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YKL-40 in Asthma and its Correlation with Different Clinical Parameters

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ABSTRACT

It has been suggested that elevated serum level of YKL-40 could be a marker for asthma and its severity. Along with few published studies, we investigated its correlation with asthma and its severity as well as spirometric indices.

114 patients with asthma and 114 healthy controls underwent the assessment of serum level of YKL-40 (by ELISA) and spirometric indices. Pearson's coefficient determined the correlation between the variables and multivariate linear regression analysis was used for adjusting the effect of different probable confounding factors.

Serum levels of YKL-40 were significantly higher in the asthmatic patients compared to those in healthy people ($p<0.001$). We also found a significant correlation between YKL-40 serum level and spirometric indices even after adjusting the effects of other variables.

We report for the first time in an Iranian population that YKL-40 may be a good diagnostic marker of asthma in serum.

Keywords: Asthma; Respiratory Indices, YKL-40

INTRODUCTION

Asthma is a chronic, complex, and heterogenic respiratory disease in which the mucosa of airways becomes abnormal and inflamed.

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Although the etiology of disease is unknown, its inflammatory entity may be accompanied with the increase of a set of inflammatory serum factors.[1,2]

Serum factor YKL-40 (also called Human Cartilage Glycoprotein 39 (Hcgp-39) or kitinase-like 1 factor), a member of 18-glycosil Hydrolase family 7 (including true kitinases and enzymaticly inactive kitinase-like factors), is a measurable serum kitinase-like protein which down/up regulates the innate immune responses in inflammatory and tissue-remodeling states.[3] In this

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regard, it may serve as a specific serologic marker of granulocyte function and macrophage activation at the site of tissue inflammation and also as a supplement to conventional acute phase proteins. For instance, it has been reported that YKL-40 probably activates interlukin-13 pathway and causes more inflammation in asthmatic patients.[4] It has also been introduced as a good biomarker of inflammatory lung diseases such as asthma. This is because of the correlation of YKL-40 level in serum with that in lining cells of respiratory system.[1,5-7] In asthmatic patients, YKL-40 is expressed by macrophages in bronchial-biopsy specimens and also cytospin of their broncho-alveolar lavage.[6]

YKL-40 is encoded by polymorphic gene of CHI3L1 whose variation in different individuals and races may influence its diagnostic as well as prognostic values in inflammatory states such as asthma.[1,6,7] This research was aimed to study the serum level of YKL-40 in an Iranian population of asthmatic patients and its correlation with asthma severity as well as spirometric parameters.

MATERIALS AND METHODS

This case-control study was conducted on asthmatics that were at remission subsequent to a course of proper treatment. All patients being visited for the first time by a pulmonologist were randomly selected at Asthma Clinic of Kashan University of Medical Sciences from July 2011 to July 2012. The minimum sample size was calculated according to serum YKL-40 levels ± SD measured in similar studies,[5] with a confidence of 95%, and 80% power of test. The protocol was approved by the local Committee of Ethics and was in accordance with the Helsinki Declaration. Written informed consent was obtained from all participants older than 16 years and in the cases of younger ones from their parents/guardians. Having clinically observed and managed the patients, the pulmonologist found none of them had any other kind of inflammatory or underlying diseases including any febrile illness, acute or chronic bronchitis, chronic obstructive pulmonary disease, bronchiectasis, respiratory tract infections, lung fibrosis, any kind of cancer and renal/hepatic/rheumatologic diseases, addiction or alcohol abuse, recent surgery, hypertension or diabetes. All patients were classified into intermittent, mild, moderate, and severe persistent asthma according to GINA (Global Initiative for Asthma)[8] criteria. Also 114 healthy individuals were considered as control group recruited from Iranian Blood Transfusion Organization who did not have a personal and family history of asthma. The spirometry test was done on all participants using Spirolab II (MIR, Italy). Serum levels of YKL-40 were measured by the commercial enzyme-linked immunoassay (ELISA) kit from Quidel (Santa Clara, CA, USA) according to kit procedure. Limit range detection of this kit was considered as 20-300 ng/ml as determined by the lowest and highest concentrations of YKL-40 in a serum specimen in our laboratory that met National Committee for Clinical Laboratory Standards and according to kit recommendations for Caucasian Asian races.

All data were analyzed in SPSS (VERSION19) using descriptive statistics (mean ± SD for quantitative, and the percentage as well as ratio for qualitative variables) and χ^2 and fisher's exact test. Analytical data were analyzed by ANOVA and t tests. Pearson's coefficient was determined to correlate between the variables. Partial correlation was also done to adjust the effect of asthma severity. Multivariate linear regression analysis with backward method was used to adjust the effect of probable different confounding variables on YKL-40. The removal criterion of variables was considered as $p>0.1$. Using Receiver Operating Characteristic (ROC) curve and Area Under Curve (AUC), we tried to determine the sensitivity and specificity of YKL-40 as a diagnostic marker of asthma.

RESULTS

Table 1 shows demographic and clinical characteristics of the patient and control groups. Serum YKL-40 level in asthmatic patients was significantly more than that in healthy controls and it remained significant (67 units more) after adjusting the effects of sex and age ($p<0.001$) (data not shown). Table 2 shows comparison of serum levels of YKL-40 at different severities as well as different spirometric parameters in asthmatic patients. Having performed such comparison, it was found that the mean of YKL-40 level was meaningful according to different types of asthma as well as spirometric parameters ($p<0.023$.

Table 1. Basic and clinical characteristics of patients and healthy subjects

Parameters		Healthy controls (n = 114)	Asthmatic patients (n = 114)	*P* value
Age (years)		43.07±9.05	42.12±12.34	0.1
Gender		53/61	54/60	0.9
Duration of asthma (months)		-	7.65±6.4	-
BMI (kg/m2)		27.44±4.1	22.87±3.25	<0.001
FEV1		3.73±0.8	2.1±0.94	<0.001
FEV1%		100.26±11	67±21.8	<0.001
FVC		4.25±0.9	2.76±1.1	<0.001
FVC%		99.8±9.9	74.5±22.3	<0.001
FEV1/FVC		87.7±4.9	75.6±12.56	<0.001
Peak Flow		9.2±5.96	4.64±1.73	<0.001
Peak Flow%		103.6±11.9	62.1±18.9	<0.001
FEF25-75		4.54±1.1	1.99±1.4	<0.001
FEF25-75%		105.9±16.4	49.5±25.8	<0.001
YKL-40 (ng/ml)	Male	52.6±28.2	133.9±66.4	<0.001
	Female	54.17±30.15	127.7±63.9	<0.001
	Total	53.42±29.14	138.42±67.13	<0.001

Table 2. Comparison of serum levels of YKL-40 at different parameters in asthmatic patients

Parameters	State	YKL-40	*P* value
Sex	Female	127.7±63.9	0.076
	Male	133.9±66.4	
Smoking	Yes (20)	136.38±75.46	0.88
	No (94)	138.84±65.65	
Family history	Yes (60)	140.25±68.87	0.76
	No (54)	136.38±65.72	
Type	Mild intermittent	167.3±68.3	0.004
	Mild persistent	107.3±47.7	
	Moderate persistent	117.9±48.6	
	Severe persistent	155.5±80.9	
FEV1%	<60 (44)	153.7±82.1	0.019
	60-80 (40)	127.7±44.9	
	>80 (30)	112.1±49.2	
FEV1/FVC	<85 (86)	138.5±67.4	0.023
	≥85 (28)	106.5±47.9	
FEF25-75%	<40 (43)	158.3±81.8	0.004
	40-60 (42)	113.9±38.5	
	61-80 (17)	119.6±50.3	
	>80 (12)	105.7±59.8	
FVC%	≤79 (65)	151.3±69.5	<0.001
	≥80 (49)	103.2±46	
Peak Flow%	≤59 (48)	154.3±70.2	0.001
	60-79 (41)	111.4±37.9	
	≥80 (25)	104.9±64.3	

Table 3. Correlation of serum levels of YKL-40 and different parameters in 2 groups

Variable	Control group (*P* value)	Asthma group (*P* value)
Age	-0.033	0.19
	(0.730)	(0.043)
BMI	-0.104	0.318
	(0.271)	(0.018)
FEV1	0.08	-0.136
	(0.4)	(0.148)
FEV1%	0.107	-0.147
	(0.258)	(0.118)
FVC	0.086	-0.01
	(0.361)	(0.914)
FVC%	0.026	0.036
	(0.784)	(0.707)
FEV1/FVC	-0.019	-0.456
	(0.838)	(0.001)
Peak Flow	0.076	-0.071
	(0.423)	(0.455)
Peak Flow%	0.014	-0.046
	(0.88)	(0.63)
FEF25-75	0.043	-0.192
	(0.647)	(0.041)
FEF25-75%	0.011	-0.24
	(0.905)	(0.01)
Disease duration	-	0.242
		(0.01)
Disease severity	-	0.157
		(0.096)

Dependent variable: YKL-40

Pearson's correlation coefficient did not show any correlations between the serum levels of YKL-40 with different parameters in control group (Table 3). However, it did show positive significant correlations with age, BMI, and disease duration ($p<0.05$) and it did show negative correlation with different spirometric indices in such a way that it was significant with FEV1/FVC, FEF25-75, and FEF25-75% (Table 3).

Adjusting the effect of variables of sex, age, BMI, FEV1, FEV1%, FVC, FVC%, FEV1/FVC, Peak Flow, Peak Flow%, FEF25-75, FEF25-75% and study group (asthmatic or healthy) on YKL-40 serum levels of all subjects by linear multiple regression analysis with backward method, we found a significant effect for age, sex, FEV1/FVC, FEV1, and the study group which all together could explain the serum YKL-40 levels as 86.4% (adjusted R squared=0.84) (Table 4). Also adjusting the effect of variables of sex, age, BMI, positive family history, smoking, duration of asthma, asthma severity, FEV1, FEV1%, FVC, FVC%, FEV1/FVC , Peak Flow, Peak Flow%, FEF25-75, FEF25-75% on YKL-40 serum levels of asthmatic patients through linear multiple regression analysis with backward method, we found a significant effect for sex, age, BMI, FEV1/FVC, FVC, Peak Flow and asthma duration which all together could explain the serum YKL-40 levels as 84.4% (adjusted R squared=0.844) (Table 5).

Using ROC curve and AUC, we tried to determine the sensitivity and specificity of YKL-40 as a diagnostic marker of asthma (Figure 1, Table 6). We found three proportion cutoff points. In the cut-off point of YKL-40=34.1, sensitivity and specificity were 97.4% and 28.9%, respectively. Increasing in the serum level of YKL-40, we found some decrease in the sensitivity, as there was a sensitivity of 89.5% at cutoff point of YKL-40=92.7.

Table 4. Linear multiple regression analysis evaluating the effect of different demographic and spirometric parameters on the serum level of YKL-40 in all subjects

Variables	Unstandardized Coefficients		t	*P* value
	B	Std. Error		
Gender	-48.36	16.29	-2.97	0.005
Age	1.28	0.712	1.8	0.079
FEV1/FVC	-3.68	0.629	-5.83	0.001
FEV1	22.33	10.2	2.19	0.033
Group	334.7	56.1	5.97	0.001

Table 5. Linear multiple regression analysis evaluating the effect of related different demographic and spirometric parameters on the serum level of YKL-40 in asthmatic patients

Variables	Unstandardized Coefficients		t	*P* value
	B	Std. Error		
Gender	-44.19	18.95	-2.376	0.022
Age	2.1	0.697	3.02	0.004
BMI	0.64	0.028	2.29	0.026
Asthma duration	3.11	1.251	2.49	0.016
FVC	29.94	8.933	3.35	0.002
FEV1/FVC	-1.156	0.436	-2.65	0.011
Peak Flow	8.407	4.57	1.84	0.072

Table 6. Validity indices of YKL-40 test for asthma diagnosis

	Cut-off point	Sensitivity	Specificity	Youden Index	AUC
YKL-40	34.1	0.974	0.289	0.263	0.876
	80.85	0.79	0.84	0.631	
	92.8	0.728	0.895	0.623	
	122	0.5	99.1	0.491	

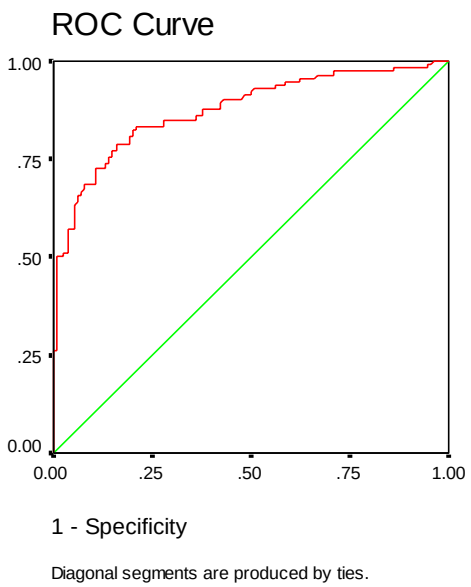

Figure 1. Receiver Operating Characteristic (ROC) of YKL-40 test for asthma diagnosis.

DISCUSSION

Our study shows that the serum level of YKL-40 in asthmatic patients is more than that in control group. There are 3 important points regarding such matter: 1) YKL-40 level is significantly higher in asthmatic males and females compared to sex-matched non-asthmatic ones. Although, in line with other studies,[9,10] we did not find significant differences in serum level of YKL-40 in asthmatics according to sex, however there were higher serum levels of YKL-40 in male asthmatics than those in females. Such concept may be due to sex-related hormones and low incidence of stress-bearing conditions in female gender, and can be examined in further studies. 2) YKL-40 level is highly correlated with age[11-13], 3) it would be distinctly applicable in each defined level of the severity. Although it has been shown that circulating YKL-40 may be a biomarker of susceptibility to the long-term effects of cigarette smoking,[14] we did not find any difference in YKL-40 levels between smoker asthmatics versus non-smoker asthmatics. Such discrepancy may be due to our younger patients with no serious smoking-related lung complications and also sample size as well as ethnicity of our smoker population. However, such declining of lung function in mild persistent asthmatics compared to mild intermittent ones has led to significantly lower YKL-40 serum levels in latter patients.

Our study endorsed the role of YKL-40 in an inflammatory state, namely asthma, a concept which has also been revealed in numerous inflammatory conditions including cancers,[15-17] infections,[18-20] and autoimmune diseases.[21-23] However, our study is unable to show a cause-and-effect relation between YKL-40 and asthma. In other words, our study can not claim that YKL-40 has a pathogenic role in asthma. Instead, the important result of our study is that YKL-40 may be

considered as a valuable diagnostic biomarker of asthma as shown for the first time by Chupp et al in 2007 [6] which was in line with what Kuepper et al.[24] approved in 2006. In his study, Kuepper et al showed that YKL-40 concentrations increased in response to allergen challenge predominantly at the site of allergen deposition; thus, it can reflect ongoing pulmonary asthma responses. An important privilege of Chupp et al. study to our study is the confirming of their results with two clinical and histopathological viewpoints. In fact, they showed that the levels of YKL-40 do increase in both serum and bronchoalveolar fluid; and such elevations have a direct correlation to the thickness of subepithelial basement membrane. Surveying the causative effect of YKL-40 in asthma, one year after Chupp et al. study, Ober et al. showed that in Huttrite tribes (inhabitant in Western America states and Canada), the level of YKL-40 in asthmatic patients is 15% more than that in normal people. In these patients Wild-type-131c allele was found and was meaningfully associated with asthma. They also found similar results in some Europeans from Germany and Americans from Chicago.[5] It has been observed that one single nucleotide polymorphism (SNP) in this allele causes the separation of transcription factors of YKL-40 gene (namely CH13L1) from its promoter and thus a diminished production of YKL-40. In fact, the natural and non-SNP form of this allele in tribes being studied by Ober caused the CH13L1 gene's promoter to be remained intact and high levels of YKL-40 to be produced. This result clearly suggests the causative role of YKL-40 at the genomic level. In other words, we may consider the level of YKL-40 as both accompanying and risk factor of asthmatic inflammation. Similar to other studies,[5,6,25-27] we found an inversely significant correlation of YKL-40 with lung function. A cut-off of 90ng/ml for YKL-40 was determined by Ober and Chupp in asthmatic patients. In our study, it was realized the best cut-off point of YKL-40 equal to80.85 analyzed by Youden index, in which the sensitivity and specificity were 79% and 84%, respectively and AUC was equal to 0.876. Showing that YKL-40 may be a diagnostic maker of asthma, we found its level more than 122ng/ml with a maximum of 1% error in all patients with each severity.

The 3 next studies, on Chinese,[25] Egyptian,[26] and Turkish[27] societies revealed that serum level of YKL-40 in asthmatic patients is more than that in control groups and also in asthmatic attacks more than that in stable asthma. They also showed a direct correlation with asthma exacerbations as well as severe asthma and a reverse correlation with lung function.

Regression models used in our study show that different demographic as well as spirometric parameters affect YKL-40 levels. Actually, the effect of group as a variable (being asthmatic or not being) was so strong in estimation of YKL-40 serum levels. It should be mentioned that the amount of adjusted R squared in our study demonstrated that the effect other factors not studied in our study would have been low.

According to our knowledge, our work is the first in an Iranian population in addition to the above-mentioned societies with similar results. Therefore, we did not find any opposing results.

Our study had some limitations. First, serial samples were not obtained to monitor the changes of YKL-40 in different clinical conditions of asthmatic patients. This limitation allowed just a cross-sectional analysis of YKL-40 profile of only limited robustness. Second, we did not correlate the serum levels of YKL-40 with its bronchoalveolar lavage levels as well as thickness of the subepithelial basement membrane in biopsy specimens of the lung. Further studies to investigate genetic polymorphism of YKL-40 in Iranian population are suggestedto be performed.

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