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RESEARCH ARTICLE

Fungal colonization and its clinical associations in pediatric cystic fibrosis: A 12-year single-center cohort study

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ABSTRACT

Fungal colonization and its clinical associations in pediatric cystic fibrosis: A 12-year single-center cohort study

Introduction: Fungal organisms are increasingly isolated from respiratory samples of patients with cystic fibrosis (CF), yet their clinical relevance remains incompletely understood. This study aimed to assess the frequency of colonization and clinical characteristics of CF patients with fungal colonization.

Materials and Methods: We retrospectively reviewed CF patients followed at the Pediatric Pulmonology Division of Ege University between January 2011 and December 2022. Those with at least one respiratory culture analyzed for fungi were included. Fungal colonization was defined as the growth of the same species in at least 50% of cultures within one year. Demographic, clinical, spirometric, and microbiological data were evaluated.

Results: Among the 98 patients, median age was 9.6 years (1.7-32.1), and 54.1% were male. Fungal growth was detected in 51 patients (52.0%), and 37 (37.7%) met the criteria for colonization. *Candida albicans* (32.7%) and *Aspergillus fumigatus* (13.3%) were the most commonly isolated species. Patients with fungal colonization were older ($p < 0.001$), more likely to have chronic *Pseudomonas aeruginosa* colonization ($p < 0.001$), had more inhaled antibiotic use ($p < 0.001$), CF-related liver disease ($p = 0.018$), and had lower FEV₁% ($p = 0.005$) and body mass index z-scores (zBMI) ($p < 0.001$). Regression analyses identified lower zBMI (95% CI: 0.527-0.948; $p = 0.021$) and *P. aeruginosa* colonization (95% CI: 1.237-8.809; $p = 0.017$) as independent predictors of fungal colonization.

Conclusion: Fungal colonization is common in children with CF and is associated with older age, impaired lung function, lower zBMI, and *P. aeruginosa* co-colonization. These findings support the need for routine fungal surveillance and further prospective studies to clarify clinical implications. Further research is needed to determine whether these relationships are causal.

Key words: Cystic fibrosis; fungal colonization; pediatric

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ÖZ

Pediyatrik kistik fibrozis hastalarında mantar kolonizasyonu ve klinik ilişkileri: 12 yıllık tek merkez deneyimi

Giriş: Kistik fibrozisli (KF) hastaların solunum yollarından alınan kültürlerde mantar etkenlerinin izolasyonu ve mantar kolonizasyonu giderek artmaktadır. Ancak bu bulguların klinik önemi hala net olarak anlaşılmamıştır. Bu çalışmada KF'li çocuk hastalarda mantar kolonizasyonu sıklığı ve ilişkili klinik özelliklerin değerlendirilmesi amaçlanmıştır.

Materyal ve Metod: Ocak 2011-Aralık 2022 tarihleri arasında Ege Üniversitesi Çocuk Göğüs Hastalıkları Bilim Dalında KF'li çocuk hastaların dosyaları retrospektif olarak incelendi ve en az bir solunum yolu örneğinden mantar kültürü gönderilen hastalar çalışmaya dahil edildi. Mantar kolonizasyonu, bir yıl içinde alınan kültürlerin $\geq 50\%$ 'inde ya da ardışık iki örnekte aynı mantar türünün üremesi olarak tanımlandı. Pulmoner alevlenme sırasında alınan kültürler çalışma dışı bırakıldı. Hastaların demografik, klinik ve mikrobiyolojik verileri analiz edildi.

Bulgular: Toplam 98 hastadan elde edilen 584 örnek değerlendirildi. Hastaların ortalama yaşı 9.6 yıl (1.7-32.1) olup hastaların %54.1'i erkekti. Mantar üremesi 51 (%52) hastada, kolonizasyon ise 37 (%37.7) hastada saptandı. En sık izole edilen türler *Candida albicans* (%32.7) ve *Aspergillus fumigatus* (%13.3) idi. Kolonize hastalar anlamlı olarak daha yaşlıydı ve %FEV₁, %FVC, %FEF₂₅₋₇₅ değerleri ile vücut kitle indeksi z-skorları (zvki) daha düşüktü ($p \leq 0.05$). Kolonize hastalarda *Pseudomonas aeruginosa* varlığı, inhale antibiyotik kullanımı ve KF'ye bağlı karaciğer hastalığı daha sık gözlemlendi. Regresyon analizinde düşük zVKİ (GA: 0.527-0.948; $p = 0.021$) ve *P. aeruginosa* kolonizasyonu (GA: 1.237-8.809; $p = 0.017$) mantar kolonizasyonu için bağımsız risk faktörleri olarak belirlendi.

Sonuç: Kistik fibrozisli çocuklarda mantar kolonizasyonu nadir bir durum olmayıp, ileri yaş, düşük FEV₁%, düşük zVKİ ve *P. aeruginosa* kolonizasyonu ile ilişkilidir. Bu ilişkilerin nedensel olup olmadığını belirlemek için daha fazla çalışmaya ihtiyaç vardır.

Anahtar kelimeler: Kistik fibrozis; mantar kolonizasyonu; pediatri

INTRODUCTION

Cystic fibrosis (CF) is a multisystemic, autosomal recessive disorder primarily affecting the cystic fibrosis transmembrane conductance regulator (CFTR) gene, which encodes a chloride channel characterize the disease. As a result, dysfunction of this membrane transporter is involved in maintaining the electrochemical balance of epithelial cells, especially in the bronchi (1). This leads to thick secretions, impaired mucociliary clearance in the respiratory tract, and chronic bacterial and fungal colonization of the airways, resulting in subsequent epithelial inflammation and damage that finally leads to progressive lung function decline (1,2).

While bacterial pathogens such as *Pseudomonas aeruginosa* and *Staphylococcus aureus* have traditionally been considered the predominant microorganisms in CF airway infections, recent studies have revealed that fungi are also commonly isolated and exhibit a broader diversity than previously recognized (3-5). Fungal species, particularly *Aspergillus fumigatus*, *Candida albicans*, *Scedosporium apiospermum*, and *Exophiala dermatitidis*, have been increasingly identified in respiratory cultures of CF patients (4,5). However, the clinical relevance of isolating fungal species in respiratory culture from a patient with CF remains controversial (4). Some studies suggest that fungal colonization is associated with increased airway inflammation, accelerated decline in lung function, and a higher frequency of pulmonary exacerbations (6,7).

Similarly, the use of chronic oral antibiotics and inhaled antibiotics is found to be associated with the isolation of fungal species in CF specimens (8,9). Furthermore, distinguishing between colonization, infection, and allergic sensitization, such as allergic bronchopulmonary aspergillosis (ABPA), remains a major diagnostic challenge, and each requires markedly different management strategies (4,6).

Given the growing recognition of fungi as potential contributors to CF lung disease, better characterization of the fungal microbiota and its clinical impact is needed. In particular, the clinical interpretation of fungal isolation in CF remains a challenge, partly due to the limited availability of national epidemiological data (4). In Türkiye, as in many countries, there is a paucity of large-scale, population-based studies assessing the prevalence, species distribution, and clinical impact of fungal organisms in CF patients (10,11). According to the 2013 Annual Report of the CF Foundation Patient Registry, the median prevalence of patients with positive respiratory cultures for *Aspergillus* species and *Candida* species was 11.9% and 7.8%, respectively (10,11). To date, the national CF registry in Türkiye lacks specific documentation regarding fungal species isolated from respiratory specimens, highlighting a critical gap in epidemiological surveillance (10). Moreover, the absence of standardized protocols for collecting, processing, and analyzing respiratory specimens further complicates inter-center comparisons and clinical decision-making. In this context, generating robust national data

constitutes a crucial first step toward understanding the true burden of fungal colonization and infection in the CF population. Establishing a reliable local evidence base will facilitate the development of standardized diagnostic approaches and improve the clinical management of affected patients.

The aim of this study was to identify the fungal species isolated from respiratory cultures in children with CF, to determine their frequency of isolation, to compare the demographic and clinical characteristics of patients with and without fungal colonization, and to evaluate potential risk factors associated with fungal colonization.

MATERIALS and METHODS

Study design and population

This retrospective cohort study was conducted at the Pediatric Pulmonology Division of Ege University. Medical records of the patients diagnosed with CF and followed between January 2011 and December 2022 were reviewed. Inclusion criteria consisted of a confirmed diagnosis of CF and at least one respiratory tract specimen [sputum, oropharyngeal (OP)], or bronchoalveolar lavage (BAL) sent for fungal culture during routine follow-up. Patients with no available fungal culture results or incomplete medical records were excluded.

Written informed consent was obtained from all parents and children older than 12 years of age. The local Ethics Committee of Ege University approved the study (23-4.1T/25), and the study was conducted in compliance with the principles of the Declaration of Helsinki.

Fungal colonization was defined as the presence of fungi in at least two respiratory cultures within a 12-month period, with at least 50% of cultures in that year testing positive for fungal growth (4). To minimize confounding by acute infection, respiratory specimens collected during episodes of pulmonary exacerbation were excluded from the analysis.

Fungal culture

Airway specimens were obtained during routine clinical care and processed in accordance with standard microbiological protocols at the university hospital's microbiology laboratory, which included testing for bacterial organisms identified based on their macroscopic and microscopic morphology. Bacterial and fungal cultures were consistently per-

formed on all sputum and BAL samples. Since OP cultures are not routinely plated on fungal media at our institution, OP results were included in the analysis only when fungal growth was reported.

Clinical characteristics

Data on demographic and clinical data included age, sex, anthropometric measurements, genotype, CF-related conditions (pancreatic sufficiency, liver disease, diabetes mellitus, ABPA), medical treatment (inhaled corticosteroid, inhaled antibiotics), and the presence of chronic bacterial colonization before the first fungal identification/colonization. For patients who were able to perform spirometry, the available acceptable-quality spirometric results were recorded at the time of the first fungal culture submission, at the first positive fungal growth (if present), and at the most recent follow-up were collected. If multiple spirometry measurements were available within the same year, the spirometry performed closest to the date of the first fungal culture submission (or the first positive fungal growth, when applicable) was included in the analysis.

Body mass index (BMI) was calculated with the weight (kilograms)/height (m^2) formula, and BMI z-scores (zBMI) were recorded according to the percentile curve for Turkish children (9). CF genotypes were grouped as the delta F508 mutation (homozygous or heterozygous) and other mutations. Pancreatic exocrine insufficiency was defined as fecal elastase levels $<200 \mu g/g$ and the use of pancreatic enzymes, and the presence of microorganism colonization was confirmed by positive respiratory cultures in at least half of the previous year's cultures (12).

Pulmonary function test

The pulmonary function test was performed using spirometry (Flowhandy ZAN 100, Germany), following the American Thoracic Society standards, to measure forced expiratory volume in 1 second (FEV_1), forced vital capacity (FVC), and forced expiratory flow during the middle half of FVC (FEF_{25-75}) (13). Results were expressed as percent predicted (%) values. For patients with fungal colonization, ΔFEV_1 was calculated as the difference between the most recent FEV_1 and the FEV_1 at the time of first fungal growth. For non-colonized patients, ΔFEV_1 was calculated as the difference between the most recent FEV_1 and the FEV_1 at the time of the first fungal culture submission.

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics 26.0. Descriptive data were presented as numbers (%) for categorical variables and as mean $\pm$ standard deviation (SD) or median [interquartile range (IQR)] for continuous variables, depending on the data distribution, as assessed by the Shapiro-Wilk test. Group comparisons were conducted using the Pearson χ^2 test or Fisher's exact test for categorical variables, and the student's t-test, Mann-Whitney U test, or Kruskal-Wallis test for continuous variables, as appropriate. Univariate and multivariate logistic regression analyses were used to identify factors associated with fungal colonization, with results reported as odds ratios (OR) and 95% confidence intervals (CIs). A $p < 0.05$ was considered statistically significant.

RESULTS

The medical records of 128 pediatric patients with a confirmed diagnosis of CF who were followed at our

department during the study period were retrospectively assessed. Exclusion criteria included insufficient clinical documentation ($n = 3$), lack of regular follow-up ($n = 5$), and absence of fungal culture data ($n = 22$).

Clinical characteristics

A total of 584 respiratory specimens—including 567 sputum or OP and 17 BAL samples—obtained from 98 children with CF were evaluated for fungal culture. Median age of the study population was 9.6 years (1.7-32.1), and 53 patients (54.1%) were male.

At least one episode of fungal growth was detected in 51 patients (52.0%), with a total of 116 fungal isolates, representing 20.4% of all samples. Median age at the time of first fungal isolation was 10.4 years (0.25-23.4). The most frequently isolated species were *C. albicans* in 32 patients (32.7%), followed by *A. fumigatus* in 13 (13.3%), *Aspergillus flavus*, *Aspergillus niger*, and *Candida parapsilosis* each in 8 (8.2%) patients (Table 1).

Table 1. Prevalence rates of molds and yeasts of CF patients with positive fungal culture results*

Fungal species	n (%)
Molds	
<i>Aspergillus fumigatus</i>	13 (13.3)
<i>Aspergillus flavus</i>	8 (8.2)
<i>Aspergillus niger</i>	8 (8.2)
<i>Aspergillus terreus</i>	7 (7.1)
<i>Scedosporium apiospermum</i>	2 (2.0)
<i>Geotrichum candidum klebahnii</i>	2 (2.0)
<i>Penicilium</i> spp.	2 (2.0)
<i>Trichosporon</i> spp.	2 (2.0)
<i>Alternaria alternata</i>	1 (1.0)
<i>Saprochaete capitata</i>	1 (1.0)
<i>Geotrichum capitatum</i>	1 (1.0)
<i>Purpureocillium lilacinum</i>	1 (1.0)
<i>Pseudallescheriae boydii</i>	1 (1.0)
Yeasts	
<i>Candida albicans</i>	32 (32.7)
<i>Candida parapsilosis</i>	8 (8.2)
<i>Candida dubliniensis</i>	7 (7.1)
<i>Candida tropicalis</i>	4 (4.1)
<i>Candida kefyr</i>	3 (3.1)
<i>Candida lusitanae</i>	3 (3.1)
<i>Candida glabrata</i>	2 (2.0)

Table 1. Prevalence rates of molds and yeasts of CF patients with positive fungal culture results* (continue)	
Fungal species	n (%)
<i>Candida famata</i>	2 (2.0)
<i>Candida boidinii</i>	1 (1.0)
<i>Candida lipolytica</i>	1 (1.0)
<i>Candida inconspicua</i>	1 (1.0)
<i>Candida pelliculosa</i>	1 (1.0)
<i>Candida krusei</i>	1 (1.0)
*Patients may have more than one isolate/sample.	

Comparison of patients based on fungal colonization

Fungal colonization was identified in 37 patients (37.7%) based on the evaluation of 288 respiratory specimens, including 278 sputum or OP and 10 BAL samples. The comparisons of patients with and without fungal colonization are shown in Table 2. Patients with fungal colonization were significantly older than those without (median age: 17.3 vs. 7.5 years,

$p < 0.001$). Mean $FEV_1\%$, FVC%, and $FEF_{25-75}\%$, as well as zBMI, were significantly reduced in fungal colonized compared to non-colonized patients ($p = 0.001$, $p = 0.003$, $p = 0.001$, and $p < 0.001$, respectively). Moreover, CF-related liver disease, colonization with *P. aeruginosa*, and use of inhaled antibiotics were more frequently observed among colonized patients ($p = 0.018$, $p < 0.001$, and $p < 0.001$, respectively).

Table 2. Comparison of patients with and without fungal colonization			
	Fungal colonization, no (n= 61)	Fungal colonization, yes (n= 37)	p
Age, years, median (IQR)	3.5 (4.7-12.4)	10.4 (5.3-15.1)	<0.001 ^a
Sex, male, n (%)	36 (60.6)	16 (26.2)	0.070 ^b
Age at diagnosis, years, median (IQR)	0.33 (0.17-0.75)	0.4 (0.1-4.5)	0.607 ^a
zBMI, median (IQR)*	-0.1 (-0.9-0.69)	-1.23 (-2.6-(-0.6))	<0.001 ^a
Mutations, n (%)			
F508del homozygous	8 (13.8)	6 (16.2)	0.339 ^b
F508del heterozygous	14 (24.1)	11 (29.7)	0.280 ^b
Colonization, n (%)			
<i>Staphylococcus aureus</i>	11 (18)	10 (27)	0.319 ^b
MRSA	3 (4.9)	4 (10.8)	0.421 ^b
<i>Pseudomonas aeruginosa</i>	12 (19.6)	21 (56.7)	<0.001 ^b
CF-related disease, n (%)			
Pancreatic insufficiency	51 (83.6)	35 (94.6)	0.108 ^b
Liver disease	3 (4.9)	8 (21.6)	0.018 ^b
Diabetes mellitus	2 (3.3)	4 (10.8)	0.195 ^b
ABPA	2 (3.3)	5 (13.5)	0.056 ^b
Medical treatment			
Inhaled corticosteroid	10 (16.4)	9 (14.7)	0.430 ^b
Inhaled antibiotic	11 (18)	25 (40.9)	<0.001 ^b

Table 2. Comparison of patients with and without fungal colonization (continue)

	Fungal colonization, no (n= 61)	Fungal colonization, yes (n= 37)	p
Spirometry, mean $\pm$ SD	n= 34	n= 29	
ppFEV ₁	86.7 $\pm$ 20.7	65.5 $\pm$ 27.4	0.005^c
ppFVC	78.1 $\pm$ 18.7	61.8 $\pm$ 22.8	0.011^c
ppFEV ₁ /FVC	111.2 $\pm$ 6.9	103.9 $\pm$ 13.5	0.014^c
ppPEF	84 $\pm$ 28.2	67.3 $\pm$ 22.2	0.028^c
ppFEF ₂₅₋₇₅	96.8 $\pm$ 37.0	62.6 $\pm$ 36.7	0.001^c
Δ FEV ₁	1.2 $\pm$ 8.6	-0.5 $\pm$ 7.4	0.417 ^c
Δ FVC	1.4 $\pm$ 7.7	1.2 $\pm$ 7.3	0.670 ^c

ABPA: Allergic bronchopulmonary aspergillosis, BMI: Body mass index, DM: Diabetes mellitus, FEF₂₅₋₇₅: Forced expiratory flow at 25-75% of forced vital capacity, FEV₁: Forced expiratory volume in the first second, FVC: Forced vital capacity, IQR: Interquartile range, MRSA: Methicillin resistant *Staphylococcus aureus*, pp: Percent predicted, SD: Standard deviation.

^aGroup comparisons were done using Mann-Whitney U test, ^bComparison of categorical data was done using χ^2 test, ^cGroup comparisons were done using Student's t test.

Twelve patients (32.4% of those colonized) harbored more than one fungal species. The most frequently colonizing organisms were *C. albicans* (n= 26, 55.3%), *A. fumigatus* (n= 8, 17.0%), *C. dubliniensis* and *A. terreus* (n= 5, 10.6% each), and *A. flavus* (n= 4, 8.5%) (Table 3).

Regression analysis

In univariate logistic regression analyses, *P. aeruginosa* colonization (OR: 5.359; 95% CI: 2.165-

13.266; p< 0.001), lower zBMI (OR: 0.646; 95% CI: 0.486-0.859; p= 0.003), and CF-Liver disease (OR: 5.333; 95% CI: 1.315-21.620; p= 0.019) were significantly associated with fungal colonization in children with CF (Table 3). In the multivariate model, zBMI (OR: 0.707; 95% CI: 0.527-0.948; p= 0.021) and *P. aeruginosa* colonization (OR: 3.301; 95% CI: 1.237-8.809; p= 0.017) remained independently associated with fungal colonization in children with CF (Table 4).

Table 3. Microbiological distribution of patients with fungal colonization

Fungal species	n	%
<i>Candida albicans</i>	15	40.54
<i>Aspergillus fumigatus</i>	4	10.81
<i>Candida dubliniensis</i>	2	5.41
<i>Aspergillus terreus</i>	2	5.41
<i>Candida albicans</i> + <i>Candida parapsilosis</i>	2	5.41
<i>Aspergillus flavus</i>	1	2.70
<i>Candida parapsilosis</i>	1	2.70
<i>Candida albicans</i> + <i>Candida dubliniensis</i>	1	2.70
<i>Candida albicans</i> + <i>Aspergillus fumigatus</i>	1	2.70
<i>Candida albicans</i> + <i>Aspergillus flavus</i>	1	2.70
<i>Candida albicans</i> + <i>Aspergillus terreus</i>	1	2.70
<i>Candida albicans</i> + <i>Candida dubliniensis</i> + <i>Aspergillus fumigatus</i>	1	2.70
<i>Candida albicans</i> + <i>Aspergillus fumigatus</i> + <i>Aspergillus terreus</i>	1	2.70
<i>Candida albicans</i> + <i>Aspergillus flavus</i> + <i>Aspergillus terreus</i>	1	2.70
<i>Candida albicans</i> + <i>Aspergillus flavus</i> + <i>Candida krusei</i>	1	2.70
<i>Candida albicans</i> + <i>Aspergillus fumigatus</i> + <i>Candida tropicalis</i> + <i>Saprochaeta capitata</i>	1	2.70
<i>Candida dubliniensis</i> + <i>Aspergillus fumigatus</i> + <i>Candida lusitanae</i>	1	2.70

Table 4. Multivariate predictors of fungal colonization in children with cystic fibrosis				
	Univariant		Multivariant	
	OR (95% CI)	p	OR (95% CI)	p
Age	1.062 (0.986-1.144)	0.112		
Age at diagnosis	1.014 (0.922-1.115)	0.779		
<i>Pseudomonas aeruginosa</i>	5.359 (2.165-13.266)	<0.001	3.301 (1.237-8.809)	0.017
zBMI	0.646 (0.486-0.859)	0.003	0.707 (0.527-0.948)	0.021
CF-Liver disease	5.333 (1.315-21.620)	0.019	3.598 (0.779-16.611)	0.101
CI: Confidence interval, BMI: Body mass index, CF: Cystic fibrosis.				

DISCUSSION

In this retrospective cohort of pediatric patients with CF, fungal species were isolated from nearly half of the participants, and over one-third met the pre-defined criteria for fungal colonization. The most commonly isolated fungi were *C. albicans* and *A. fumigatus*, mirroring prior studies reporting these organisms as dominant members of the CF airway mycobiome. Notably, fungal colonization was associated with older age, worse FEV₁%, CF-liver disease, lower zBMI, and increased prevalence of *P. aeruginosa* colonization — findings that align with the growing literature suggesting fungi may contribute to CF lung disease. Additionally, lower zBMI and *P. aeruginosa* colonization are independent predictors of fungal colonization in children with CF.

The prevalence of fungal colonization among the children with CF in our cohort was 37.7%, which is nearly three times higher than rates reported in many developed countries (10,11). This discrepancy may be attributed to several context-specific factors commonly observed in developing nations, including suboptimal standards of care, limited access to advanced antifungal diagnostics and therapies, inadequate nutritional support, and a higher prevalence of chronic *P. aeruginosa* colonization—all of which may contribute to an increased risk of fungal persistence and overgrowth in the airways. Similarly, a prior study by Haiko et al. also observed the coexistence of *Candida* and *P. aeruginosa* in adult CF patients, suggesting that age-related airway remodeling and cumulative antimicrobial exposure may favor fungal persistence (14). Our data support this view, as fungal colonization was more frequent among patients with chronic *P. aeruginosa* colonization and those receiving inhaled antibiotics.

The use of inhaled antibiotics and decreased FEV₁% are associated with the isolation of fungi from

sputum samples (7,15). Consistent with these findings, our study demonstrated that children with CF who were colonized by fungi were more likely to be on inhaled antibiotics and had lower FEV₁% values. In contrast, the relationship between older age and fungal isolation remains inconclusive, as existing studies have reported both increased and decreased rates of fungal detection associated with this factor (15,16). In our cohort, colonized children were significantly older than their non-colonized peers; however, age was not identified as an independent predictor of fungal colonization in regression analysis. These findings underscore the need for prospective studies to clarify the potential roles of age, lung function, and inhaled antibiotic exposure in the development and persistence of fungal colonization in CF populations.

Prior studies showed no significant association between BMI and fungal colonization (5,6). Although not entirely unexpected, our study presents a relatively underexplored finding by demonstrating that CF children with fungal colonization exhibited significantly lower zBMI compared to non-colonized peers. This association suggests that impaired nutritional status may not only be a marker of disease severity but could also predispose patients to fungal persistence in the airways. Malnutrition may compromise mucosal immunity and alter the local airway environment, thereby facilitating fungal colonization. On the other hand, CF patients with malnutrition were significantly more frequently colonized by *P. aeruginosa* and fungi (17). While existing literature has primarily focused on the link between lung function and fungal isolation, the relationship between nutritional deficits and fungal burden in the absence of bacterial colonization remains insufficiently studied. Our findings highlight the need to consider nutritional status as a potential risk factor in future investigations on airway mycobiota in CF.

Notably, our study demonstrated that CF-related liver disease was more frequently observed in children with fungal colonization. This finding aligns with a retrospective study by Cabaret et al., which reported that co-colonization with fungal and bacterial pathogens was independently associated with liver disease in CF patients (18). Similarly, a study conducted in Türkiye among adult CF patients found a significantly higher prevalence of fungal colonization in those with hepatobiliary disease compared to patients with other comorbidities (6). Despite these emerging associations, current evidence remains limited, a plausible explanation may involve the interplay between gut dysbiosis and dysregulated innate immunity, both commonly described in CF (19). Prolonged intestinal transit, frequent antibiotic exposure, and pancreatic enzyme therapy can exacerbate dysbiosis, leading to increased systemic inflammation. Fungal colonization may serve as an additional inflammatory trigger, potentially promoting hepatic inflammation and fibrosis through the gut–liver axis. Further epidemiological studies are warranted to explore this relationship.

Although *A. fumigatus* remains the most frequently studied filamentous fungus in CF, our study also identified multiple other species, including *A. flavus*, *A. niger*, and *A. terreus*, reinforcing the need for species-level identification and broader fungal surveillance in CF microbiology. Previous studies have demonstrated that different fungal species exhibit varying levels of virulence, immune interactions, and responses to treatment, underscoring the clinical significance of accurate fungal speciation (3,20,21). Therefore, identifying fungal species at the species level is not just a laboratory detail but may be a clinically important step.

This study has several limitations. First, its retrospective design limits causal inference regarding the observed associations between fungal colonization and clinical outcomes. Although our results suggest a correlation between colonization and pulmonary deterioration, the temporal sequence remains unclear. Second, fungal cultures were obtained during routine clinical visits, rather than through standardized protocols, which introduces potential variability and underestimation of fungal burden. Additionally, we excluded cultures obtained during pulmonary exacerbations to minimize bias; however, this may have limited our ability to detect clinically relevant fungi. Third, fungal identification was based solely on culture-based methods, which are known to have limited sensitivity for some

filamentous and non-culturable species. The use of molecular diagnostic techniques, such as next-generation sequencing or polymerase chain reaction, might have revealed a broader spectrum of fungal organisms and clarified fungal-bacterial dynamics in polymicrobial infections. We also lacked data on fungal-specific immune responses (e.g., IgE, eosinophilia, or skin testing), ABPA status, or antifungal treatment history, all of which could influence clinical expression and outcomes. Finally, the sample size, while reasonable, limits subgroup analyses by specific fungal species or coinfections.

Despite these limitations, our study contributes to the growing body of evidence that fungal colonization in children with CF is not benign. The observed associations between colonization and reduced lung function, nutritional impairment, and increased prevalence of *P. aeruginosa* support a potential role of fungi in disease progression.

CONCLUSION

In conclusion, our findings suggest that fungi may make a meaningful contribution to CF airway disease. Routine fungal surveillance may help identify high-risk patients who might benefit from closer monitoring or antifungal interventions. Future multicenter, prospective studies incorporating biomarkers of fungal activity, host immune response, and antifungal efficacy are essential to develop risk-stratification tools and guide evidence-based treatment decisions in comprehensive CF care.

Ethical Committee Approval: This study was approved by Ege University Faculty of Medicine Clinical Research Committee (Decision no: 757, Date: 25.01.2023).

Peer-review: Externally peer-reviewed.

CONFLICT of INTEREST

The authors declare that they have no conflict of interest.

AUTHORSHIP CONTRIBUTIONS

Concept/Design: EO, GKÖ

Analysis/Interpretation: EH, BGD

Data acquisition: FÇ, MB

Writing: EO

Clinical Revision: MMÖ, AÖ

Final Approval: FG

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