

ORIGINAL ARTICLE

Iran J Allergy Asthma Immunol
September 2013; 12(3):247-253.

The Relationship between Serum YKL-40 Levels and Severity of Asthma

Serap Duru¹, Gülbahar Yüce², Sevinc Sarinc Ulasli³, Melike Erdem¹, Murat Kizilgün⁴, Fatma Kara⁴, and Sadık Ardıc¹

¹ *Dışkapı Yıldırım Beyazıt Education and Research Hospital, Chest Diseases Clinic, Ankara, Turkey*

² *Etlik İhtisas Education and Research Hospital, Chest Diseases Clinic, Ankara, Turkey*

³ *Afyon Kocatepe University, School of Medicine, Department of Pulmonary Diseases, Afyon, Turkey*

⁴ *Ankara Pediatrics Hematology and Oncology Hospital, Department of Biochemistry, Ankara, Turkey*

Received: 28 June 2012; Received in revised form: 10 October 2012; Accepted: 28 October 2012

ABSTRACT

YKL-40 (chitinase-3-like-1) has been introduced as a marker of inflammation in asthma. The aim of this study was to determine the role of YKL-40 in asthma and to evaluate the relationship between YKL-40 and asthma severity.

In the study, 60 non-smoker asthma patients without additional diseases (aged between 20-60 years, female: 34) were grouped [Group I: Well controlled asthma patients (n: 30), Group II: Patients during acute exacerbation of asthma (n: 30)]. Healthy non-smoker female individuals were included in Group III (n: 30) as a control group. The level of serum YKL-40 of all groups were determined by ELISA. Also, serum YKL-40 level was correlated with age, asthma duration in years, body mass index (BMI), forced expiratory volume in first second/ forced vital capacity (FEV₁/FVC, %), FEV₁ (%), and total IgE levels of asthma patients.

Mean serum YKL-40 level was highest in patients during acute exacerbation of asthma (36.36±10.49 ng/ml) while mean serum YKL-40 level was the lowest (13.20±5.60 ng/ml) in the control group. There was a negative significant correlation between the serum YKL-40 levels and FEV₁ (%) in patients during acute exacerbation of asthma. There were no significant correlations between the serum YKL-40 levels and other variables in group II.

We found that increased serum YKL-40 levels may be used as a marker for evaluation of asthma severity and genetic polymorphism.

Keywords: Asthma; Marker; YKL-40

INTRODUCTION

Asthma is a chronic inflammatory lung disease characterized by recurrent airway obstruction.¹

Nowadays, exact pathogenesis of asthma is still

unknown. As a result of increasing exposure to environmental allergens, immune response plays an important role in the pathogenesis as well as genetic predisposition. In addition to inflammatory response in airways, structural changes as remodelling also occur.^{2,3} Subepithelial fibrosis develops due to accumulation of collagen fibers and proteoglycans under basal membrane in asthma.^{4,5} These changes may

Corresponding Author: Serap DURU, MD;
Diskapı Yıldırım Beyazıt Training and Education Hospital, Ankara,
Turkey. Tel: (+90 31)2596 2776, E-mail: akcalis@hotmail.com

affect asthma severity, and end up in relative irreversible obstruction of airways. Conventional follow up methods may not be reliable in disease control. Some patients can not evaluate their symptoms, and also some patients can not perform convenient peak expiratory flow (PEF) monitoring. In recent years, monitoring of inflammation in asthma is believed to have contribution to disease follow up and treatment.

YKL-40 (Human cartilage gp-39, chitinase 3-like 1 protein) is a glycopeptide, produced and secreted from chondrocytes, osteoblasts, macrophages, neutrophils, epithelial cells and smooth muscle cells.⁶ The chitinase-like protein YKL-40 lacks chitinase activity but binds to ubiquitously expressed chitin and has been implicated in inflammation and tissue remodeling.⁷ Exact physiological functions of YKL-40 are unknown and YKL-40 functions as a growth factor for synovial fibroblasts. YKL-40 has angiogenic role for vascular endothelial cells and is involved in inflammation and remodelling. Previous studies have demonstrated that the expression level of YKL-40 increased in T-helper cell (Th) type 2 inflammation.⁸

YKL-40 induced by the proinflammatory cytokines tumor necrosis factor- α and interleukin (IL)-1 as well as by interleukin-13 is a potential key regulator of asthma.⁹ In asthma, chitinases like YKL-40 are secreted from macrophages and airway epithelial cells with IL-13 related mechanism. Th-2 associated inflammatory response due to allergen exposure, which results in airway hyperresponsiveness and smooth muscle contraction, plays role in tissue remodeling.^{7,10,11} Increased serum YKL-40 levels were demonstrated in severe asthmatic patients in a study.¹² Serum YKL-40 levels also increase in exacerbation period of asthma.¹³ In this study, the serum YKL-40 levels of stable asthmatic patients, as classified according to criteria of the Global Initiative for Asthma (GINA) 2011, were measured and compared with each other and the control group.

In recent years, YKL-40 expression in some tumors and also patients with active arthritis¹⁴ has been demonstrated.¹⁵⁻¹⁷ Further more, it is accepted as a biomarker in inflammatory bowel diseases and fibrosis marker in chronic liver diseases.¹⁸

The aim of this study was to examine a possible relationship between the serum YKL-40 levels and asthma severity.

MATERIALS AND METHODS

Patients

The study included patients with asthma diagnosis according to the guidelines.¹ Sixty nonsmoker asthmatic patients (without additional diseases aged between 20 to 60 years, (female/male ratio: 43/47) were followed up between June 2010 and December 2011 in Diskapi Yildirim Beyazit Education and Research Hospital Pulmonary Diseases Clinic and Etlik Ihtisas Training and Research Hospital, Ankara. Demographic data and the smoking history of patients were recorded. Our exclusion criteria were other infections within the last month, malignancy, other chronic diseases. The body mass index (BMI: kg/m²) of each patient was calculated. Chest radiographs were evaluated to exclude other pathologies. The patients also underwent physical examinations. One or more positive results to allergens in skin prick test (Allergopharma, Germany) was accepted as atopy. According to previous skin prick test results of asthma patients regularly followed in our clinics, 10 well controlled asthma patients and 11 asthma patients during exacerbation had atopy.

Our patients were divided into two groups according to the GINA criteria as follows: Group I: well controlled asthma patients (n:30, female/male:12/18), Group II: Asthmatic patients during acute exacerbation (n: 30, female/male:16/14). Thirty healthy non-smoker females with no chronic diseases were included as control group (Group III, n: 30, female/male:15/15).

Fourteen of well controlled asthmatic patients were categorized as mild persistent, 16 of them as moderate persistent according to GINA before treatment. The treatment protocols of asthmatic patients were arranged according to GINA criteria.¹ Mild persistent asthmatic patients received inhaled short acting β_2 agonists as needed, moderate persistent asthmatic patients received fluticasone (250-500 μ g/day) or generic and long acting β_2 agonists. Acute exacerbation was defined as symptoms like dyspnea, cough, wheezing, shortness of breath or chest tightness together with decrease in PEF and forced expiratory volume in first second (FEV₁) values according to GINA in last month. During acute exacerbation period, patients received inhaled short acting β_2 agonists, 1-4puffs/hour [inhaler salbutamol 100 mcg or nebulized 0.15 mg/kg (2.5 mg)/20 minute (x3)], systemic corticosteroids (0.5/2 mg/kg/day prednisolone), and/or oxygen therapy.

Laboratory

Fasting serum samples were taken in the morning from all patients with asthma and healthy individuals 6-12 hours after physical examination. Blood samples from patients during acute exacerbation of asthma were obtained before initiation of exacerbation therapy. Serum C-reactive protein (CRP) level (0-6 mg/l, Advia 2400, Latex enhanced immunoturbidimetric, Siemens Healthcare Diagnostics Inc., USA), total serum IgE (1-83 IU/ml, Immulite 2000XPi, chemiluminescent immunometric assay, Siemens Healthcare Diagnostics Inc., USA) levels were assessed using standard laboratory methods. Serum samples for YKL-40 (ng/ml) were centrifuged for 10 minutes at 3000 rpm and stored at -70°C . Human YKL-40 enzyme-linked immunosorbent assay (ELISA) kit (Adipobiotec, 20110516, Santa Clara, USA) was used for the serum YKL-40 level measurement.

All patients performed respiratory function tests at the respiration laboratory of our clinic using a Jaeger spirometer according to American Thoracic Society (ATS) guidelines. FEV1(%), forced vital capacity (FVC, %) were measured and the values were recorded.¹⁹ The study was planned in accordance with the suggestions of the Helsinki Document and Diskapi Yildirim Beyazit Training and Research Hospital's ethic commission. Signed consent forms were obtained from all subjects.

Statistical Analysis

SPSS 15.0 (SPSS Inc; Chicago, III) package program was used for statistical analysis of the data. As the measure of the average, mean \pm SD, or, when normal distribution was not the case, median (min-max) was used. Least Square Deviation (LSD) was performed to determine the group that was different. Dunnett T3 test was performed to determine the group that was different. Different groups were determined by Mann-Whitney U test and Bonferroni correction. In order to correlation between the variables, Pearson correlation coefficient and Spearman correlation coefficient was used.

RESULTS

Demographic and laboratory data for the 60 patients and control group monitored at our clinic were demonstrated in Table 1. The mean serum YKL-40 level of the control group was found to be 13.20 \pm 5.60 ng/ml. Mean serum YKL-40 level of well controlled asthmatic patients was 19.84 \pm 6.19 ng/ml while the mean serum

YKL-40 level of the patients during acute exacerbation was found to be 36.36 \pm 10.49 ng/ml. There was a significant difference between the serum YKL-40 levels of well controlled asthma patients and the acute exacerbation group ($p<0.0001$). Figure 1 shows the box plot graphs of the serum YKL-40 level in asthmatic patients and the control group.

Relationships between the serum YKL-40 levels and other variables were demonstrated in table 2. Although patients with well controlled asthma had more duration of asthma in years, no significant correlation was found between other parameters with the serum YKL-40 levels ($p>0.05$) of well controlled asthmatic patients. FEV1 values and the serum YKL-40 levels of asthma patients during acute exacerbation period showed significant negative correlation ($r=-0.421$, $p=0.02$). Figure 2 shows the box plot graphs of the relationship the serum YKL-40 level and FEV1 (%) in asthmatic patients during acute exacerbation period. No significant correlations between age, asthma duration in years, BMI, FEV1/FVC (%), serum total IgE levels and serum YKL-40 levels were found in all groups ($p>0.05$).

DISCUSSION

In this study, we found that the serum YKL-40 level was higher in the acute exacerbation group than the well controlled asthmatic patients and the control group. Moreover, in the acute exacerbation group, significant negative correlation was found between FEV1 (%) and the serum YKL-40 levels. In recent years, the serum YKL-40 levels in inflammation, endothelial dysfunction, angiogenesis and remodeling have been reported to increase.¹⁰

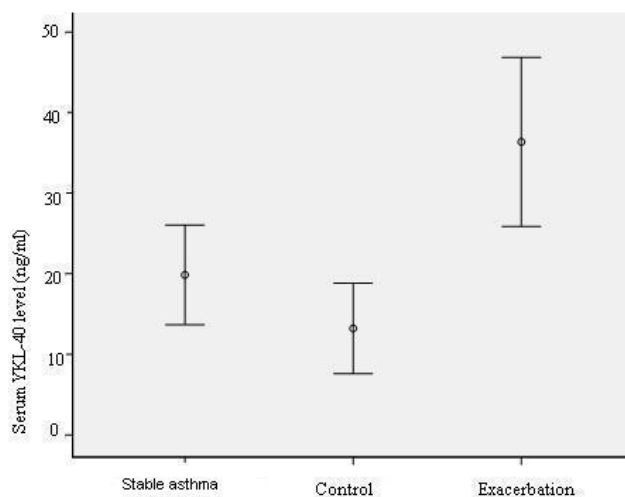
In studies with chronic diseases such as diabetes,^{20, 21} neurological disease,²² malignancies,²³ YKL-40 may be used as a noninvasive prognostic marker.

There has been limited number of studies related with YKL-40 in lung diseases. In a study by Johansen *et al*,²⁴ the serum YKL-40 levels were increased and YKL-40 levels as a biomarker for disease activity and fibrosis were suggested in patients with sarcoidosis. YKL-40 may play a role in differentiation and proliferation of cancer cells and YKL-40 has been accepted as a poor prognostic biomarker in patients with lung carcinoma.^{25, 26} In another study, asthma severity, the thickness of the subepithelial basal membrane, and pulmonary function tests were correlated with the serum YKL-40 levels.¹²

Table 1. Demographic and laboratory data of asthma and control groups.

Groups Variables	Group I Patients with well controlled asthma (n:30)	Group II Patients during acute exacerbation of asthma (n:30)	Group III Control group(n: 30)	P value
Age (years)	38.80±11.31 (20-55)	39.06±11.57 (27-60)	38.10±10.98 (23-60)	0.421
Asthma in years	13.76±5.06	11.03±5.62	-	0.067
BMI (kg/m ²)	24.19±4.64	25.19±4.09	25.34±3.70	0.234
FEV1/FVC(%)	76.34±12.08	51.17±11.67	95.45±7.65	<0.0001
FEV1(%)	74.70±8.61	44.76±6.24	96.86±3.73	<0.0001
Total IgE (1-83 IU/ml)	156.43±103.95	155.91±79.03	49.60±68.39	<0.0001
CRP (0-6 mg/l)	5.77±1.31	6.06±1.35	3.82±1.34	0.245
YKL-40 (ng/ml)	19.84±6.19	36.36±10.49	13.20±5.60	<0.0001

BMI: Body mass index, FEV1/FVC: Forced expiratory volume in 1 second/ Forced vital capacity, CRP: C-reactive protein.

**Figure 1. Serum YKL-40 level in asthmatic patients and control group.****Table 2. The relationship between variables and serum YKL- 40 level in asthmatic patients.**

Variables	Patients with well controlled asthma (n:30)		Patients during acute exacerbation of asthma (n:30)	
	r	P	r	P
Age (years)	0.335	0.070	-0.158	0.404
Asthma duration in years	0.100	0.600	-0.314	0.091
BMI (kg/m ²)	0.067	0.724	0.160	0.400
FEV1/FVC(%)	0.319	0.086	0.103	0.588
FEV1(%)	0.193	0.307	-0.421*	0.020
Total IgE (1-83 IU/ml)	0.174	0.357	0.099	0.601

BMI: Body mass index, FEV1: Forced expiratory volume in 1 second, FVC: Forced vital capacity.

YKL-40 and Asthma

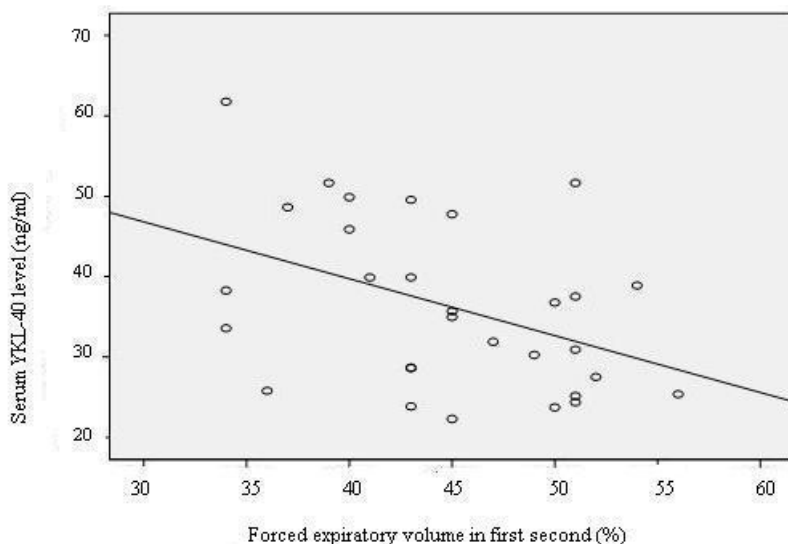


Figure 2. The relationship between serum YKL-40 level and forced expiratory volume in 1 second (FEV1, %).

In a recent study, serum YKL-40 level has been significantly higher in asthmatic patients than control subjects, however no significant correlation were found between the serum YKL-40 level with asthma severity and total serum IgE level.²⁷ In our study, when severity of asthma increased, the serum YKL-40 level also increased especially in the acute exacerbation group. This relationship can suggest that the serum YKL-40 level can be used as a biomarker for monitoring of inflammation. Recently Otsuka *et al.*⁹ reported in patients with asthma, sputum YKL-40 levels were positively correlated with disease severity and sputum neutrophil counts and were negatively correlated with measures of pulmonary function test (with pre- and postbronchodilator %FEV1). These findings showed that elevated sputum YKL-40 levels were reflecting airflow obstruction in asthma. In a study, total serum IgE levels of asthmatic patients in the acute exacerbation period were found higher than the well controlled asthma and the control groups and significantly correlated with the serum YKL-40 level.¹³

Total serum IgE level in all asthmatic patients were high in our study. We found no correlation between the serum YKL-40 level and total serum IgE level among asthmatic patients. In a recent study, a positive correlation between serum total IgE and the serum YKL-40 level was demonstrated in atopic patients related to genotype.²⁸ No correlation between the serum YKL-40 level, total serum IgE levels and asthma duration in years might be due to genotypic

structure of Turkish patient population. In recent years, attention to YKL-40 regarding the role in the pathogenesis of asthma is increasing. Improvement of health related quality of life and decreased mortality rates can be provided with the detection of factors underlying disease etiology. Determination of relationship between asthma and YKL-40 is important in the diagnosis and treatment of the disease. Moreover, plasma levels of YKL-40 can be cost effective in follow up of the asthma severity. Therefore, the results of the present study can suggest that YKL-40 can be used as a follow up marker.

The major limiting factor in our study was that it was difficult to find patients having asthma without any additional diseases.

In conclusion, although the exact role of the serum YKL-40 levels in asthma is not known, increase of the serum YKL-40 levels especially in the acute exacerbation group can indicate the relationship between the serum YKL-40 levels and severity of asthma according to the results of the present study. Further multicentric studies with larger number of subjects are needed to explore these associations.

REFERENCES

1. Global Strategy for Asthma Management and Prevention 2011 (update). www.ginasthma.org.
2. James A. Airway remodeling in asthma. *Curr Opin Pulm Med* 2005; 11(1):1-6.

3. Vignola AM, Mirabella F, Costanzo G, Di Giorgi R, Gjomarkaj M, Bellia V, et al. Airway remodeling in asthma. *Chest* 2003; 123 (3 Suppl):417S-22S.
4. Hirst SJ, Martin JG, Bonacci JV, Chan V, Fixman ED, Hamid QA, et al. Proliferative aspects of airway smooth muscle. *J Allergy Clin Immunol* 2004; 114 (2 Suppl):S2-17.
5. Black JL. Asthma-more muscle cells or more muscular cells? *Am J Respir Crit Care Med* 2004; 169(9):980-1.
6. Johansen JS. Studies on serum YKL-40 as a biomarker in diseases with inflammation, tissue remodelling, fibroses and cancer. *Dan Med Bull* 2006; 53(2):172-209.
7. Ober C, Tan Z, Sun Y, Possick JD, Pan L, Nicolae R, et al. Effect of Variation in *CHI3L1* on Serum YKL-40 Level, Risk of Asthma, and Lung Function. *N Engl J Med* 2008; 358(16):1682-91.
8. Lee CG, Hartl D, Lee GR, Koller B, Matsuura H, Da Silva CA, et al. Role of breast regression protein 39 (BRP-39)/chitinase 3-like-1 in Th2 and IL-13-induced tissue responses and apoptosis. *J Exp Med* 2009; 206(5):1149-66.
9. Otsuka K, Matsumoto H, Niimi A, Muro S, Ito I, Takeda T, et al. Sputum YKL-40 Levels and Pathophysiology of Asthma and Chronic Obstructive Pulmonary Disease. *Respiration* 2012; 83(6):507-19.
10. Bara I, Ozier A, Girodet PO, Carvalho G, Cattiaux J, Begueret H, et al. Role of YKL-40 in bronchial smooth muscle remodeling in asthma. *Am J Respir Crit Care Med* 2012; 185(7):715-22.
11. Shuhui L, Mok YK, Wonk WS. Role of mammalian chitinase in asthma. *Int Arch Allergy Immunol* 2009; 149(4):369-77.
12. Chupp GL, Lee CG, Jarjour N, Shim YM, Holm CT, He S, et al. A chitinase-like protein in the lung and circulation of patients with severe asthma. *N Engl J Med* 2007; 357(20):2016-27.
13. Tang H, Fang Z, Sun Y, Li B, Shi Z, Chen J, et al. YKL-40 in asthmatic patients, and its correlations with exacerbations, eosinophils and immunoglobulin E. *Eur Respir J* 2010; 35(4):757-60.
14. Volck B, Johansen JS, Stoltenberg M, Garbarsch C, Price PA, Ostergaard M, et al. Studies on YKL-40 in knee joints of patients with rheumatoid arthritis and osteoarthritis. Involvement of YKL-40 in the joint pathology. *Osteoarthritis Cartilage* 2001; 9(3):203-14.
15. Johansen JS, Jensen BV, Roslind A, Nielsen D, Price PA. Serum YKL-40, a new prognostic biomarker in cancer patients? *Can Epidemiol Biomarkers Prev* 2006; 15(2):194-202.
16. Cinton C, Johansen JS, Christensen IJ, Price PA, Sørensen S, Nielsen HJ. High serum YKL-40 level after colorectal carcinoma is related to short survival. *Cancer* 2002; 95(2):267-74.
17. Schmidt H, Johansen JS, Gehl J, Geertsen PF, Fode K, von der Maase H. Elevated serum level of YKL-40 is an independent prognostic factor for poor survival in patients with metastatic melanoma. *Cancer* 2006; 106(5):1130-9.
18. Berres ML, Papen S, Pauels K, Schmitz P, Zaldivar MM, Hellerbrand C, et al. A functional variation in *CHI3L1* is associated with severity of liver fibrosis and YKL-40 serum levels in chronic hepatitis C infection. *J Hepatol* 2009; 50(2):370-6.
19. Rabe KF, Hurd S, Anzueto A, Barnes PJ, Buist SA, Calverley P, et al. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. *Am J Respir Crit Care Med* 2007; 176(6):532-55.
20. Røndjberg A, Omerovic E, Vestergaard H. YKL-40 levels are independently associated with albuminuria in type 2 diabetes. *Cardiovasc Diabetol* 2011; 10:54.
21. Nielsen AR, Erikstrup C, Johansen JS, Fischer CP, Plomgaard P, Krogh-Madsen R, et al. Plasma YKL-40: a BMI-independent marker of type 2 diabetes. *Diabetes* 2008; 57(11):3078-82.
22. Bonneh-Barkay D, Wang G, Starkey A, Hamilton RL, Wiley CA. In vivo *CHI3L1* (YKL-40) expression in astrocytes in acute and chronic neurological diseases 2010; 7:34.
23. Shao R, Hamel K, Petersen L, Cao QJ, Arenas RB, Bigelow C, et al. YKL-40, a secreted glycoprotein, promotes tumor angiogenesis. *Oncogene* 2009; 28(50):4456-68.
24. Johansen JS, Milman N, Hansen M, Garbarsch C, Price PA, Graudal N. Increased serum YKL-40 in patients with pulmonary sarcoidosis-a potential marker of disease activity? *Respir Med* 2005; 99(4):396-402.
25. Thöm I, Andritzky B, Schuch G, Burkholder I, Edler L, Johansen JS, et al. Elevated pretreatment serum concentration of YKL-40-An independent prognostic biomarker for poor survival in patients with metastatic non-small cell lung carcinoma. *Cancer* 2010; 116(17):4114-21.
26. Choi IK, Kim YH, Kim JS, Seo JH. High serum YKL-40 is a poor prognostic marker in patients with advanced non-small cell lung cancer. *Acta Oncol* 2010; 49(6):861-4.
27. Specjalski K, Jassem E. YKL-40 protein is a marker of asthma. *J Asthma* 2011; 48(8):767-72.

YKL-40 and Asthma

28. Sohn MH, Lee JH, Kim KW, Kim SW, Lee SH, Kim KE, et al. Genetic variation in the promoter region of chitinase

3-like 1 is associated with atopy. *Am J Respir Crit Care Med* 2009; 179(6):449-56.