Increased Plasma YKL-40 Level and Chitotriosidase Activity in Cystic Fibrosis Patients

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Abstract—We investigated plasma YKL-40 levels and chitotriosidase (CHIT1) activity in patients with cystic fibrosis (CF) lung disease and evaluated clinically relevant factors that may affect their levels. Plasma samples were obtained from pediatric (n = 19) and adult patients (n = 15) during exacerbation, discharge, and stable period of the disease. YKL-40 levels and chitotriosidase activity were measured by enzyme-linked immunosorbent assay and fluorometric assay, respectively. Data were compared with healthy children and adults of similar age. YKL-40 levels of pediatric and adult CF patients at all periods were significantly higher than controls (p < 0.001 and p < 0.05). CHIT1 activities of adult patients at all periods were significantly higher compared to controls (p < 0.05). On the other hand, CHIT1 activities of pediatric CF patients were similar with controls. YKL-40 levels of exacerbation period of adult CF patients were negatively correlated with forced vital capacity (FVC) (r = −0.800, p = 0.014) and forced expiratory volume in 1 s (FEV1) (r = −0.735, p = 0.008). YKL-40 levels in the exacerbation period of pediatric CF patients were negatively correlated with FVC (r = −0.697, p = 0.0082) and FEV1 (r = −0.720, p = 0.006). CHIT1 activity may be a valuable marker of chronic inflammation in adult CF patients who suffer from CF for a longer period compared to pediatric patients. Increased YKL-40 levels in both pediatric and adult patients compared to controls may point to a role in between CF pathology.

KEY WORDS: Cystic fibrosis; YKL-40; Chitotriosidase activity; Exacerbation; Inflammation; Respiratory function test.
INTRODUCTION

Cystic fibrosis (CF) is an inherited autosomal recessive disorder that is characterized by disrupted epithelial transport of chloride due to a mutation in cystic fibrosis transmembrane conductance regulator, CFTR, gene. This results with dehydration, increased viscosity of the mucus, and obstruction of the ductal tract in various organs including lung, pancreas, liver, intestine, sweat glands, and epididymis. Hyperviscosity and airway obstruction increase the propensity for bacterial colonization, resulting with infection and inflammation particularly in the lung tissue where intermittent inflammation and frequent infections lead to gradual development of fibrosis and bronchiectasis [1]. The terminal progression of the disease is frequently characterized by respiratory failure; about 80% of deaths are caused by pulmonary insufficiency in CF patients [2].

There is no consensus on the diagnostic criteria for pulmonary exacerbation of CF [3]. Although bronchoalveolar lavage is defined as the best sample to diagnose pulmonary infection in CF, bronchoscopy is an invasive technique. On the other hand, blood is presumably an easily obtained sample. Therefore, investigating the blood during pulmonary inflammation and remission in CF may give clues on the pathology of the disease and help to find novel biomarkers for monitoring CF.

YKL-40 (also known as the chitinase 3-like protein 1 or the human cartilage glycoprotein-39) is a member of the glycosyl hydrolase protein family [4]. Macrophages, chondrocytes, and some cancer cells secrete YKL-40. It is thought to play a role in chronic inflammation, tissue regeneration, and cell growth [5]. There are reports of increased YKL-40 in CF patients [4, 6, 7]. According to recent studies; increased sputum YKL-40 levels of CF patients were correlated with pulmonary functions [8]. In addition, serum YKL-40 levels of CF patients were reported to be higher than healthy controls and it was suggested to be a useful biomarker in CF lung disease [6, 9, 10].

CHIT1 activity has been reported to be elevated in some hereditary lysosomal storage diseases (especially as a marker in Gaucher) [13]. Additionally, it is increased in atherosclerosis, hematological diseases, chronic inflammatory diseases, neurodegenerative diseases, and various lung diseases (such as sarcoidosis, idiopathic pulmonary fibrosis, asthma, cystic fibrosis) in which active macrophages are involved [12, 14, 15, 16].

CHIT1 activity was particularly shown to increase in CF patients with fungal Candida albicans colonization and was suggested as a disease biomarker for fungal infections [17]. The relationship between CHIT1 and CF pathology has not been understood, yet.

Both CHIT1 and YKL-40 are members of 18-glycosyl-hydrolase family, and they are good indicators of neutrophil infiltration in CF [11]. There is no study in the literature evaluating circulating CHIT1 activity and YKL-40 levels simultaneously in CF patients. In this study YKL-40 levels and CHIT1 activity were measured in the plasma of pediatric and adult CF patients who were hospitalized with pulmonary exacerbation.

MATERIAL AND METHODS

Study Group

We enrolled a total of 34 CF patients including pediatric and adult individuals who were hospitalized due to pulmonary exacerbation. The diagnosis of CF was performed according to the most recent diagnostic criteria [18, 19]. Despite the importance of identifying and managing a pulmonary exacerbation, there is no standardized definition of exacerbation. Some attempts have been made to clarify pulmonary exacerbation criteria in CF with the use of various scoring systems, but no common agreement was reached [20–22]. Pulmonary exacerbation was defined in patients with changes in sputum volume or color, increased coughing, increased fatigue, loss of appetite, and weight loss, 10% or more reduction in pulmonary function tests and at least two of the signs of increased dyspnea. Dyspnea is a complex and hardly understood sensation that can be described only by the individual who is experiencing it. Therefore, its definition and expression may vary from person to person. Although dyspnea is a subjective sensation, Medical
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Research Council Scale and Revised Borg Scale for Grading Severity of Dyspnea applied to the patients [23]. Patients with at least two of the above symptoms were diagnosed with pulmonary exacerbation and included in the study [24]. All patients suffered with pulmonary exacerbation were hospitalized and received IV antibiotic therapy for 14 days.

Exclusion criteria were non-compliance with medication, being younger than 6 years, receiving systemic steroid and immunosuppressive treatment, patients with identified *Burkholderia cepacia* complex in sputum culture, and presence of allergic bronchopulmonary aspergillosis (ABPA). Glucocorticosteroids have been employed as the fundamental therapy of ABPA. This situation could affect due to change/inhibit inflammation pathways our study results. Also, the pathophysiology of ABPA is complex; many different immune pathways and factors play a role.

Age-matched healthy individuals (n = 32) were involved in the study. They did not have acute upper or lower respiratory tract infections and were not under any medical treatment.

### Blood Sampling

Blood samples were obtained from pediatric and adult CF patients in three separate occasions: during pulmonary exacerbation, at discharge after appropriate treatment, and in the stable period of disease (after discharge; at least 1 month later and latest within 3 months). Exacerbation samples were collected from all pediatric and adult patients before medication within 24 h after admission. Blood was obtained once from healthy participants at the time of their inclusion.

Whole blood samples collected into EDTA-containing tubes were centrifuged at 3000 rpm for 10 min, and the plasma was stored at −80 °C until measurements were performed.

We were unable to collect blood from some of the patients at discharge and during the stable period of the disease for various reasons. Some patients were discharged without blood withdrawal or refused sampling, while some who resided in other cities did not return for their scheduled follow-up studies. Thus, the number of patients in the discharge and stable periods was lower.

### Clinical and Laboratory Investigation

Pulmonary function tests were performed in the patients at the exacerbation and stable periods. All spirometric tests were performed in accordance with the American Thoracic Society and European Respiratory Society (ATS/ERS) standards [25] by the same certified spirometry technicians. Indices of spirometry were analyzed using the reference values of Quanjer et al. [26]. Spirometry curves were re-evaluated by a single senior pediatric pulmonologist following the recently published update of the ATS/ERS standardization of spirometry [27]. The same spirometer (Vyntus SPIRO, CareFusion, Germany) was used for each participant, and it was calibrated daily with a single 3-L discharge syringe. The patients were seated in an upright position and a nose clip was applied during the measurement. At least three acceptable and repeatable spiromgrams were obtained for each patient and the best one was selected for analysis.

Forced expiratory volume in 1 s (FEV1) and forced vital capacity (FVC) were presented as % predicted of normal values adjusted for age, gender, sex, height, and weight. The spirometric measurements in adult patients were grouped into two: mild to moderate (FVC > 50% of predicted) and severe (FVC < 50%).

We measured YKL-40 levels in duplicate by using an ELISA based Human Chitinase 3-Like 1/YKL-40 Picokine Kit (Boster Biological Technology, CA, USA) according to the manufacturer’s instructions. YKL-40 levels were optimized to a linear calibration range from 62.5 to 4000 pg/mL, and dilution factor was 100.

We determined chitotriosidase activity by a fluorometric method using 4-methylumbelliferyl β-D-N,N′,N″-triacetylchitotrioside as the substrate [28]. CHIT1 activity analyses were optimized via standards to a linear calibration range from 0 to 1.670 nmol/mL, and dilution factor was 243.1. Four of the patients (3 children, 1 adult) and 6 of the healthy controls’ (3 children, 3 adults) plasma did not show any CHIT1 activity. There is a previous report of a polymorphism (24-bp long duplication in the encoding gene) that resulted with the absence of enzyme activity [29]. About 1/3 of the Caucasian population are heterozygotes and as a result have reduced CHIT1 activity, and up to 6% of homozygotes have no CHIT1 activity. Although we have not done any genetic testing for these 10 individuals, we excluded their data from the analysis for their possibility of being homozygotes for the inactivating polymorphism.
Ethics Statement

Ethical approval was obtained from the Clinical Research Ethical Committee of Hacettepe University (2016/14). All the patients, controls, and caretakers of the pediatric patients provided informed consent. All steps of the study conformed to the Helsinki Declaration and Good Clinical Practice guidelines.

Statistics

Statistical analysis was performed via Graphpad Prism 6. Data was expressed as either mean ± standard deviation (SD) or median (minimum–maximum) with regard to normality of distribution that was checked by the D'Agostino-Pearson test. 

RESULTS

The pediatric CF patients included 19 children at pulmonary exacerbation. Blood samples were collected from all the patients at exacerbation, from 11 of them at discharge and from 9 of them at the stable period. The pediatric control group included 17 healthy children. The adult CF patients included 15 individuals at

| Table 1 Demographic Characteristics of the Participants |
|--------------------------------------------------------|
| Adult                                               | Pediatric                                            |
| CF patients \(n = 15\)                                | Healthy controls \(n = 15\)                          |
| Sex (M/F)                                           | Sex (M/F)                                           |
| 11/4                                                | 6/9                                                 |
| Age (years)                                         | 22.0 (20.0–38.0)                                   |
| 25.4 ± 3.2                                          | 12.0 (6.0–17.0)                                    |
| BMI (kg·m\(^{-2}\))                                 | 14.2 ± 1.7                                         |
| 17.3 ± 2.8                                          | 18.8 ± 2.9                                         |
| Nicotine use (%)                                    | None                                                |
| 13.3                                                | None                                                |

Data were presented as \(n\), \(n/n\), or \(n\ (%\)), unless otherwise stated

Data were expressed as mean ± standard deviation and median (minimum–maximum) with regard to normality of distribution that was checked by the D’Agostino-Pearson normality test.

BMI body mass index, \(M\) male, \(F\) female

| Table 2 Clinical Characteristics of the Patients |
|-------------------------------------------------|
| Current clinical situation/all patients \((n/n)\) | Adult CF patients \(n/15\) | Pediatric CF patients \(n/19\) |
| Pancreatic insufficiency                        | 14                      | 19                        |
| CF-related diabetes mellitus                    | 1                       | 1                         |
| Pulmonary hypertension                           | 2                       | None                      |
| Asthma                                           | 1                       | 1                         |
| Inhaled corticosteroid use                       | 3                       | 1                         |
| Chronic colonization*                             | 14                      | 18                        |
| Colonization with \(P.\ aeruginosa\)            | 6                       | 6                         |
| Colonization with \(S. aureus\)                | 4                       | 4                         |
| Colonization with \(P. aeruginosa + S. aureus\) | 3                       | 7                         |
| Bronchiectasis                                   | 10                      | 19                        |
| Atelectasis**                                    | 1                       | 19                        |
| Homozygous delta F508 mutation                   | 2                       | 9                         |

* The diagnosis of chronic colonization is made by \(P. aeruginosa\) growth in 3 consecutive samples taken at least 1 month apart in the last 6 months or in more than 50% of the samples taken in the last year in microbiological samples (deep throat swab or sputum) obtained from the lungs [30, 31]

** In the study, patients with new atelectasis (lobar and segmental) on the control X-ray during the acute pulmonary exacerbation period were mentioned. X-rays of patients with chronic atelectasis were not included in the radiological worsening criteria within the pulmonary exacerbation criteria
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Blood samples were collected from all the patients at exacerbation, from 13 of them at discharge and from 11 of them at the stable period. The adult control group included 15 healthy individuals. None of our patients had fungal colonization and gone lung transplantation.

Demographic characteristics of the participants are presented in Table 1. Clinical characteristics and routine laboratory data of the participants are presented in Tables 2 and 3.

Almost all the patients had pancreatic insufficiency, chronic bacterial colonization, and bronchiectasis in the lungs. Colonization with *Pseudomonas aeruginosa* was the most frequent among all other bacterial colonizations. Inflammation was apparent by increased CRP levels during the exacerbation of the CF patients.

YKL-40 levels of pediatric and adult CF patients at the exacerbation, discharge, and stable periods were significantly higher when compared to their respective healthy control groups (*p* < 0.001 and *p* < 0.05) (Fig. 1a, b). On the other hand, both pediatric and adult patients had similar YKL-40 levels among the exacerbation, discharge, and stable periods of their disease.

CHIT1 activities of pediatric and adult CF patients were not significantly different among the exacerbation, discharge, and stable periods of the disease. Although CHIT1 activities of adult CF patients at each period were higher compared to that of healthy controls (*p* < 0.05

### Table 3 Laboratory Data of the Patients

|                      | Adult CF patients |                      | Pediatric CF patients |                      |
|----------------------|-------------------|----------------------|-----------------------|----------------------|
|                      | Pulmonary exacerbation | Stable | Pulmonary exacerbation | Stable |
| FVC (% predicted)    | 42.58 ± 20.19 (n = 12) | 45.36 ± 18.35 (n = 11) | 56.08 ± 22.41 (n = 13) | 64.13 ± 22.79 (n = 8) |
| FEV1 (% predicted)   | 34.83 ± 14.68 (n = 12) | 35.45 ± 13.68 (n = 11) | 61.08 ± 26.85 (n = 13) | 65.25 ± 26.51 (n = 8) |
| sO2                 | 96.00 (85.00–98.00) (n = 7) | 93.82 ± 3.94 (n = 11) | 92.50 (80.00–96.00) (n = 18) | 95.36 ± 2.37 (n = 14) |
| CRP (mg/dL)         | 7.61 ± 5.20 (n = 9) | 3.80 ± 4.05 (n = 8) | 3.13 (0.80–19.00) (n = 15) | 1.55 (0.10–5.91) (n = 10) |
| WBC (10^3/µL)       | 11.12 ± 4.13 (n = 9) | 12.82 ± 4.66 (n = 8) | 12.14 ± 4.69 (n = 17) | 11.27 ± 2.74 (n = 11) |

Data were expressed as mean ± standard deviation and median (minimum–maximum) with regard to normality of distribution that was checked with the D’Agostino-Pearson test.

FVC forced vital capacity, FEV1 forced expiratory volume in 1 s, CRP C-reactive protein, WBC white blood cell, sO2 artery oxygen saturation

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**Fig. 1** YKL-40 levels of adult (a) and pediatric (b) CF patients at exacerbation, discharge, and stable periods compared to healthy controls.
(Fig. 2a), CHIT1 activities of pediatric patients at the three periods of the disease were similar to that of healthy controls (Fig. 2b).

Also, plasma YKL-40 levels and chitotriosidase activities of CF patients at exacerbation, discharge, and stable periods and comparisons with healthy individuals are summarized in Table 4.

### Table 4: Plasma YKL-40 Levels and Chitotriosidase Activities of CF Patients at Exacerbation, Discharge and Stable Periods, and Comparisons with Healthy Individuals

|                              | Plasma YKL-40 levels (ng/mL) | p         | Chitotriosidase activity (nmol/h/mL) | p         |
|------------------------------|-----------------------------|-----------|--------------------------------------|-----------|
| **Adults**                   |                             |           |                                      |           |
| Pulmonary exacerbation period| 45.0 (17.6–190.0) (n = 15)  | < 0.0001  | 55.7 ± 32.9 (n = 14)                 | 0.0013    |
| Discharge period             | 21.9 (12.8–149.1) (n = 13)  | 0.0025    | 52.2 ± 25.7 (n = 11)                 | 0.0070    |
| Stable period                | 42.1 (11.9–216.4) (n = 11)  | 0.0002    | 61.6 ± 29.1 (n = 9)                  | 0.0132    |
| Healthy controls             | 14.6 (7.4–35.7) (n = 15)    |           | 21.4 ± 13.4 (n = 12)                 |           |
| **Children**                 |                             |           |                                      |           |
| Pulmonary exacerbation period| 34.3 ± 20.1 (n = 19)        | < 0.0001  | 50.5 ± 35.6 (n = 16)                 | n.s       |
| Discharge period             | 21.9 (8.0–86.3) (n = 11)    | 0.0162    | 31.9 ± 21.9 (n = 10)                 | n.s       |
| Stable period                | 32.8 ± 13.6 (n = 9)         | 0.0003    | 56.7 ± 51.9 (n = 9)                  | n.s       |
| Healthy controls             | 12.4 ± 6.6 (n = 17)         |           | 22.9 (6.4–113.9) (n = 14)            |           |

Data were expressed as mean ± standard deviation and median (minimum–maximum) with regard to normality of distribution that was checked by the D’Agostino-Pearson test.

n.s. not specific
YKL-40 levels in the exacerbation period of pediatric CF patients were negatively correlated with FVC \( r = -0.697, p = 0.0082 \) (Fig. 4a) and FEV1 \( r = -0.720, p = 0.006 \) (Fig. 4b) in the same period.

Plasma YKL-40 levels and CHIT1 activities at the exacerbation and stable periods of both adult and pediatric patients did not correlate with gender, age, BMI, WBC, CRP, and were not associated with the presence or absence of \( P. aeruginosa \) colonization and other clinical conditions.

Exacerbation plasma YKL-40 levels measured in pediatric CF patients with homozygous phe508del
mutation \((n = 9)\) were significantly higher than in patients with other mutations \((n = 9)\) \((p < 0.05)\).

**DISCUSSION**

To the best of our knowledge, there are no previous studies that investigated the levels of plasma YKL-40 and CHIT1 activity simultaneously in pediatric and adult CF patients. We, for the first time, investigated and compared CHIT1 activity and YKL-40 levels in three different clinical occasions including pulmonary exacerbation, discharge, and stable periods of the disease. Additionally, this is the first report on YKL-40 levels in pediatric CF patients. YKL-40 levels are correlated with FEV1 and FVC in patients.

In this study, adult CF patients had higher plasma CHIT1 activity in all three periods of the disease compared to healthy controls. However, CHIT1 activity did not differ significantly among the exacerbation, discharge, and stable periods. CHIT1 activity was similar between pediatric CF patients compared to healthy controls.

Harlander et al. found a significant positive correlation between CHIT1 activity and age in chronic obstructive pulmonary disease (COPD) patients [32]. Also, there was another report that described such a correlation in healthy subjects [33]. Although the underlying mechanisms of this relationship are unclear, it has been suggested to be a result of age-associated chronic macrophage activation due to chronic inflammation [32]. We did not find a correlation between age and CHIT1 activity, most likely due to the relatively small sample size in this study. CHIT1 activity may be a valuable marker of chronic inflammation in adult CF patients who have suffered from CF for a longer period with an increased disease burden compared to pediatric patients, according to findings of this study.

There was no correlation between CHIT1 activity and lung function in both pediatric and adult patients. The lack of correlation between CHIT1 activity and lung function is in agreement with the latest reports in COPD [32–34] one study that reported a weak correlation [35]. A latest study indicated that CHIT1 activity has been found to increase in active sarcoidosis patients and correlated with radiological findings. They showed that CHIT1 was correlated with disease activity, severity, and multiorgan dissemination [36].

The work by Hector et al. demonstrated that CHIT1 levels were elevated in patients with cystic fibrosis especially in patients with fungal infections. Additionally, this study demonstrated the susceptibility of CHIT1 to neutrophil elastase mediated cleavage. Given the persistent neutrophilic inflammation in cystic fibrosis lungs, it is not clear how this cleavage of CHIT1 alters its biological activity or its possible contribution to disease progression [37].

Chitin is found in the walls of fungi. Fungal colonization and presence of ABPA could alter chitinase activity and elevate chitinase-like protein YKL-40 levels. For these reasons, we think that the absence of fungal colonization and ABPA turned into an advantage in our study. Thus, we aimed to focus only on cystic fibrosis without other interferences.

Vicencio et al. (2019—C61 Pediatric Asthma/Thematic Poster) found in two patients with allergic bronchopulmonary aspergillosis (ABPA) high YKL-40/
chitinase activity ratios compared to all other asthma patients. There is no similar study in patients with ABPA with cystic fibrosis.

We did not find any correlations between CHIT1 activity and CF-related factors, sex, genotype, bacterial/fungal colonization status, body mass index, CRP, and WBC levels. There is only one study that showed a statistically significant association between Candida albicans growth in culture and serum CHIT1 levels in CF patients [17].

Circulating YKL-40 could come from more than one source and might be a biomarker of not only systemic inflammatory response but also a biomarker of a proliferative and tissue remodeling response. Neutrophils are a potential source of YKL-40 in the systemic circulation of patients with CF; however, other cellular types, such as hepatocytes or fibrotic tissues, could contribute to the circulating level of YKL-40 [38].

Plasma YKL-40 levels of both our pediatric and adult CF patients were significantly higher than healthy controls at all the three different periods of CF. However, there was no significant difference among YKL-40 levels at exacerbation, discharge, and stable periods of the disease. Therefore, we suggest YKL-40 as a marker of chronic lung pathology in CF rather than being a marker associated with acute exacerbation in our study group.

Although the literature concerning YKL-40 levels in lung diseases is rather substantial, the studies are insufficient in terms of explaining its exact role. A previous study reported increased serum YKL-40 and CHIT1 in patients with asthma and COPD compared to healthy controls [35]. They found that YKL-40 and CHIT1 were negatively correlated with various parameters of the pulmonary function test. They also measured serum YKL-40 and CHIT1 levels during the exacerbation period in selected asthma and COPD patients and reported no significant difference compared to baseline levels. On the other hand, there is another study that investigated YKL-40 expression in the lung tissue from COPD patients. They reported that YKL-40 expression was increased in stable patients and was correlated with exacerbation attacks. A recent study reported sputum YKL-40 and CHIT1 as valuable markers of inflammation in COPD [40].

YKL-40 levels were investigated in various lung diseases complicated with inflammation and tissue remodeling in the pediatric age group. An early study reported increased serum YKL-40 levels in children with severe asthma compared to healthy controls [41]. However, another study reported YKL-40 levels were not correlated to asthma severity in the pediatric patients [42]. A third study reported significantly higher values of YKL-40 in children with intermittent asthma and persistent asthma than healthy controls. However, no correlation had been found with duration and severity of asthmatic disease [43]. Considering YKL-40 in asthma pathology, a recent study reported that increased YKL-40 levels were found in patients either with irreversible airway obstructions or with severe exacerbations [44]. Regarding the biomarker potential of YKL-40 in asthma, a recent study reported that serum YKL-40 levels in asthmatic patients might be important in assessing inflammatory phenotypes with clinical relevance [45].

After those many reports on circulating YKL-40 levels in COPD and asthma pathology, researchers suggested that YKL-40 levels may provide valuable clinical information in CF patients as a potential biomarker [9]. YKL-40 levels were found to be further elevated in severe exacerbations of CF [6]. A later study reported that serum YKL-40 levels were not elevated enough to detect the early phase of CF lung disease [8]. It is apparent that blood sampling is easier to obtain and non-invasive compared to obtaining BAL. Therefore, novel studies with larger number of patients that employ phenotype clustering (and account for disease severity) may provide valuable data for the potential of circulating YKL-40 as a biomarker in CF.

Interestingly, YKL-40 levels during exacerbation were negatively correlated with FVC% and FEV1 values of the same period in adult CF patients. FVC values which were < 50% were included in the statistical evaluation because the disease was more advanced in adult patients than in pediatric patients. Also, YKL-40 levels in the stable period were negatively correlated with % FEV1 in the same period.

In a similar manner, YKL-40 levels during exacerbation in pediatric CF patients were negatively correlated with FVC% and FEV1 values of the same period. Leonardi et al. showed that both sputum and serum YKL-40 levels in adult patients with CF were higher in the patients compared to healthy individuals. However, they suggested that sputum YKL-40 levels were more sensitive than serum levels for lung injury [6]. Coriati et al. found that lung function (FEV1) was strongly associated with high YKL-40 concentrations. They identified CF patients two distinct groups, according to YKL-40 serum concentration, as high and low YKL-40 group. The patients in the high YKL-40 group consisted...
of patients with a higher rate of *P. aeruginosa* colonization, deltaF508 homozygosity, CF-related disease and have received more systemic antibiotic treatment. Additionally, they found that lung function (FEV1) was strongly associated with YKL-40 concentrations. The patients in the high YKL-40 group with a worse clinical profile had lower FEV1 [46]. In our study, we found significantly higher YKL-40 levels in patients with homozygous phe508del mutation during exacerbation periods compared to other mutations like Coriati et al. Our findings suggest that plasma YKL-40 could be a potential biomarker of CF disease severity. In addition, while BAL is an invasive and difficult procedure, it is much easier to take a blood sample from the patient.

There was no correlation between CRP and WBC levels of the exacerbation period in adult and pediatric CF patients.

The low sample size and missing data from some of the patients are the biggest limitations of this study. Unfortunately, we were unable to perform some pulmonary function tests and collect blood from some of the patients for various reasons. Some patients were discharged without blood withdrawal or refused sampling, while some who resided in other cities did not return for their scheduled follow-up studies. In addition, pulmonary function tests could not be performed because some patients were unstable. For these reasons, we performed statistics with the available data.

The other important limitation of the study was a gene polymorphism that resulted with the absence of CHIT1 enzyme activity [29]. Ten plasma samples from the participants did not show any CHIT1 activity. Although we have not done any genetic testing for these 10 individuals, we excluded their data from the analysis for any potential possibility of being homozygotes for the inactivating polymorphism.

In conclusion, CHIT1 activity may be a valuable marker of chronic inflammation in adult CF patients who suffer from CF for a longer period compared to pediatric patients. The increase in YKL-40 levels in all patients compared to controls may point to a relationship between CF pathophysiology and YKL-40.

**AUTHOR CONTRIBUTION**

DBT and YO designed the study; clinicians evaluated the patients, collected clinical data, and obtained the blood samples; DBT did the experiments; YO and IL supervised the experiments; DBT and YO evaluated the data and wrote the manuscript.

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**DATA AVAILABILITY**

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**CODE AVAILABILITY**

Not applicable.

**DECLARATIONS**

**Ethical Approval** Ethical Approval was obtained from the Clinical Research Ethical Committee of Hacettepe University (2016/14). All the patients, controls, and caretakers of the pediatric patients provided informed consent. All the steps of the study conformed to the Helsinki Declaration and Good Clinical Practice guidelines.

**Consent to Participate** Approved by all authors.

**Consent for Publication** Approved by all authors.

**Conflict of Interest** The authors declare no competing interests.

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