Journal of Cystic Fibrosis 15 (2016) 583 – 586

www.elsevier.com/locate/jcf

Short Communication

YKL-40 as marker of severe lung disease in cystic fibrosis patients

Salvatore Leonardi [a,*], Giuseppe Fabio Parisi [a], Antonino Capizzi [a], Sara Manti [b], Caterina Cuppari [b], Maria Grazia Scuderi [c], Novella Rotolo [a], Angela Lanzafame [a], Maria Musumeci [d], Carmelo Salpietro [b]

[a] Department of Clinical and Experimental Medicine, University of Catania, Catania, Italy
[b] Department of Pediatrics, Unit of Pediatric Genetics and Immunology, University of Messina, Messina, Italy
[c] Department of Medical and Surgical Sciences and Advanced Technologies, University of Catania, Catania, Italy
[d] Department of Laboratory Medicine and Microbiology, Center for Integrated Research, Campus Bio-Medico University of Rome, Rome, Italy

Received 12 October 2015; revised 19 December 2015; accepted 22 December 2015
Available online 8 January 2016

Abstract

Background: YKL-40 is a chitinase-like protein present in serum of healthy subjects and its levels are increased in several human inflammatory diseases. The aim of this study was to evaluate the levels of both serum and sputum YKL-40 in cystic fibrosis (CF) patients.
Methods: Serum and sputum YKL-40 levels were measured in a cohort of twenty-eight patients with a diagnosis of CF and twenty healthy controls.
Results: Serum YKL-40 levels were significantly higher in CF patients (88.8 ± 56.7 vs 18.6 ± 2.9 ng/ml, $P < 0.001$), as well as sputum YKL-40 levels (138.5 ± 132.7 vs 28.2 ± 24.34, $P < 0.001$) than in healthy controls. Serum YKL-40 levels were closely related to YKL-40 levels assessed in sputum samples ($r = 0.71$; $P < 0.01$).
Conclusions: YKL-40 is elevated in CF patients and is further elevated during severe exacerbations. Longitudinal studies in infant are needed to establish its role in disease pathogenesis.

Keywords: YKL-40; Cystic fibrosis; Pulmonary pseudomonas infection; Acute pulmonary exacerbation; Lung function decline

1. Introduction

YKL-40 (also named chitinase 3-like 1) is a chitinase-like protein first discovered in mouse breast cancer cells; in healthy humans it is produced by neutrophils, monocytes, macrophages, cultured chondrocytes and synovial cells, where it regulates cell proliferation and survival [1]. It increases in some severe human diseases, such as cancer [2,3], osteoarthritis [4,5], cardiovascular diseases [6,7], neurological diseases [8] and injury [9], infections [10], chronic obstructive pulmonary disease [11,12], asthma [13,14,15] and recently even in serum and sputum of cystic fibrosis (CF) adults [16].

The aim of this study was to evaluate the levels of serum and sputum YKL-40 in CF patients, both younger and older than 18 years, to examine the relationship between serum and sputum YKL-40 levels with different phenotypes or genotypes of disease and, finally, to analyze its sensitivity as a marker of lung disease severity.

2. Materials and methods

2.1. Patients population and study design

We enrolled a cohort of twenty-eight patients (CF group – 16 males and 12 females – age range 7–41 years) with a diagnosis

* Corresponding author at: Department of Clinical and Experimental Medicine, University of Catania, Catania, Italy, Via S. Sofia n.78, 95123. Tel.: +39 0953782764; fax: +39 0953782895.
E-mail address: leonardi@unict.it (S. Leonardi).

http://dx.doi.org/10.1016/j.jcf.2015.12.020
1569-1993/

of CF followed in the Cystic Fibrosis Hospital Centre of Catania University. To increase the homogeneity of the CF population, patients with clinical signs of exacerbation (decline of lung > 10%, start of antibiotic therapy, increase in coughing, clinical signs of infection, or signs on infection in terms of C-reactive protein elevation of differential counts) were excluded from analysis.

Twenty healthy controls (HS group – 11 males and 9 females – age range 8–35) were also recruited. None of these subjects was under medical treatment.

2.2. Collection of blood, sputum, procedures and definitions

Blood samples were drawn from all patients in fasting state in the morning during their regular visits.

All the spirometric tests used in the present study were performed in our Pulmonary Physiology Unit. Values of forced expiratory volume in 1 s (FEV1) and forced vital capacity (FVC) were expressed as % predicted of normal values adjusted for age, gender, sex height and weight. Lung disease severity was established according to FEV1 values. In particular, FEV1 > 80% of predicted was considered as normal, FEV1 > 50% < 80% of predicted as indicative of a mild respiratory disorder; FEV1 < 50% of predicted as indicative of a severe respiratory disorder.

We defined as severe, a phenotype characterized by the presence of a FEV1 permanently < 50% of predicted, a condition of chronic airways colonization by *Pseudomonas aeruginosa* and underweight status; conversely we identified as mild a phenotype characterized by a FEV1 > 80% of predicted, the absence of *P. aeruginosa* colonization in airways and a normal weight status.

In CF patients and healthy controls sputum induction and processing were performed as described by Sagel et al. [17]. In brief, the subjects were premedicated with inhaled salbutamol (200g). They then inhaled hypertonic (3%) saline solution. Adequate sputum plugs were separated from saliva. After centrifugation, sputum supernatants were stored at −80 °C.

Serum and sputum YKL-40 levels were measured using an enzyme-linked immunosorbent assay kit (Quidel, San Diego, Calif, USA) using the manufacturer's instruction. The detection limit of this assay was 15.6 ng/ml.

The body mass index (BMI) was used for the clinical definition of the nutritional status according to recommendations of international consensus [18]. According to BMI patients were classified as underweight if BMI was < 18.5; as normal weight if BMI was ≥ 18.5 ≤ 24.9; as overweight if BMI was ≥ 25.0 ≤ 29.9; as obese if BMI was 30.0 and above.

The presence of pancreatic insufficiency was defined when fecal elastase values < 200 mg/g. Cystic fibrosis-related diabetes mellitus (CFRD) was defined by the presence of 2-hour glycemia > 200 mg/dL in the OGTT or two fasting glycemia measurements greater than 126 mg/dL [19].

The study was approved by our local Committee for Clinical Investigation and informed consent was obtained by each patient or parent.

2.3. Statistical analyses

Statistical analyses were performed with Minitab software (version 16.0). Differences between groups were established by unpaired t test or by ANOVA followed by Bonferroni's test. Correlations were verified with Spearman rho test. A P value of < 0.05 was considered to be significant.

3. Results

Baseline clinical characteristics of our study subjects are summarized in Table 1.

Table 1
Baseline clinical characteristics of cystic fibrosis patients and healthy controls.

	CF patients	Healthy controls
N.	28	20
Age [yrs]	17.4 ± 9.4	17.9 ± 9.9
Sex [m/f]	16/12	11/9
Patients younger 18 years	17 (61%)	12 (60%)
Patients older 18 years	11 (39%)	8 (40%)
Pancreatic insufficiency	24 (86%)	NA
Cystic fibrosis-related diabetes mellitus	11 (39%)	NA
Normal weight status (BMI ≥ 18.5 ≤ 24.9)	12 (42%)	20 (100%)
Underweight status (BMI ≤ 18.5)	16 (57%)	0 (0%)
FEV1 < 50% of predicted	8 (29%)	0 (0%)
FEV1 > 50% < 80% of predicted	7 (25%)	0 (0%)
FEV1 > 80% of predicted	12 (46%)	20 (100%)
Chronic *Pseudomonas aeruginosa* airways colonization	14 (50%)	NA
Fungal airways colonization	3 (11%)	NA
Homozygosis ΔF508/ΔF508	7 (25%)	NA
Severe phenotype (FEV 1 <50% + chronic *Pseudomonas aeruginosa* airways colonization + underweight status)	6 (21%)	NA
Mild phenotype (FEV 1 >80% + no colonization of the airways + normal weight status)	7 (25%)	NA
Serum YKL-40 levels [ng/ml]	88.8 ± 56.7	18.6 ± 2.9
Sputum YKL-40 levels [ng/ml]	138.5 ± 132.7	28.2 ± 24.3

NA: not available.

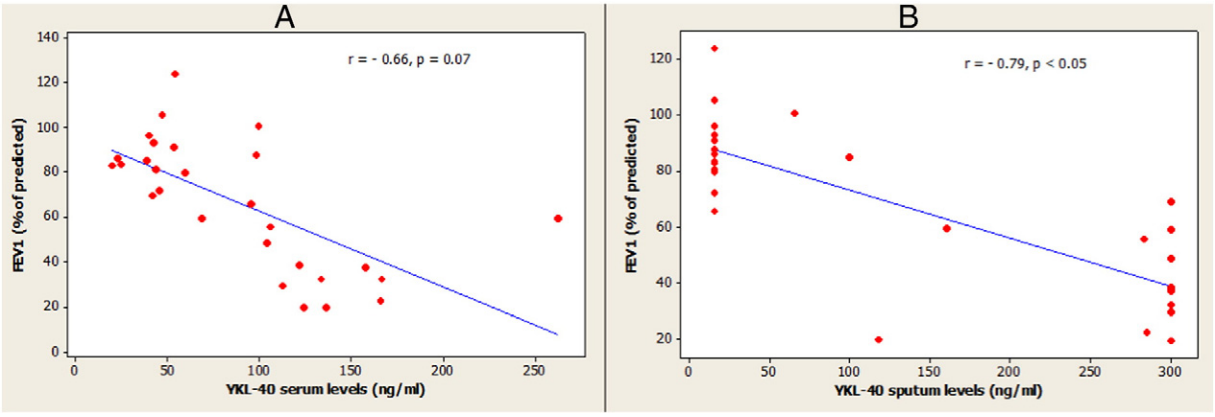

Fig. 1. FEV1 and YKL-40 serum (A) and sputum (B) level correlation.

Serum YKL-40 levels were significantly higher in CF patients, as well as sputum YKL-40 levels than in healthy controls. Serum YKL-40 levels were closely related to YKL-40 levels assessed in the sputum samples ($r = 0.71$; $P < 0.01$).

Within CF patients, adults showed significantly higher YKL-40 levels both in serum and sputum whereas YKL-40 levels do not seem to be related neither to pancreatic insufficiency nor to nutritional status nor to the presence of the homozygosis ΔF508/ΔF508 genotype.

In patients with chronic *P. aeruginosa* or fungal airway colonization we found higher levels of YKL-40 as well as in those with CFRD. According to pulmonary function, we found that patients with a severe respiratory disorder had significantly higher YKL-40 levels in sputum but not in serum (Fig. 1). In severe phenotype clinically defined patients, we found YKL-40 higher levels than in mild phenotype clinically defined patients. Finally, among the fourteen patients with *P. aeruginosa* airway colonization we found significantly higher levels of YKL-40 in the sputum of the patients with FEV1 < 50% than those with FEV1 > 50% but not in the serum. Data are detailed in Table 2.

4. Discussion

We demonstrated that CF patients are characterized by higher levels of both serum and sputum YKL-40 when compared with healthy subjects, supporting the hypothesis that this protein could play a critical role in CF pathogenesis. Moreover, sputum YKL-40 levels seem to be more sensitive for lung damage than serum levels in patients with chronic *P. aeruginosa* airways colonization. In fact we showed that patients with a severe

Table 2
Serum and sputum YKL-40 levels (ng/ml) related to different clinical phenotypes.

	Serum YKL-40 levels (ng/ml)		Sputum YKL-40 levels (ng/ml)	
	Mean ± SD	P value	Mean ± SD	P value
≥18 yrs	131.1 ± 55.2	<0.001	239.4 ± 103.7	<0.01
<18 yrs	61.4 ± 38.7		93.1 ± 121.0	
Pancreatic insufficiency	93.8 ± 59.2	ns *	169.9 ± 135.0	ns *
Pancreatic sufficiency	58.7 ± 25.2		36.7 ± 42.2	
Underweight status	87.9 ± 51.2	ns *	164.9 ± 140.0	ns *
Normal weight status	89.9 ± 65.6		106.4 ± 122.0	
Homozygosis ΔF508/ΔF508	101.8 ± 86.3	ns *	144.6 ± 146.4	ns *
Other genotypes	84.4 ± 44.9		151.2 ± 133.1	
Chronic *Pseudomonas aeruginosa* airways colonization	108.1 ± 65.8	<0.03	204.6 ± 132.8	<0.03
No chronic *Pseudomonas aeruginosa* airways colonization	67.4 ± 40.6		86.9 ± 112.2	
Fungal airways colonization	149.3 ± 21.9	<0.03	295.1 ± 8.5	<0.02
No fungal airways colonization	81.5 ± 55.3		121.2 ± 129.1	
Cystic fibrosis-related diabetes mellitus	136.4 ± 51.7	<0.01	259.5 ± 80.5	<0.01
No cystic fibrosis-related diabetes mellitus	57.9 ± 34.3		60.1 ± 95.5	
FEV1 < 50%	138.8 ± 22.7	ns *	269.7 ± 74.3	<0.05
50% < FEV1 < 80%	97.1 ± 76.9		155.8 ± 139.5	
FEV1 > 80%	48.7 ± 25.9		26.8 ± 27.2	
Severe phenotype	143.3 ± 23.1	<0.001	297.5 ± 6.0	<0.001
Mild phenotype	59.1 ± 29.0		34.8 ± 34.6	
Chronic *Pseudomonas aeruginosa* airways colonization and FEV1 < 50%	136.1 ± 19.6	ns *	274.0 ± 68.8	<0.05
Chronic *Pseudomonas aeruginosa* airways colonization and FEV1 > 50%	80.1 ± 84.6		135.1 ± 149.1	

* ns: not significant.

respiratory disorder (i.e. characterized by chronic *P. aeruginosa* airways colonization and FEV1 < 50%) had significantly higher YKL-40 levels in sputum than in serum and the difference with healthy controls resulted significant only for sputum.

Furthermore in CF patients older than 18 years we found both in serum and in sputum significantly higher YKL-40 levels than in CF patients younger than 18 years, confirming the relationship between this marker and the progression of the disease. On the contrary in patients with homozygosis ΔF508/ΔF508, YKL-40 levels resulted not significantly higher than in patients with other different genotypes. Our results confirm Hector's datum that YKL-40 level in CF patients is not modulated directly by the genetic CF defect [16].

About YKL-40 serum levels, we also hypothesized that this protein has a lower sensitivity for a mild systemic involvement and a major sensitivity only for advanced disease with severe lung damage and CFRD.

Interestingly, in the fourteen patients with chronic *P. aeruginosa* airways colonization we found significantly higher levels of YKL-40 in the sputum of patients with FEV1 < 50% than those with FEV1 > 50%. Conversely, serum YKL-40 levels were not significantly higher in patients with *P. aeruginosa* airways colonization and FEV1 < 50% suggesting that the dosage of this marker on sputum is more sensitive than on serum for lung damage.

5. Conclusions

In summary, we demonstrated that CF patients are characterized by higher levels of both serum and sputum YKL-40 when compared with healthy subjects, supporting the hypothesis that this protein could play a critical role in CF pathogenesis especially in severe lung disease but longitudinal studies in infant are needed to establish this hypothesis.

Funding source

This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

Financial disclosure

The authors have no financial relationship relevant to this article to disclose.

Conflict of interest

The authors have no conflicts of interest to disclose that could be perceived as prejudicing the impartiality of the research reported.

Acknowledgments

We would like to thank all the participants and their families who took part in this research.

References

[1] Johansen JS, Schultz NA, Jensen BV. Plasma YKL-40: a potential new cancer biomarker? Future Oncol 2009;5:1065–82.
[2] Hamilton G, Rath B, Burghuber O. Chitinase-3-like-1/YKL-40 as marker of circulating tumor cells. Transl Lung Cancer Res 2015;4:287–91.
[3] Huang K, Wu LD. YKL-40: a potential biomarker for osteoarthritis. J Int Med Res 2009;37:18–24.
[4] Lorenz H, Wenz W, Ivancic M, et al. Early and stable upregulation of collagen type II, collagen type I and YKL40 expression levels in cartilage during early experimental osteoarthritis occurs independent of joint location and histological grading. Arthritis Res Ther 2005;7:156–65.
[5] Mathiasen AB, Henningsen KM, Haratyunyan MJ, et al. YKL-40: a new biomarker in cardiovascular disease? Biomark Med 2010;4:591–600.
[6] Wu S, Hsu LA, Cheng ST, et al. Circulating YKL-40 level, but not CHI3L1 gene variants, is associated with atherosclerosis-related quantitative traits and the risk of peripheral artery disease. Int J Mol Sci 2014;15:22421–37.
[7] Bonneh-Barkay D, Wang G, Starkey A, et al. In vivo CHI3L1 (YKL-40) expression in astrocytes in acute and chronic neurological diseases. J Neuroinflammation 2010;7:34.
[8] Bonneh-Barkay D, Zagadailov P, Zou H, et al. YKL-40 expression in traumatic brain injury: an initial analysis. J Neurotrauma 2010;27:1215–523.
[9] Kromborg G, Ostergaard C, Weis N, et al. Serum level of YKL-40 is elevated in patients with *Streptococcus Pneumoniae bacteriemia* and is associated with the outcome of the disease. Scand J Infect Dis 2002;34:323–6.
[10] Sarma NJ, Tiriveedhi V, Subramanian V, et al. Hepatitis C virus mediated changes in miRNA-449a modulates inflammatory biomarker YKL40 through components of the NOTCH signaling pathway. PLoS One 2012;7, e50826.
[11] Holmgaard DB, Mygind LH, Titlestad IL, et al. Plasma YKL-40 and all-cause mortality in patients with chronic obstructive pulmonary disease. BMC Pulm Med 2013;13:77.
[12] Filippelli M, Cuppari C, Giacchi V, et al. Serum and BAL YKL-40 levels in different inflammatory lung disease: an update. J Biol Regul Homeost Agents 2015;29S:130–6.
[13] Chupp GL, Lee CG, Jarjour N, et al. A chitinase-like protein in the lung and circulation of patients with severe asthma. N Engl J Med 2007;357:2016–27.
[14] Ahangari F, Sood A, Ma B, et al. Chitinase 3-like-1 regulates both visceral fat accumulation and asthma-like Th2 inflammation. Am J Respir Crit Care Med 2015;191:746–57.
[15] Leonardi S, Filippelli M, Lanzafame A, et al. Serum YKL-40 in children with asthma. J Biol Regul Homeost Agents 2015;29S:114–9.
[16] Hector A, Kormann MS, Mack I. The chitinase-like protein YKL-40 modulates cystic fibrosis lung disease. PLoS One 2011;6, e24399.
[17] Sagel SD, Kapsner R, Osberg I, et al. Airway inflammation in children with cystic fibrosis and healthy children assessed by sputum induction. Am J Respir Crit Care Med 2001;164:1425–31.
[18] Sinaasappel M, Stern M, Littlewood J, et al. Nutrition in patients with cystic fibrosis: a European Consensus. J Cyst Fibros 2002;1:51–75.
[19] Moran A, Hardin D, Rodman D, et al. Diagnosis, screening and management of cystic fibrosis related diabetes mellitus: a consensus conference report. Diabetes Res Clin Pract 1999;45:61–73.