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The Spectrum of Fungal Colonization and Their Attributable Effects on Cystic Fibrosis Patients with Rare CFTR Genetic Mutations

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Abstract: Chronic airway colonization by bacteria and fungi is very common in CF patients, causing irreversible lung damage. It is known that rates of fungal infections are much lower than those of bacterial infections, however they can worsen the medical condition of CF patients. In this study, we identify the most common fungal species isolated from 31 adult CF patients in Qatar and analyze their correlation with lung function, pulmonary exacerbations, bronchial asthma, and pancreatic insufficiency. Mycological evaluation, as well as medical records, were reviewed for the patients regularly under the adult CF service at Hamad General Hospital in the period between 2017–2019. All CF patients included in this study carry rare CFTR mutations. The majority of those patients (n = 25) carried the c.3700A>G; I1234V mutation, whereas three patients carried the heterozygous mutation (c.1657C>T and c.1115A>T) and the remaining three carried the homozygous mutation (c.920G>A). Twenty-two of the adult CF participants (70.9%) were colonized with fungal species regardless of the type of the CFTR mutation. *Candida* and *Aspergillus* species were the most common, colonizing 81% and 45% of the patients, respectively. For *Candida* colonized patients, *Candida dubliniensis* was the most frequently reported species (55.6%), whereas *Aspergillus fumigatus* colonization was the most common (50.0%) among *Aspergillus* colonized patients. These identified fungal pathogens were associated with poor lung function, pancreatic insufficiency, and asthma in this cohort. Such colonization could possibly aggravate the most known CF complications, notably pulmonary exacerbations, asthma, and pancreatic insufficiency.

Keywords: cystic fibrosis; CF; I1234V CFTR mutation; fungal colonization; bronchial asthma; pancreatic insufficiency

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1. Introduction

Cystic fibrosis (CF) is an inherited autosomal recessive disease due to specific mutations within the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene. The gene encodes a protein responsible for regulating chloride ion transportation in the epithelium. Over 2000 mutations within the CF gene have been identified, with ΔF-508 being the most common mutation [1]. The Middle East is heavily affected by the disease, seemingly due to the high consanguinity rate as traditional of tribes. While rare worldwide, pIle1234Val (I1234V) mutation has been reported to be the most common mutation in the Bedouin tribes in the Middle East, and the most common in Qatar [2,3]. In Qatar, the most updated number of CF patients is estimated to be 82 patients, of which 34 are adults and the

rest are children under 18 years old [4]. Although the majority of those CF patients possess the same mutation, they show striking variations in their clinical manifestations [4,5].

The CFTR protein dysregulation significantly affects the lungs in addition to other organs such as the pancreas. Pulmonary and pancreatic insufficiency results from reduced airway surface liquid, leading to the accumulation of thick desiccated mucus that blocks airway passage and pancreatic ducts, respectively. Such sticky and thick mucus results from the dysfunction of the chloride channel, which lead to a decrease in water content in the mucus layer lining the mucosal cells, causing mucosal dehydration and mucous plug formation [6]. Notably, the thick and dry mucus will create a low oxygen environment, which is ideal for microbial infections. Bacterial colonization, biofilm formation, and resistance development are very common in CF patients, however, fungal colonization has also been reported in several cases [7]. Certain fungal pathogens commonly cause pulmonary infections in CF patients such as *Candida* and *Aspergillus* species [8]. Due to the chronic bacterial and fungal infections, CF patients experience multiple pulmonary exacerbations leading to pulmonary morbidity and mortality.

While the persistence of *Candida dubliniensis*, and its association with lung function and nutritional status in CF patients, had been addressed in a microbiological study performed in Qatar, the other common types of fungal colonization and their association with the disease parameters and risk factors have not been elucidated to date. Therefore, in the present study, we aim to describe the spectrum of fungal colonization in adult CF patients, predominantly carrying the homozygous 1234V mutation, as well as other rare mutations, and explore its association to the clinical course of CF.

2. Materials and Methods

2.1. Patient Population and Ethical Approvals

We conducted a descriptive study on data collected on adult CF patients at Hamad Medical Corporation (HMC), the main provider of tertiary CF care in Qatar. IRB approvals for the use of patients' data were received from both HMC and Qatar University with approval numbers 17105/17 and QU-IRB 1227-E/20.

2.2. Data Source

Data from HMC Cerner medical records were extracted from the period of 2017–2019. This data covered demographic, microbiological colonization, pulmonary exacerbations, the coexistence of bronchial asthma, and pancreatic insufficiency of all adult CF patients in the registry.

2.3. Variables of Interest

The majority of patients included in this study had two to four sputum cultures in one year, of which more than 50% grew fungal isolates. These fungal isolates were reported as colonization, as their persistence was confirmed. Persistence was based on the definition from Brandt et al. (2018), which is the detection of fungi culture once or more within a year [9]. The majority of the sputum cultures were taken during routine outpatient follow up, while others were taken at inpatient admission. Identification of *Candida* species was through matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), whereas *Aspergillus* species were majorly identified by morphology (a few identified by MALDI-TOF MS). These data were then analyzed to understand the relationship between fungal status and explanatory variables of interest.

2.4. Lung Function Data

Spirometry was performed using the standard protocol recommended by the American Thoracic Society [10] for each patient. Data included percentage predicted forced vital capacity (ppFVC), percentage predicted forced expiratory volume in one second (ppFEV1), and maximum expiratory flow at 50% and 25% of vital capacity (FEF25–75). Spirometry results were described as the percentage of the predicted values based on reference values

of pulmonary function tests, with the cut-off for normal lung function defined at 80% for ppFEV1 and ppFVC [11]. The highest pulmonary function recorded over the past two years, excluding measurements taken during a pulmonary exacerbation, was chosen. An exacerbation was defined as a new or increased cough, sputum production, dyspnea on exertion or rest, increased fatigue, decreased appetite, change in sputum appearance, fever, and/or an isolated drop in ppFEV1 of 10% from baseline [12]. The frequency of exacerbation was calculated over a period of one year for the study.

2.5. Genetics and Diagnosis of Complications

All suspected CF patients in Qatar are first screened for I1234V, as it is the predominant genetic mutation. In the case of negative screening, an extended panel of 12 and 34 gene variants is carried out. Finally, complications were diagnosed by clinicians at HMC and extracted as part of patient medical records.

2.6. Statistical Methods

This study described the characteristics of the CF adult population in Qatar. It outlined the different variables associated with fungal colonization in order to understand the nature of this disease and its clinical outcomes in the given population. Additionally, subgroup analysis was conducted to identify any associations between different subgroups of interest within this population. To do this, we used Fisher's exact test to examine statistical significance in categorical data. For the majority of patients with fungal colonization (n = 12, 54.5%), more than one fungal species was isolated, which was referred to as fungal co-colonization, and was considered as separate isolates during analysis. Categorical variables were summarized and presented as frequencies and proportions. When presenting them in the form of charts, the proportion was used instead of the frequency as percentages give a better understanding of the population as well. Both can be misleading, as mentioned late in the discussion. Numerical data, such as lung function, was not normally distributed, thus we reported medians and interquartile ranges (IQRs) in the format of median (IQR). To present such data, box plots were used to show medians, upper and lower quartiles (thus illustrate IQR), and ranges. The Wilcoxon rank-sum test was used to generate the associated *p*-value and a *p*-value lower than 0.05 was considered statistically significant.

3. Results

3.1. Demographic Data of Adult CF Patients in Qatar

A total of 31 adult patients with CF were under the regular care of adult CF service at HMC, Qatar. The demographic analysis was performed on this cohort, where we found that of these patients, only three were non-Qataris, while the rest were nationals belonging to a single Qatari tribe. Moreover, our analysis showed that the median age of patients was 24 years old, where the youngest patient was 18 and the eldest was 42 (Table 1). Furthermore, the vast majority of patients (n = 12) fell in the 20 to 24-year-old cohort. In addition, the median Body Mass Index (BMI) of the adult CF patients was found to be 26.7 (IQR: 20.5–29.6) (Table 1). Importantly, the majority of these patients (n = 25; 81%) harbored the I1234V (c.3700A>G) CFTR mutation, whereas the rest carried other, rarer CFTR mutations. Three patients carried a heterozygous CFTR mutation (c.1657C>T and c.1115A>T), whereas another three carried the homozygous mutation (c.920G>A) (Table 1).

Table 1. Demographic data of adult CF patients with rare CFTR mutations in Qatar.

Demographic Data	Patients Colonized with Fungi	Patients Not Colonized with Fungi	All CF Patients
Genetic mutation: n (%)			
I1234V (c.3700A>G)	18 (81.8)	7 (77.8)	25 (80.6)
Heterozygous R553X (c.1657C>T and c.1115A>T)	3 (13.6)	0	3 (9.7)
Homozygous (S307N(c.920G>A))	1 (4.5)	2 (22.2)	3 (9.7)
Nationality: n (%)			
Qatari	19 (86.4)	9 (100.0)	28 (90.3)
Non-Qatari	3 (13.6)	0	3 (9.7)
Age: median	24.5	24	25.5
Age group: n (%)			
≤20	3 (13.6)	2 (22.3)	5 (16.2)
>20–25	9 (40.9)	3 (33.3)	12 (38.7)
>25–30	5 (22.7)	4 (44.4)	9 (29.0)
>30–35	2 (9.1)	0	2 (6.5)
>35–40	2 (9.1)	0	2 (6.5)
>40–45	1 (4.5)	0	1 (3.2)
BMI: median	26.6	29.8	26.6
BMI classes: n (%)			
Underweight	2 (9.1)	0	2 (6.5)
Normal	7 (31.8)	4 (44.4)	11 (35.5)
Overweight	11 (50.0)	1 (11.1)	12 (38.7)
Obese (class I)	2 (9.1)	2 (22.2)	4 (12.9)
Obese (class II)	0	2 (22.2)	2 (6.5)
Total	22	9	31 (100)

3.2. Fungal Colonization in CF Patients

Twenty-two patients (70.9%) in our study population tested positive for fungal colonization. Eighteen of these patients carried the I1234V CFTR mutation, three carried the heterozygous (c.1657C>T and c.1115A>T) mutation, and one patient carried the homozygous (c.920G>A) mutation (Table 1). The two most common fungal pathogens were the *Candida* species (18 patients; 58.0%) followed by the *Aspergillus* species (10 patients; 32.2%) (Figure 1; Table S1). Within the 18 *Candida* patients, most cases were positive for *C. dubliniensis* (55.6%) followed by *C. albican*s (44.4%), *C. tropicalis* (11.1%), and *C. parapsilosis* (5.5%). Several other *Candida* species were also sporadically reported in 27.8% of the cases (Figure 1; Table S1). Conversely, the 10 patients who presented with *Aspergillus* colonization showed the highest frequency for *A. fumigatus* (50.0%) and *A. nige*r (20%). The rest of the patients (40%) had different species of *Aspergillus* (Figure 1; Table S1). Additionally, only two patients (9%) had positive cultures with *Curvularia* and *Exserohilum* species (Figure 1; Table S1). Upon further investigation, our analysis revealed that more than one fungal species is colonized within individual patients (n = 12, 38.7%). Eight patients had two co-colonizing fungal species, whereas three patients had three fungal co-colonizing species, and one patient had four co-colonizing fungal species (Table S2). No specific combination of fungal co-colonization was noticed. In addition, we discovered that the majority of patients with fungal colonization (n = 11) were overweight with a BMI > 25, whereas most patients (n = 4) who did not have fungal colonization were within the normal BMI range (Table 1).

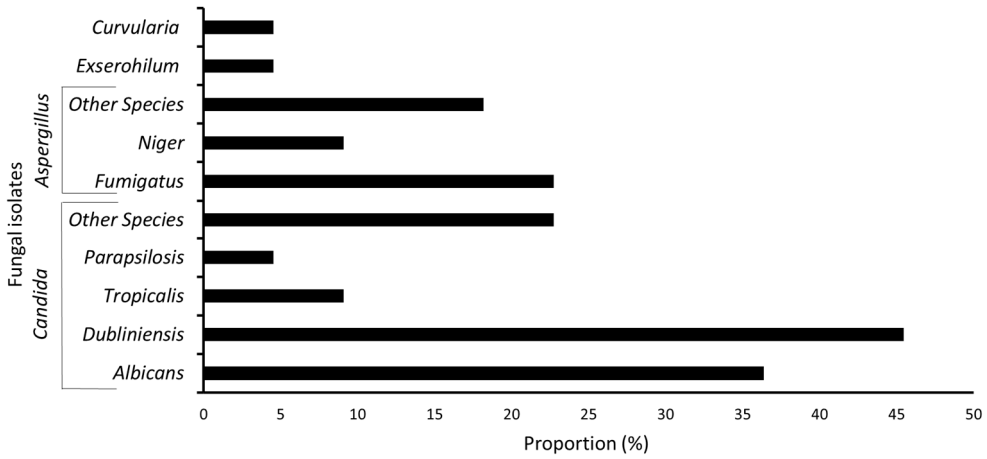

Figure 1. Identified fungal species within CF patients carrying rare genetic mutations. Bar chart illustrating the proportions of fungal species identified within the adult CF population in Qatar.

3.3. Lung Function in the Presence of Fungal Colonization

Our analysis showed differences in ppFEV1, ppFVC, and the number of pulmonary exacerbations values between the fungal and non-fungal group (Figure 2). Both ppFEV1 and ppFVC were lower in the fungal colonized group as compared to the non-colonized one. Indeed, the median ppFEV1 in both *Candida* and *Aspergillus* patients was 71%, while that of the non-fungal colonization group was 74% (Table S3). Similarly, the non-fungal group showed a better ppFVC profile (higher IQRs; upper quartile: 86.5%; lower quartile: 70.0%) in comparison to the fungal group (lower IQRs; upper quartile: 82.3; lower quartile: 67.8%) (Figure 2; Table S3). Specifically, *Candida* colonized patients showed the worst ppFVC median (73%), while the non-fungal group median was at 75% for example (Figure 2A Left Panel).

Further analysis of fungal subspecies showed that patients colonized with *Candida parapsilosis* and *tropicalis* had the lowest overall median ppFEV1 of 46.0% and 52.5%, respectively (Figure 2A Middle Panel, Table S3). Likewise, the ppFVC values in those patients were 45.0% for *Candida parapsilosis* and 54.0% for *Candida Tropicalis* (Figure 2B Middle Panel, Table S3). On the other hand, among the *Aspergillus* colonized patients, *A. niger* colonization caused the lowest median ppFEV1 and ppFVC values of 61.5% and 67%, respectively (Figure 2B Right Panel, Table S3).

With regard to pulmonary exacerbations (Figure 2C), surprisingly, patients with no fungal colonization had the highest number of pulmonary exacerbations (n = 7), followed by those colonized with *Aspergillus* (n = 6.5), *Candida* (n = 5.5), and finally other fungal species (n = 4.5) (Figure 2C Left Panel). Despite the fungal colonized patients having a better median frequency of pulmonary exacerbations, there were higher upper quartile values in this group as compared to the non-fungal group, indicating a trend of worse lung function and more frequent exacerbations in these patients (Figure 2C and Table S3). Indeed, the highest frequency of pulmonary exacerbations (n = 8) was recorded in patients colonized with *Candida dubliniensis* and other *Aspergillus* species (Figure 2C Right Panel).

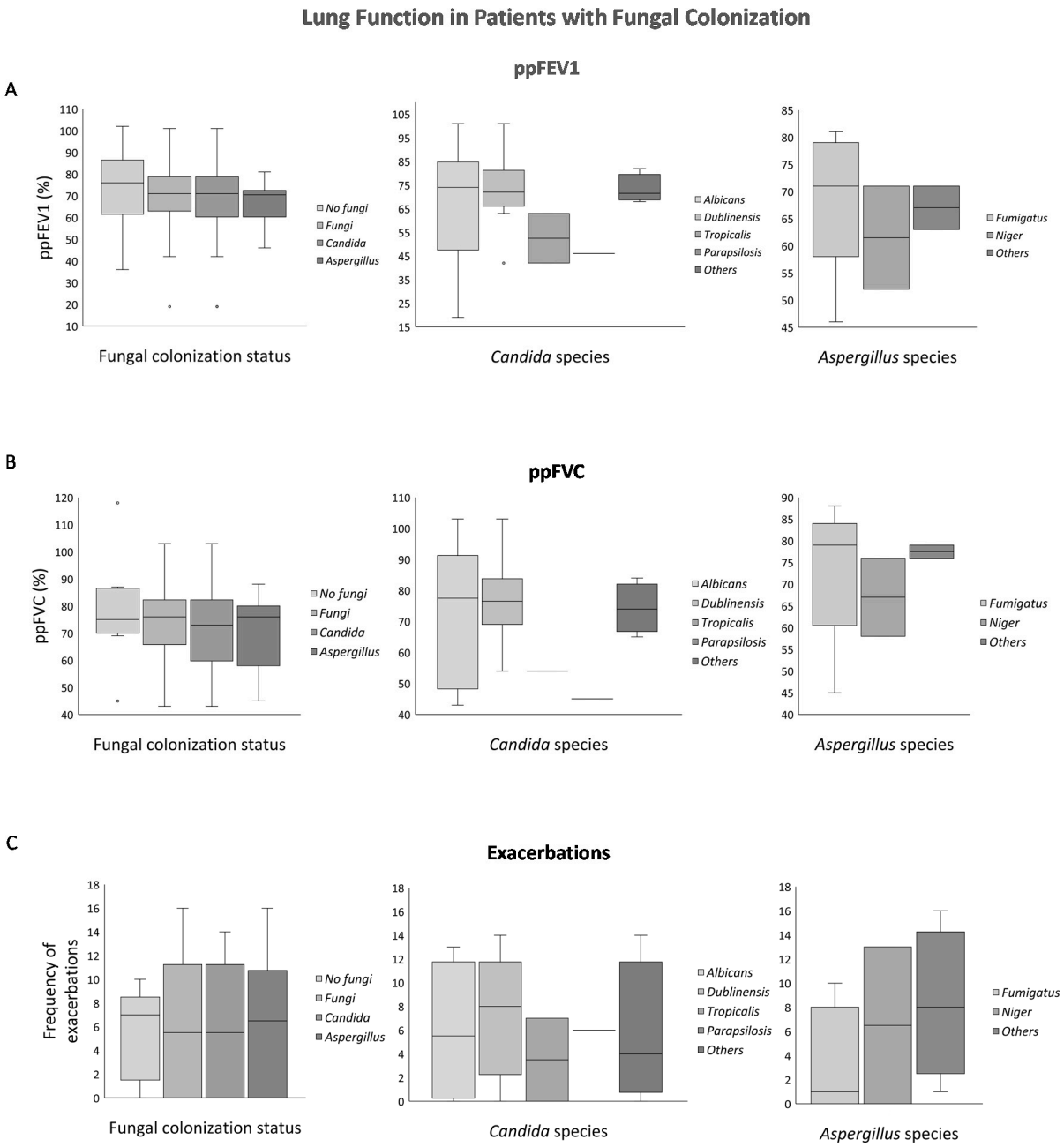

Figure 2. Lung function in adult CF patients in Qatar. Box plots illustrating the medians, upper quartile, lower quartile, interquartile range, and overall ranges for the lung function within the adult CF patients in Qatar, categorized by lung function indicator (**A**–**C**) and status of fungal colonization (left panels: absence/presence/genus of colonizing fungi; middle panels: colonizing *Candida* species; right panels: colonizing *Aspergillus* species). Dots outside of the box plots illustrate the outliers. The left panels compare lung function in patients colonized with fungi (n = 22) and per genus (to the nine patients who were not colonized with fungi. (**A**) ppFEV1 levels (%), (**B**) ppFVC levels (%), and (**C**) Pulmonary exacerbations frequency. Left panels: compare lung function between patients with positive (n = 22) and negative (n = 9) fungal culture, and genus of fungi (*Candida* and *Aspergillus*); middle panels: compare lung function in patients colonized by different *Candida* species; and right panels compare lung function in patients colonized with different *Aspergillus* species.

Lung Function in the Presence of Multiple Fungal Co-Colonization

As the majority of CF patients were found to have been colonized with more than one fungal species **(**Table S2), we were interested to further analyze the effect of an increase in the number of colonizing species on lung function (Figure 3; Table S2). We found that patients (n = 10) colonized with one fungal species had a median ppFEV1 of 71% and

ppFVC of 76%, whereas patients (n = 3) who were colonized with three fungal species had a drastically reduced median ppFEV1 (63%), as well as an even more significant decrease in ppFVC (45%). Contrastingly, we observed that the nine patients without fungal colonization had the best median ppFEV1 (76%) and ppFVC of 75%. Significantly, those patients who were free of fungal colonization, as well as those who were colonized with one or three different fungal species, had an equal median of exacerbations frequency (n = 7) (Figure 3 right panel; Table S2). While none of these values was statistically significant ($p > 0.05$), it is important to note that the clinical relevance of the noticeable reduction in ppFEV1 that is associated with the fungal co-colonization of three different species indicates some influence of these species on lung function of CF patients (Figure 3; Table S2).

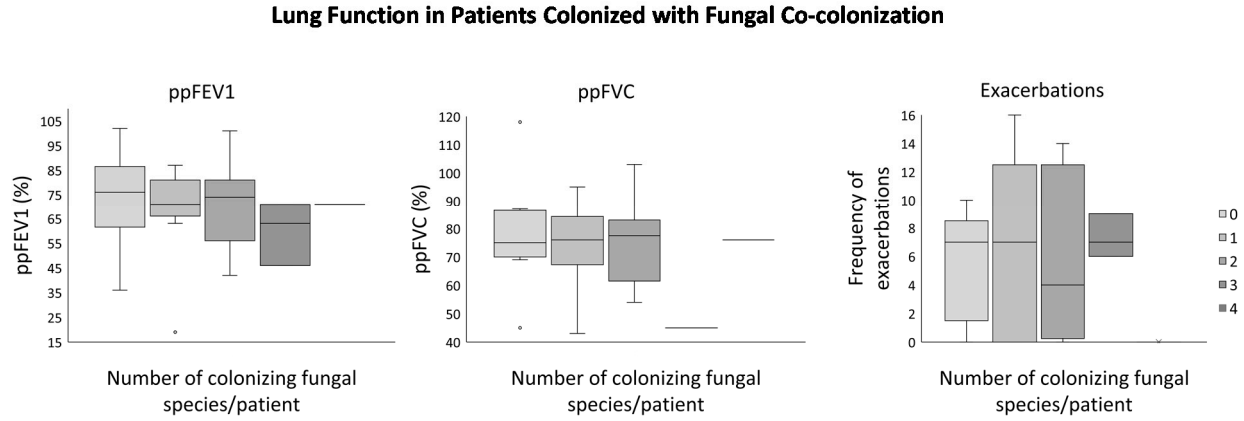

Figure 3. Lung function in CF patients with fungal co-colonization. Box plots illustrating the medians, upper quartile, lower quartile, interquartile range, and overall ranges for the lung function within the 31 adult CF patients in Qatar categorized by the number of fungal species isolated per patient, from zero to four. Dots outside of the box plots illustrate the outliers. Left panel: ppFEV1 values (%). Middle panel: ppFVC values (%). Right panel: frequency of exacerbations.

Although lung function was variable in patients carrying I1234V mutation co-colonized with different fungal species, patients with the other two mutations showed a trend of low lung function during fungal co-colonization (Table S4). A patient who harbored the homozygous c.1657C>T and c.1115A>T mutation, and was colonized with *Candida albicans*, *Candida parapsilosis*, and *Aspergillus fumigatus,* showed very low ppFEV1 and ppFVC values of 46% and 45%, respectively. Similarly, another patient who carried the heterozygous mutation R553X (c.1657C>T and c.1115A>T), and had two different *Candida* colonization, *C. dubliniensis,* and *C. tropicalis,* showed markedly compromised lung function with regard to the ppFEV1 and ppFVC values (42% and 54%, respectively).

3.4. Asthma and Pancreatic Insufficiency in the Presence of Fungal Colonization

Out of the 31 CF patients tested in this study, 17 (55%) were reported to suffer from asthma. The majority of these patients, (n = 15; 88%), were colonized with fungus, and only two patients had no fungal colonization (Figure 4A). *Candida* was the leading species in the fungal colonized group (n = 12), followed by *Aspergillus* in seven patients, and only two patients with other fungal species (Table 2). Importantly, all asthmatic patients carry the I1234V mutation (Table S4).

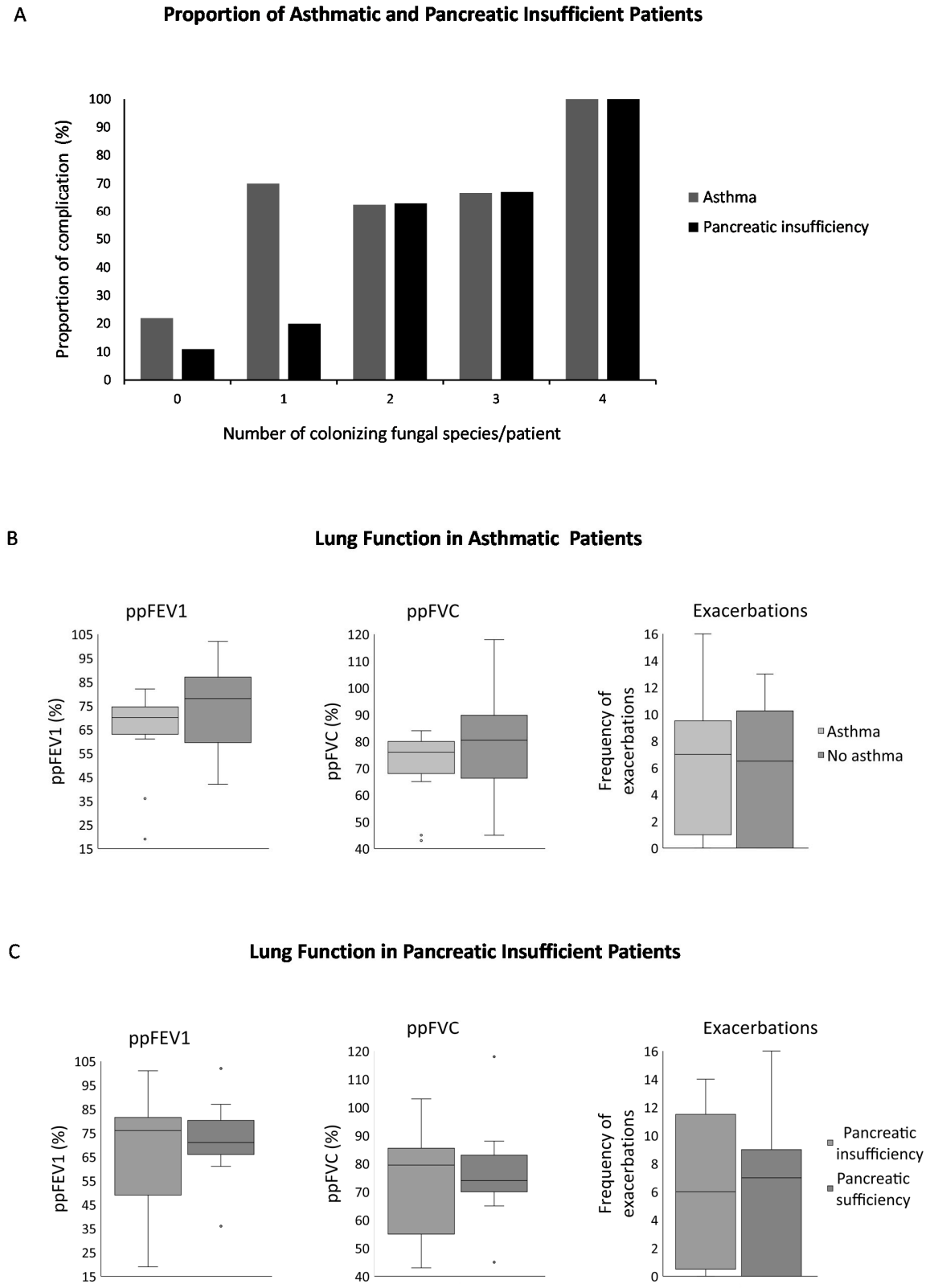

Figure 4. Complications in CF patients colonized with fungi species. (**A**): Bar graph illustrating the relationship between the number of fungal colonization isolated per adult CF patients in Qatar, and the proportion of complications (asthma and pancreatic insufficiency). (**B**,**C**): Box plots illustrating the medians, upper quartile, lower quartile, interquartile range, and overall ranges for the lung function within adult CF in Qatar, sorted by asthma (**B**) and pancreatic insufficiency (**C**). Dots outside of the box plots illustrate the outliers. Left panels: ppFEV1 levels (%). Middle panels: ppFVC levels (%). Right panels: exacerbations frequency.

Table 2. Complications in adult CF patients in Qatar who were colonized with fungi species, in comparison to those who were not.

	Complications n (%)			
Microbial Colonization (n)	**Asthma (n = 17)**	***p*-Value**	**Pancreatic Insufficient (n = 13)**	***p*-Value**
Fungi colonization (n = 22)	15 (88.0)	0.044	12 (54.5)	0.045
Candida species (n = 18)	12 (66.7)	0.157	12 (66.7)	0.002
Aspergillus species (n = 10)	7 (70.0)	0.280	5 (50.0)	0.701
Other species (n = 2)	2 (100.0)	0.488	1 (50.0)	1.000

Further analysis of CF complications, in the presence of fungal colonization, revealed that 41.9% of the study population was pancreatic insufficient. Similar to asthma, the complication was almost exclusive to patients colonized with fungi (all but one patient), yielding a statistical significance of $p = 0.045$ (Table 2). Additionally, all patients with pancreatic insufficiency and positive fungal culture (n = 12) were significantly colonized by *Candida* ($p = 0.002$). The dominance of *Candida* in both groups could have possible clinical significance, and may be a prognostic factor that is associated with worse outcomes, thus requiring further management. When looking specifically at the relationship between these complications and fungal co-colonization, it seemed that, as the number of fungal species isolated per patient increased, a higher proportion had asthma and pancreatic insufficiency (Figure 4A, Table 3).

Table 3. Lung function in adult CF patients in Qatar who were colonized with fungal species and were complicated or not by asthma and pancreatic insufficiency.

Complication and Number of Colonizing Fungal Species/Number of Patients (n)	**ppFEV1 (%)**	**ppFVC (%)**	**Frequency of Exacerbations**
Asthma (17)	70	76	7
0 (2)	48.5	58	8
1 (7)	68	73	9
2 (5)	77	79	3
3 (2)	67	N/A *	8
4 (1)	71	76	0
No asthma (14)	78	80.5	6.5
0 (7)	80	86	7
1 (3)	81	88	0
2 (3)	52	58	11
3 (1)	46	45	6
4 (0)			
Pancreatic insufficiency (13)	76	79.5	6
0 (1)	76	86	10
1 (3)	81	83	12
2 (6)	77.5	79.5	4
3 (2)	54.5	45	6.5
4 (1)	71	76	0
Pancreatic sufficiency (18)	71	74	7
0 (8)	75.5	74.5	7
1 (7)	70	73	5
2 (2)	69.5	74	7.5
3 (1)	71	ND	9
4 (0)			

* N/A: not available.

Furthermore, we analyzed the lung function in the presence or absence of these complications. As anticipated, lung function indicators ppFEV1 and ppFVC were noticeably lower in asthmatic patients (70% and 76%, respectively) compared to non-asthmatic patients (78% and 80.5%, respectively) (Figure 4B; Table 3). This data illustrated that a statistically significant number of patients with fungal colonization suffered from asthma as compared to those without fungal colonization and asthma ($p = 0.044$). Specifically, asthmatic patients with negative fungal culture showed a remarkably worse lung function, as reflected by the lowest ppFEV1 and ppFVC values (48.5% and 58%, respectively), compared to patients with multiple fungal species, such as those with two co-colonizing fungal species, with ppFEV1 and ppFVC of 77% and 79%, respectively (Table 3).

Contrastingly, pancreatic insufficient patients seemed to actually have better lung function on average in comparison to pancreatic sufficient patients, with ppFEV1 and ppFVC values of 76.0% and 79.5%, as compared to 76.0% and 79.5%, respectively (Figure 4C, Table 3).

3.5. Prophylactic Antibiotics and Inhaled Steroids Management in Patients with or without Fungal Colonization

An important confounding factor in CF exacerbations is the use of antibiotics. Therefore, it was of our interest to analyze the management of this CF cohort in the presence or absence of fungal colonization. Two types of management were applied in this cohort, prophylactic antibiotics, and inhaled steroids. Our analysis showed that prophylactic antibiotics were more commonly administered by the CF patients with fungal colonization (n = 14; 63.6%) compared to those without fungal colonization (n = 2; 22.2%). A higher proportion of patients with *Aspergillus* colonization (n = 8; 80.0%) were given prophylactic antibiotics more often than those with *Candida* colonization (n = 11; 61.1%) (Figure 5A, Table 4). Similar to prophylactic antibiotics, inhaled steroids were found to be highly used among the fungi group (n = 15; 68.2%) as opposed to the non-fungal group (n = 4; 44.4%) (Figure 5, Table 4). Notably, a higher proportion of patients with *Aspergillus* colonization (n = 8; 80.0%) received inhaled steroids compared to *Candida* (n = 12; 66.7%) (Figure 5A, Table 4). When looking at how the type of management differed with fungal co-colonization, we noticed that the proportion of the administration of both inhaled steroids and prophylactic antibiotics increased with the number of colonizing fungal species per patient (Figure 5B). However, there was no statistical significance between the use of antibiotics or inhaled steroids and fungal colonization.

Table 4. Treatment of adult CF patients in Qatar (2017–19).

		Treatment-n(%)			
Microbial Colonization	**Culture (n)**	**Prophylactic Antibiotics**	***p*-Value**	**Inhaled Steroid**	***p*-Value**
Fungal species	P (22)	14 (63.6)	0.054	15 (68.2)	0.253
	A (9)	2 (22.2)		4 (44.4)	
Candida species	P (18)	11 (61.1)	0.285	12 (66.7)	0.710
	A (13)	5 (38.5)		7 (53.8)	
Aspergillus species	P (10)	8 (80.0)	0.054	8 (80.0)	0.240
	A (21)	8 (38.1)		11 (52.3)	
Other species	P (2)	1 (50.0)	1.000	2 (100)	0.510
	A (29)	15 (51.7)		17 (58.6)	

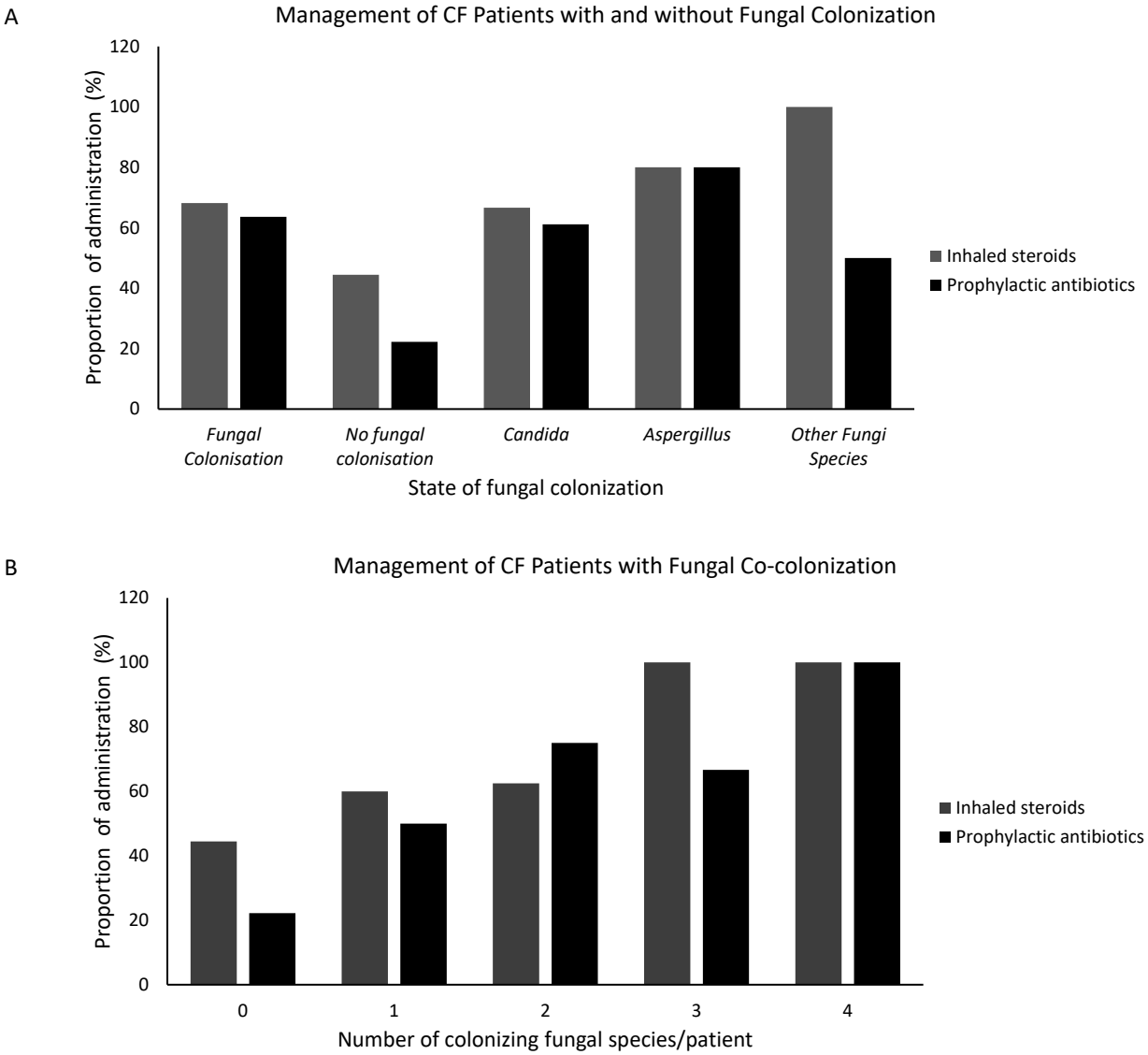

Figure 5. Management of adult CF patients colonized with fungi. Bar charts demonstrating the proportion of inhaled steroids and prophylactic antibiotics administration amongst the 31 adult CF patients categorized by (**A**): the status of fungal colonization and colonizing genus, and (**B**): the number of fungal species isolated per patient.

4. Discussion

In the present study, we showed that 70% of the adult CF patients in Qatar (majority carrying I1234V CF mutation) are colonized with different fungal species. The most common isolates are *Candida* and *Aspergillus* reported in 81% and 45% of the colonized patients, respectively. Importantly, *Candida dubliniensis* was found to be the most common yeast colonization (45%), whereas *Aspergillus fumigatus* (18%) is the dominant *Aspergillus* species. Interestingly, while we found *C. dubliniensis* to be the most common, followed by *C. albicans*, many studies reported the opposite order of prevalence. For instance, one of the largest mycology studies in 637 patients from CF center in Germany, where 75% of the CF patients were colonized with *Candida*, reported that *Candida albicans* (38%) was the most common isolate, followed by *Candida dubliniensis* (12%) [13]. In Qatar, however, *Candida dubliniensis* was also the most common *Candida* isolated in the pediatric CF population, followed by *Candida albicans*, which shows consistency with our findings [14]. Interestingly, such fungal colonization in the lungs of patients with CF is different than that reported in healthy individuals [15].

Additionally, the majority of the patients with fungal colonization were receiving prophylactic antibiotics, a finding echoed in previous studies [16,17]. Although this data was not found to be statistically significant, it is important to note the clinical relevance of the noticeable increase in the proportion of administration of antibiotics and inhaled steroids among CF patients with fungal colonization as compared to without fungal colonization, in addition to its increase with fungal co-colonization, possibly indicating a relationship between the frequency of antibiotics use and fungal load. This can be attributed to the fact that frequent antibiotic use can raise fungal load and predisposes patients to diseases associated with fungi, as in the case with *Candida* and *Aspergillus* species [18]. Therefore, it can lead to a cycle of patients having poor lung function administering antibiotics, leading to the colonization of fungi, leading to worse lung function.

The identified fungal species are associated with poor lung function, pancreatic insufficiency, and asthma in CF patients with 1234V mutation. These complications did not only constitute a major risk on those CF patients in the presence of fungal colonization, but they also appeared to occur in combination with one another that may lead to a serious impact in this cohort. The effect of fungal colonization on pancreatic insufficiency is of clinical importance. Notably, our data showed that as the number of colonizing fungal pathogens increases, pancreatic insufficiency increases. Moreover, all pancreatic insufficient patients were significantly found to be colonized with *Candida* (Figure 3). In accordance with our data, fungal infections have been previously reported to cause a high prevalence of acute pancreatitis that reaches up to 41%, with *Candida* as the most common fungi [13,19,20]. Importantly, treating fungal infections was shown to improve the clinical outcome of these cases [19,21]. Indeed, if left untreated, exocrine pancreatic insufficiency will develop. A recent study showed that 62% of acute pancreatitis patients develop exocrine pancreatic insufficiency, regardless of the severity of the admission case [22]. In CF patients, exocrine pancreatic insufficiency has been found to occur in 90% of the cases [23]. Not only is the exocrine part of the pancreas that has been shown to be affected in those patients, but the endocrine also exhibits some level of impairment [24–26]. Interestingly, deficiency in insulin secretion causes a noticeable decrease in muscle mass of the intercostals and diaphragm, which in turn leads to a decline in lung function [27–29] Our data also showed an observable decline in lung function in pancreatic insufficient patients with fungal colonization (Figure 3 and Table 3). Indeed, two pancreatic insufficient patients colonized with three different fungal species were found to have the worst lung function in terms of ppFEV1 and ppFVC (54.5% and 45%, respectively) (Table 3). Therefore, it is possible that fungal colonization may worsen the status of pancreatic insufficiency in this cohort, which may lead to other severe complications such as a marked deterioration in lung function.

Our analysis also indicated a unique characteristic in this cohort, where the majority of patients with fungal colonization were overweight with BMI > 25, whereas most patients who did not have fungal colonization were within the normal BMI range (Table 1). This is not a common trend seen in CF patients, where the majority are not obese, and they are more likely to be within the normal BMI [30]. The association of fungal infections with high BMI had been illustrated before. It has been shown that during pulmonary infections, obesity negatively affects the ability of the patients to respond well to fungal infections, possibly by diminishing pulmonary host defense against those pathogens [31]. Moreover, other reports classified obesity as a risk factor for nosocomial infections including pneumonia [32,33]. Obesity complications had also been shown to impair host defense against fungal pathogens [31]. In line with those effects, we observed that the majority of high BMI patients colonized with fungus are pancreatic insufficient and asthmatic. Altogether our data, as well as others, suggest that CF patients with high BMI may be at high risk for more fungal infections, and may lead to developing other complications. This idea is supported by a previous report, which showed that a significant number of CF patients who are either overweight or obese were also pancreatic insufficient [34]. Furthermore, data from obese patients showed that loss in pancreatic function is associated with the increase in BMI due to improved nutritional supplements and pancreatic enzyme

replacement therapy [14,35]. Such association was also seen in asthmatic patients, where obesity has been previously reported as a risk factor for asthma [36–38].

This study provides important insights into the fungal isolates in Qatari adult CF patients, and presents a detailed description of the association of these isolates with CF complications. However, the study was limited by the small sample size. While all data available on adult CF patients in Qatar were included within the analysis—which ensured the study was representative of and generalizable to the sample population—the number of participants was still too small to yield major statistically significant results and conclusions. The small sample size is attributed to the fact that pediatric CF patients—that constitute half of the CF population—could not be included in the study, as they are treated in a different facility and records could not be obtained, limiting our sample size to only adult CF patients with the inherent limitation of an underpowered study. Thus, the insignificant *p* values generated may not reflect the reality of these associations. For example, while analysis of lung function in relation to fungal colonization did not yield statistically significant differences, there were notable findings within these groups. While patients with *C. albicans*, *C. dubliniensis*, and *A. fumigatus* had FEV1 values in the 70% range, patients with *C. parapsilosis* and *C. tropicalis* species had values in the 45–55% range, which could be of clinical relevance. The illustrated relationships between fungal colonization, lung function, asthma status, and pancreatic insufficiency may be used to implement clinical and public health measures to optimize CF patient care. Importantly, the fungal colonization status discussed in this study requires further research with a larger sample size to fully understand the scope of this disease and the multitude of factors involved in its prognosis.

5. Conclusions

This is the first report that presents an exhaustive analysis of fungal colonization and its attributable effects on CF patients with rare CFTR genetic mutations. A total of 31 adult patients with CF in Qatar with uncommon CFTR mutations were studied. The majority of these patients (n = 25) carried the I1234V mutation, while the remaining six patients were equally divided between the heterozygous (c.1657C>T and c.1115A>T) and the homozygous (c.920G>A) mutations. In spite of the type of mutation, 22 patients were colonized with fungal species, out of which 81% had *Candida* species, and 45% had *Aspergillus* species. Specifically, *Candida dubliniensis* and *Aspergillus fumigatus* were the most common fungal pathogens identified. Such colonization could aggravate the most known CF complications, notably pulmonary exacerbations, asthma, and pancreatic insufficiency. In conclusion, further investigations on fungal infections and colonization in the rest of CF patients in Qatar are needed for prompting adequate management of the disease.

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Informed Consent Statement: Patient consent was waived due to the study being purely retrospective. Relevant data were collected from medical records, and the anonymity of the patients were protected. No names were recorded, and instead, data were identified by an ID.

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

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References

1. Elborn, J.S. Cystic fibrosis. *Lancet* **2016**, *388*, 2519–2531. [CrossRef]
2. WHO Human Genetics Programme. The Molecular Genetic Epidemiology of Cystic Fibrosis: Report of a Joint Meeting of WHO/IECFTN/ICF(M)A/ECFS, Genoa, Italy, 19 June 2002. World Health Organization. 2004. Available online: https://apps.who.int/iris/handle/10665/68702 (accessed on 12 July 2021).
3. Wahab, A.A.; Al Thani, G.; Dawod, S.T.; Kambouris, M.; Al Hamed, M. Heterogeneity of the cystic fibrosis phenotype in a large kindred family in Qatar with cystic fibrosis mutation (I1234V). *J. Trop. Pediatr.* **2001**, *47*. [CrossRef]
4. Hammoudeh, S.; Gadelhak, W.; Abdulwahab, A.; Al-Langawi, M.; Janahi, I.A. Approaching two decades of cystic fibrosis research in Qatar: A historical perspective and future directions. *Multidiscip. Respir. Med.* **2019**, *14*. [CrossRef]
5. Hammoudeh, S.; Gadelhak, W.; AbdulWahab, A.; Al-Langawi, M.; Janahi, I.A. An Overview of the Homozygous Cystic Fibrosis Transmembrane Conductance Regulator Mutation c.3700 A>G (p.Ile1234Val) in Qatar. *Curr. Genet. Med. Rep.* **2019**, *7*. [CrossRef]
6. Ehre, C.; Ridley, C.; Thornton, D.J. Cystic fibrosis: An inherited disease affecting mucin-producing organs. *Int. J. Biochem. Cell Biol.* **2014**, *52*, 136–145. [CrossRef]
7. Lopes, S.P.; Ceri, H.; Azevedo, N.F.; Pereira, M.O. Antibiotic resistance of mixed biofilms in cystic fibrosis: Impact of emerging microorganisms on treatment of infection. *Int. J. Antimicrob. Agents* **2012**, *40*, 260–263. [CrossRef] [PubMed]
8. Pihet, M.; Carrere, J.; Cimon, B.; Chabasse, D.; Delhaes, L.; Symoens, F.; Bouchara, J.P. Occurrence and relevance of filamentous fungi in respiratory secretions of patients with cystic fibrosis—A review. *Sabouraudia* **2009**, *47*, 387–397. [CrossRef]
9. Barreiro, T.; Perillo, I. An Approach to Interpreting Spirometry. *Am. Fam. Physician* **2004**, *69*, 1107–1114. Available online: https://www.aafp.org/afp/2004/0301/p1107.html (accessed on 12 September 2020).
10. Brandt, C.; Roehmel, J.; Rickerts, V.; Melichar, V.; Niemann, N.; Schwarz, C. Aspergillus bronchitis in patients with cystic fibrosis. *Mycopathologia* **2018**, *183*, 61–69. [CrossRef]
11. Crapo, R.O.; Hankinson, J.L.; Irvin, C.; MacIntyre, N.R.; Voter, K.Z.; Wise, R.A.; Graham, B.; O'Donnell, C.; Paoletti, P.; Roca, J.; et al. Standardization of spirometry: 1994 update. *Am. J. Respir. Crit. Care Med.* **1995**, *152*, 1107–1136.
12. Maria, I.; Jar, N. Cystic Fibrosis Pulmonary Exacerbation—Natural History, Causative Factors and Management. In Respiratory Disease and Infection—A New Insight. Available online: https://www.intechopen.com/books/respiratory-disease-and-infection-a-new-insight/cystic-fibrosis-pulmonary-exacerbation-natural-history-causative-factors-and-management (accessed on 12 July 2021).
13. De Waele, J.J.; Vogelaers, D.; Blot, S.; Colardyn, F. Fungal Infections in Patients with Severe Acute Pancreatitis and the Use of Prophylactic Therapy. Clinical Infectious Diseases Virginia. Available online: https://pubmed.ncbi.nlm.nih.gov/12856213/ (accessed on 12 July 2021).
14. Wahab, A.A.; Taj-Aldeen, S.J.; Kolecka, A.; ElGindi, M.; Finkel, J.S.; Boekhout, T. High prevalence of Candida dubliniensis in lower respiratory tract secretions from cystic fibrosis patients may be related to increased adherence properties. *Int. J. Infect. Dis.* **2014**, *24*. [CrossRef] [PubMed]
15. Charlson, E.S.; Diamond, J.M.; Bittinger, K.; Fitzgerald, A.S.; Yadav, A.; Haas, A.R.; Bushman, F.D.; Collman, R.G. Lung-enriched organisms and aberrant bacterial and fungal respiratory microbiota after lung transplant. *Am. J. Respir. Crit. Care Med.* **2012**, *186*. [CrossRef]
16. Bargon, J.; Dauletbaev, N.; Köhler, B.; Wolf, M.; Posselt, H.G.; Wagner, T.O.F. Prophylactic antibiotic therapy is associated with an increased prevalence of Aspergillus colonization in adult cystic fibrosis patients. *Respir. Med.* **1999**, *93*, 835–838. [CrossRef]
17. Reece, E.; Segurado, R.; Jackson, A.; McClean, S.; Renwick, J.; Greally, P. Co-colonisation with Aspergillus fumigatus and Pseudomonas aeruginosa is associated with poorer health in cystic fibrosis patients: An Irish registry analysis. *BMC Pulm. Med.* **2017**, *17*, 70. [CrossRef] [PubMed]
18. Leclair, L.W.; Hogan, D.A. Mixed bacterial-fungal infections in the CF respiratory tract. *Med. Mycol.* **2010**, *48*. [CrossRef]
19. Hoerauf, A.; Hammer, S.; Müller-Myhsok, B.; Rupprecht, H. Intra-abdominal Candida infection during acute necrotizing pancreatitis has a high prevalence and is associated with increased mortality. *Crit. Care Med.* **1998**, *26*. [CrossRef] [PubMed]
20. Boucher, B.A.; King, S.R.; Wandschneider, H.L.; Hickerson, W.L.; Hanes, S.D.; Herring, V.L.; Canada, T.W.; Hess, M.M. Fluconazole pharmacokinetics in burn patients. *Antimicrob. Agents Chemother.* **1998**, *42*. [CrossRef]
21. Kochhar, R.; Ahammed, S.K.M.; Chakrabarti, A.; Ray, P.; Sinha, S.K.; Dutta, U.; Wig, J.D.; Singh, K. Prevalence and outcome of fungal infection in patients with severe acute pancreatitis. *J. Gastroenterol. Hepatol. Aust.* **2009**, *24*. [CrossRef]
22. Huang, W.; de la Iglesia-García, D.; Baston-Rey, I.; Calviño-Suarez, C.; Lariño-Noia, J.; Iglesias-Garcia, J.; Shi, N.; Zhang, X.; Cai, W.; Deng, L.; et al. Exocrine Pancreatic Insufficiency Following Acute Pancreatitis: Systematic Review and Meta-Analysis. *Dig. Dis. Sci.* **2019**, *64*. [CrossRef] [PubMed]
23. Gibson-Corley, K.N.; Meyerholz, D.K.; Engelhardt, J.F. Pancreatic pathophysiology in cystic fibrosis. *J. Pathol.* **2016**, *238*. [CrossRef]

24. Olivier, A.K.; Yi, Y.; Sun, X.; Sui, H.; Liang, B.; Hu, S.; Xie, W.; Fisher, J.T.; Keiser, N.W.; Lei, D.; et al. Abnormal endocrine pancreas function at birth in cystic fibrosis ferrets. *J. Clin. Investig.* **2012**, *122*. [CrossRef]
25. Navis, A.; Bagnat, M. Loss of cftr function leads to pancreatic destruction in larval zebrafish. *Dev. Biol.* **2015**, *399*. [CrossRef] [PubMed]
26. Meyerholz, D.K.; Stoltz, D.A.; Pezzulo, A.A.; Welsh, M.J. Pathology of gastrointestinal organs in a porcine model of cystic fibrosis. *Am. J. Pathol.* **2010**, *176*. [CrossRef] [PubMed]
27. Milla, C.E.; Warwick, W.J.; Moran, A. Trends in pulmonary function in patients with cystic fibrosis correlate with the degree of glucose intolerance at baseline. *Am. J. Respir. Crit. Care Med.* **2000**, *162*. [CrossRef] [PubMed]
28. Arora, N.S.; Rochester, D.F. Respiratory muscle strength and maximal voluntary ventilation in undernourished patients. *Am. Rev. Respir. Dis.* **1982**, *126*. [CrossRef]
29. Arora, N.S.; Rochester, D.F. Effect of body weight and muscularity on human diaphragm muscle mass, thickness, and area. *J. Appl. Physiol.* **1982**, *52*. [CrossRef]
30. Alvarez, J.A.; Ziegler, T.R.; Millson, E.C.; Stecenko, A.A. Body composition and lung function in cystic fibrosis and their association with adiposity and normal-weight obesity. *Nutrition* **2016**, *32*, 447–452. [CrossRef] [PubMed]
31. Mancuso, P. Obesity and respiratory infections: Does excess adiposity weigh down host defense? *Pulm. Pharmacol. Ther.* **2013**, *26*. [CrossRef]
32. Huttunen, R.; Karppelin, M.; Syrjänen, J. Obesity and nosocomial infections. *J. Hosp. Infect.* **2013**, *85*. [CrossRef]
33. Falagas, M.E.; Kompoti, M. Obesity and infection. *Lancet Infect. Dis.* **2006**, *6*. [CrossRef]
34. Hanna, R.M.; Weiner, D.J. Overweight and obesity in patients with cystic fibrosis: A center-based analysis. *Pediatr. Pulmonol.* **2015**, *50*. [CrossRef] [PubMed]
35. Teichmann, J.; Riemann, J.F.; Lange, U. Prevalence of Exocrine Pancreatic Insufficiency in Women with Obesity Syndrome: Assessment by Pancreatic Fecal Elastase. *ISRN Gastroenterol.* **2011**, *2011*. [CrossRef] [PubMed]
36. Dixon, A.E.; Holguin, F.; Sood, A.; Salome, C.M.; Pratley, R.E.; Beuther, D.A.; Celedón, J.C.; Shore, S.A.; Boulet, L.P.; O'Donnell, C.; et al. An Official American Thoracic Society Workshop Report: Obesity and Asthma. Proceedings of the American Thoracic Society New York, United States of America 7, No. 5. 2010, pp. 325–335. Available online: https://www.atsjournals.org/doi/10.1513/pats.200903-013ST (accessed on 8 April 2020).
37. Farah, C.S.; Kermode, J.A.; Downie, S.R.; Brown, N.J.; Hardaker, K.M.; Berend, N.; King, G.G.; Salome, C.M. Obesity is a determinant of asthma control independent of inflammation and lung mechanics. *Chest* **2011**, *140*. [CrossRef]
38. Mosen, D.M.; Schatz, M.; Magid, D.J.; Camargo, C.A. The relationship between obesity and asthma severity and control in adults. *J. Allergy Clin. Immunol.* **2008**, *122*. [CrossRef]