attached to the spirometer and was placed in the mouth in such a way that there were no gap around it. Then the patients were asked to breathe normally, and the reading in the spirometer were recorded. As a next step the subject was asked to take a deep breath and expire out forcefully into the spirometer, giving the FEV1 recording. At least three readings were taken from each subject and the best of three recordings were considered for the statistical analysis. Consequently, the sample was stratified into three groups, using severity of asthma as the stratifying factor. Severity of asthma was categorized as mild, moderate and severe based on GINA (Global Initiative for Asthma) guidelines.^[8]

After taking the necessary aseptic precautions from the median cubital vein, two ml of venous blood was collected from each patient using vacutainers. After collecting the blood samples they were left undisturbed for about half an hour for complete clot formation. The sample was then centrifuged to separate the serum from the clot. After centrifugation the serum was stored at -20°C in eppendorf tubes till the analysis was done.

Serum hsCRP levels were measured by turbidometry method. The principle of the test is serum C-reactive protein (CRP) causes agglutination of latex particles coated with anti-human C-reactive protein. The agglutination of the latex particles is proportional to the CRP concentration and can be measured by turbidometry. All subjects had their pulmonary function tests (PFTs) done before the blood sample collection.

Statistical analysis

The Statistical software namely SPSS 11.0, used for the analysis of the data. Microsoft word and Excel have been used to generate graphs, tables etc. Student t test has been used to find the significance of hsCRP between controls and cases. P-value less than 0.05 was considered as statistically significant.

RESULTS AND DISCUSSION:

Asthma is a triad of intermittent airway obstruction, bronchial smooth muscle cell hyper reactivity to bronchoconstrictors, and chronic bronchial inflammation. From an etiological standpoint, asthma is a heterogeneous disease, but often appears as a form of immediate hyper reactivity with airway inflammation. Chemokines, which are produced by many cell types from inflamed lungs, play a major role in recruiting the mediators of asthmatic inflammation^[9] Previous studies have reported airway epithelial cells (AEC) may play a crucial role in processes of airway remodeling found in chronic airway inflammatory diseases and suggest that AEC are actively involved as regulators of airway inflammatory responses playing an important role in the pathogenesis of airway disorders.^[10]

All such reports lead to the thought that inflammatory response in asthma is limited to lungs and airways.

The present study consisted of 54 asthma patients with varying degree of severity and 15 healthy controls to assess and compare the levels of hsCRP. We have estimated serum hsCRP levels in bronchial asthma patients and compared with normal subjects to study the role of systemic inflammation in pathogenesis of asthma. It is found that hsCRP is significantly elevated in asthma patients suggesting that systemic inflammation may play a role in bronchial asthma. However the mean values of hsCRP showed no significant difference among mild, moderate & severe groups of asthma. Similar finding has been reported by Olafsdottir who observed no significant relationship between hsCRP and allergic asthma or bronchial responsiveness.^[6]

Table 1: Comparison of serum levels of hsCRP in healthy controls and asthma patients.

Study parameter	Normal Mean \pm SD	Cases Mean \pm SD	P value	Effect size (d)(95% CI)
hsCRP	2.63 \pm 1.99 (1.50-9.40)	10.52 \pm 2.19 (4.00-13.90)	<0.001**	3.63 (2.80-4.46)

P value is obtained by student t test and Effect size (d) is computed using the Cohen and its 95% confidence Interval.

Table 2: Serum levels of hsCRP with the severity of Asthma.

Severity of Asthma	hsCRP levels	
	Min-Max	Mean \pm SD
Normal	1.50-9.40	2.63 \pm 1.99
Mild	4.00-13.90	9.98 \pm 2.67
Moderate	6.20-13.00	10.30 \pm 2.14
Severity	8.80-13.90	11.20 \pm 1.52
Significance	F= 54.722,<0.001**	
Pair wise significance	Normal vs Mild <0.001** Normal vs Moderate <0.001** Normal vs Severity <0.001** Mild vs Moderate P=0.976 Mild vs Severity P=0.288 Moderate vs severity p=0.622	

P values obtained by ANOVA and Post-hoc test Tukey for pair wise significance.

Buyukozturk S examined the blood concentrations of three acute-phase proteins namely C-reactive protein (CRP), serum amyloid A(SAA) and fibrinogen in patients with asthma and found mean CRP and fibrinogen values in the asthma groups were not significantly different when compared to the control group. However, the mean SAA levels were found to be significantly high. Estimating SAA & fibrinogen may throw more light on the pathogenesis of

asthma. IL-6, a Th2 cytokine is also proved to be a marker of systemic inflammation. [4] Serum IL-6 has also been estimated in these subjects of the present study. Statistical analysis has revealed no significant increase in serum IL-6 in these groups of asthma patients (unpublished data). Therefore the present study suggests that inflammation alone is not responsible for severity of asthma, as serum hsCRP levels showed no significant change with severity of asthma. The other possibility is inflammation is not solely responsible for the raise in serum hsCRP levels because the levels do not match to the severity of the disease.

CONCLUSION

We conclude that systemic inflammation dose play a role in pathogenesis of bronchial asthma and the Inflammation dose not reflect to the severity of the disease because the levels of hsCRP do not match to the severity of the disease. Further studies on the possible role of elevated hsCRP and other cardiovascular risk factors in the pathogenesis of asthma could lead to a recognition of new biomarkers, other mechanisms which we have not corrected for, or even new therapeutic possibilities. hsCRP might in the near future be used as a risk factor marker for lung diseases.

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CONFLICT OF INTEREST:

Authors have no conflict of interest to declare

REFERENCES

1. <http://www.who.int/mediacentre/factsheets/> accessed on 19/05/2014

2. <http://www.aaaai.org/allergist/allergies/types/dust-allergy> accessed on 19/05/2014
3. Reckess GZ. What causes Asthma? Beyond allergens, other culprits—viruses—also may pose a risk. <http://mpaweb1.wustl.edu/> accessed on 19/05/2015
4. Büyüköztürk S, Gelincik AA, Genç S, et al., Acute phase reactants in allergic airway disease. *Tohoku J Exp Med.*2004; 204(3): 209-13.
5. AL Obaidi AHA, Al Samarai AGM, Jawad AKY, et al. Association Between C Reactive Protein and Asthma. *Turkish Thoracic Journal*2010; 11 (3): 098-104
6. Olafsdottir IS, Gislason T, Thjodleifsson B, et al., C reactive protein levels are increased in non-allergic but not allergic asthma: a multicentre epidemiological study. *Thorax* 2005; 60: 451–454
7. Shelbaya S, Amer H, Seddik S, et al., Study of the role of Interleukin-6 and highly sensitive C-reactive protein in diabetic nephropathy in type 1 diabetic patients. *European Review for Medical and Pharmacological Sciences* 2012; 16: 176-182
8. Bethesda. Global strategy for asthma management and prevention. National Institutes of Health. NHBLI Workshop Report 2002; 2: 3659.
9. Renauld JC, New insights into the role of cytokines in asthma. *J Clin Pathol.* 2001; 54(8): 577-589
10. Takizawa H. Airway epithelial cells as regulators of airway inflammation (review) *Intl J Mol Med* 1998 ; 1(2): 367 -378