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EPIDEMIOLOGY OF PULMONARY ASPERGILLOSIS: IDENTIFYING ASPERGILLUS SPECIES AND THEIR CORRELATION WITH COEXISTING MEDICAL CONDITIONS IN A LOCAL HEALTHCARE SETTING

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Abstract

Background: Invasive fungal infections (IFIs) not only result in increased rates of mortality and morbidity in humans but also present a substantial global burden due to the growing resistance to antifungal treatments.

Objective: This research was conducted to ascertain the occurrence of *Aspergillus* species in our local area and investigate the coexisting medical conditions in the cases of pulmonary aspergillosis.

Materials and Methods: This cross-sectional investigation enrolled seventy three clinical cases of pulmonary aspergillosis. *Aspergillus* species were identified in the specimens of patients who attended the Out Patient Department or were admitted to the Pulmonology Department of Lahore General Hospital, Lahore.

Results: The study disclosed that the most frequently isolated *Aspergillus* species from the pulmonary specimens was *Aspergillus flavus* (39 cases, 53.4%), followed by *Aspergillus niger* (21 cases, 28.8%), *Aspergillus fumigatus* (12 cases, 16.4%), and *Aspergillus terreus* (1 case, 1.4%). The prevailing comorbidities among the patients included asthma (21 cases, 30%), tuberculosis (22 cases, 30%), and chronic obstructive pulmonary disease (30 cases, 40%).

Conclusion: The results obtained from this research can serve as a foundation for monitoring the clinically significant and widespread species of *Aspergillus* in future. Establishing a connection with comorbidities may serve as a pivotal part in enhancing our comprehension of pathophysiology of the disease.

Introduction

Fungal infections in humans can be classified as superficial and invasive. Superficial fungal infections (SFI) typically affect the outer layers of the skin, hair, and nails. These are caused by dermatophytes, yeast and non-dermatophyte filamentous fungi (Gupta et al., 2021; Khodadadi et al.,

2021). Invasive fungal infections (IFIs), although being less common than invasive bacterial infections, are of major concern because of their high mortality rate, which often crosses over 50%, resulting in the loss of approximately 1.5 million lives per year (Armstrong et al., 2020). There are many different types of fungi that can cause invasive fungal infections, including *Aspergillus*, *Candida*, *Cryptococcus*, *and Mucorales*. These fungi attribute to almost 90% of the fungal infections. These can affect many different organs and systems in the body, including lungs, brain, liver, and kidneys (Leroy-Freschini et al., 2018). The filamentous fungi consist of about 250 species and *Aspergillus* is the largest genera which causes human diseases. Globally, the most common cause of invasive aspergillosis is *Aspergillus fumigatus* and it has been studied and reviewed widely. Infection due to *A. flavus* is more common in Africa, Asia, and the Middle East because it has the ability to survive in dry climatic and hot conditions as compared to other *Aspergillus* species (Friedman & Schwartz, 2019). Pulmonary Aspergillosis is a type of invasive fungal infection that affects the lungs. There are several types of pulmonary aspergillosis, including allergic bronchopulmonary aspergillosis (ABPA), chronic pulmonary aspergillosis (CPA), and invasive pulmonary aspergillosis (IPA) (Moldoveanu et al., 2021). Pulmonary aspergillosis (PA) is caused by *A. fumigatus, A. niger, A. flavus* and *A. terreus. A. flavus* is mainly reported to cause invasive forms of aspergillosis (Zanganeh et al., 2018). Chronic pulmonary aspergillosis (CPA) is a type of pulmonary aspergillosis caused by infection with *Aspergillus* species, most commonly *Aspergillus fumigatus complex.* Unlike invasive pulmonary aspergillosis (IPA), which is typically an acute and rapidly progressive infection that affects immunocompromised patients, CPA is a chronic and slowly progressive infection that can occur in people with relatively minor immunosuppression and pre-existing co-morbidities (Laursen et al., 2020). Chronic Pulmonary Aspergillosis (CPA) has an effect on individuals with previous mycobacterial lung disease or chronic structural lung disease. *Aspergillus* bronchitis has an effect on individuals with bronchial lung disease such as bronchiectasis (Hosseini-Moghaddam et al., 2020). Multiple co-morbidities can exist along with CPA like Tuberculosis TB, Diabetes, Asthma and any malignancy but the most common is TB worldwide (Akram et al., 2021). In nations with a high prevalence of tuberculosis (TB), the rate of chronic pulmonary aspergillosis (CPA) is growing at an exceptionally alarming rate. In spite of the fact that Pakistan is thought to have a high pulmonary aspergillosis burden, there is lack of actual data. A recent CPA burden assessment from Pakistan indicated a high frequency of 39 cases for every 0.1 million people in the country (Iqbal et al., 2020). This study has focused on overall prevalence of *Aspergillus* species endemic in our area in suspected cases of pulmonary aspergillosis and the co-morbidities in patients along with pulmonary aspergillosis.

Methodology

This cross-sectional research was conducted in the Microbiology department of Lahore General Hospital, spanning from January, 2022 to October, 2022. The study protocol obtained approval from the Advanced Studies and Research Board of the University of Health Sciences, Lahore, as well as the Ethical Committee of the Postgraduate Medical Institute, Lahore.

Data collection and analysis

The patient's required details were collected via profoma. The parameters included in the proforma were the type of specimen, date of sample collection, patient's name, patient's age and gender, medical record number (MR number), presenting complaints of the patient, previous medical history and the provisional diagnosis. The data was analyzed statistically using SPSS 25.0. The descriptive statistics including mean and standard deviation were calculated for numeric data. Frequency percentage was used for *Aspergillus* species and their antifungal susceptibility patterns.

Sampling Methods:

Convenient sampling techniques were utilized to obtain specimens from patients admitted to the Pulmonology ward or visiting the Out Patient Department (OPD) at the tertiary care hospital of

Lahore. Pulmonary specimens were taken including bronchoalveolar lavage fluid, tracheal aspirate and sputum.
Samples were collected under the supervision of a qualified physician in a sterile environment and promptly transported to the Microbiology laboratory within a two-hour window. Each sample was processed in accordance with the predefined Standard Operating Procedures in the laboratory.
Sample Processing: All respiratory samples were mixed with a sputasol reagent to liquefy mucus components. Subsequently, the samples were centrifuged at 1200 RCF for ten minutes, and a direct examination was performed to observe fungal hyphae through the preparation of a KOH (Potassium hydroxide) solution.

Culture Inoculation (Waqas et al., 2021)
Sabouraud Dextrose Agar was used for culture inoculation. The culture plates were incubated at 35 °C for ten days. Daily observations were made to detect any growth of fungus, and plates showing no growth after an incubation period of ten days were discarded.

Cellophane Tape Preparation (Bailey et al., 2021)
Lactophenol Cotton Blue Staining:
Tape preparations were made from the culture plates after growth of the fungus to identify the *Aspergillus* upto species level. Lactophenol Cotton Blue stain was used for the tape preparations for easier identification of the microscopic characteristics. Preparations were observed microscopically first under lower power 10X objective and then under high power 40X.

Growth and morphology (Bailey et al., 2021; Larone, 2018)
Regarding *Aspergillus flavus*, its appearance was characterized by a velvety texture, with colors spanning from yellow to green or brown. On the reverse side, it displayed a golden to red-brown hue. Microscopically, the vesicles showed a spherical shape, and the phialides covering the entire vesicle projected outward in multiple directions (Figure 1).

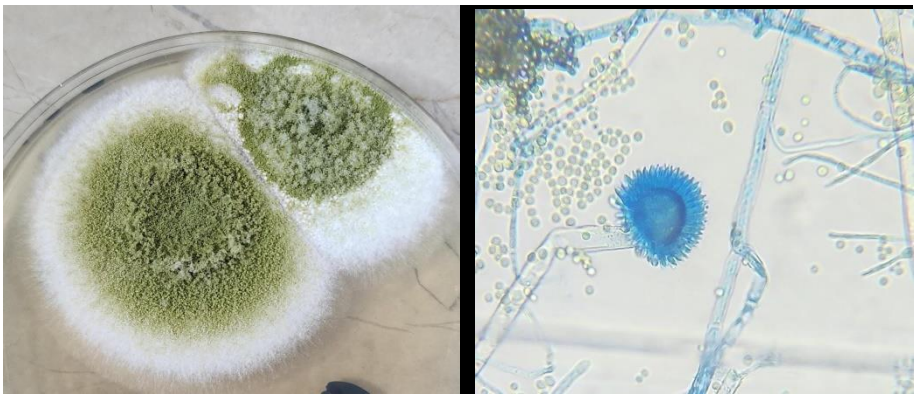

***Figure 1: A. flavus*; A front texture of colony, B Cellophane Tape preparation**

In the case of *Aspergillus niger*, its appearance on the culture plate presented as a deep cottony texture, initially appearing white to yellow and later turning black. On the reverse side, it exhibited a white to yellow coloring. Under microscopic examination, it displayed a biseriate arrangement with smaller phialides covering the entire vesicle (Figure 2).

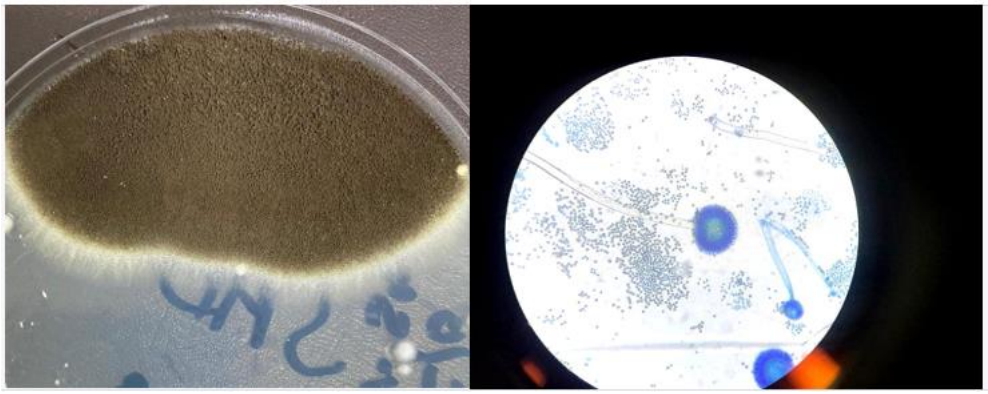

***Figure 2: A. niger*; A front texture of colony, B Cellophane Tape preparation**

Regarding *Aspergillus fumigatus,* it displayed a texture either velvety or powdery when inoculated on the culture plate. Initially, it presented as white and subsequently transformed into a dark greenish to gray shade. On the reverse side, it showed a white to tan appearance in color. When subjected to microscopic examination, conidiophores extended into sizable, dome like vesicles with phialides which are bottle shaped and predominantly concentrated on the upper two-thirds of the surface (Figure 3).

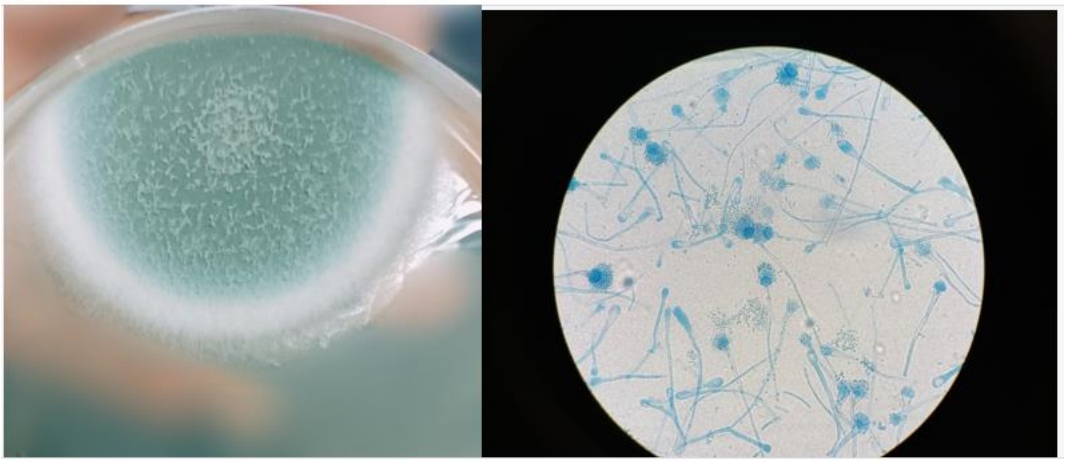

***Figure 3: A. fumigatus*; A front texture of colony, B Cellophane Tape preparation**

As for *Aspergillus terreus*, its culture plate appearance was characterized by a velvety texture with a cinnamon-brown coloration. On the reverse side, it displayed shades of yellow or brown. Microscopically, the vesicles gave a hemispherical shape, and phialides that covered the entire surface (Figure 4).

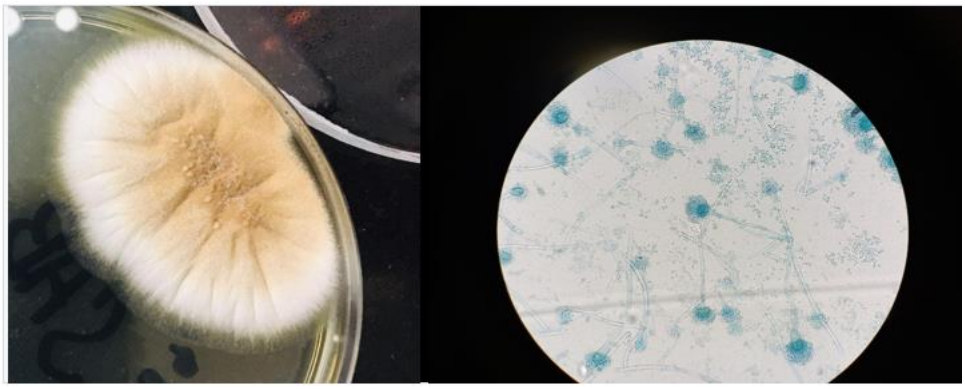

***Figure 4: A. terreus*; A front texture of colony, B Cellophane Tape preparation**

Results:

This study comprised of 73 clinical isolates of *Aspergillus* species, all sourced from the pulmonary specimens of patients visiting the Outpatient Department or admitted to the Pulmonology Department of LGH, Lahore. Among these 73 *Aspergillus* isolates detected in positive specimens, 47 (64.4%) were from male patients, while 26 (35.6%) were from female patients, as represented (Figure 5).

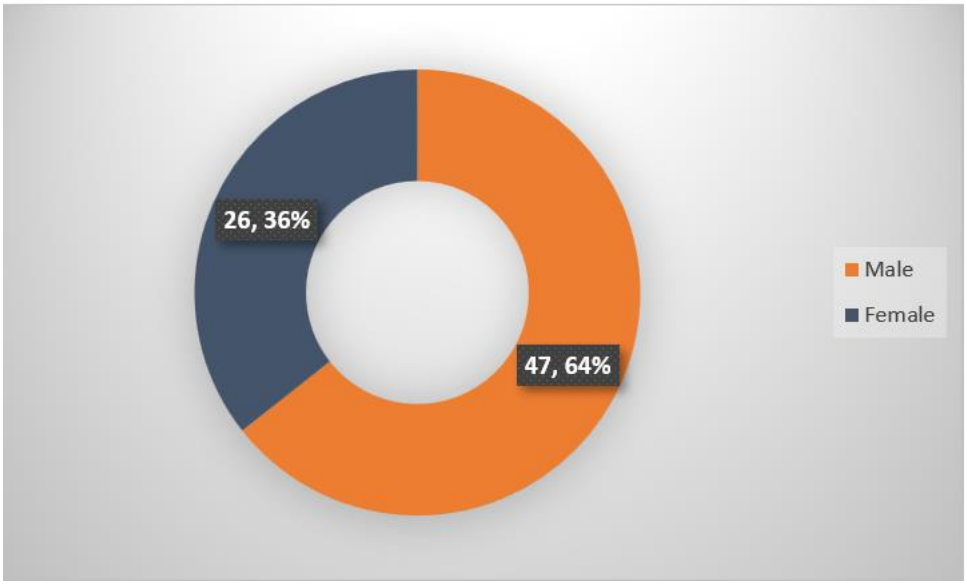

Figure 5: Frequency of gender distribution in *Aspergillus* isolates (n=73)

The patients in this study were stratified into five distinct age groups for analysis. The mean age of these individuals was 56.4±17.1, covering a broad age range from 18 to 90 years. Furthermore, the majority of patients were clustered in the 60-79 years age bracket, constituting a total of 27 individuals, as depicted (Figure 6).

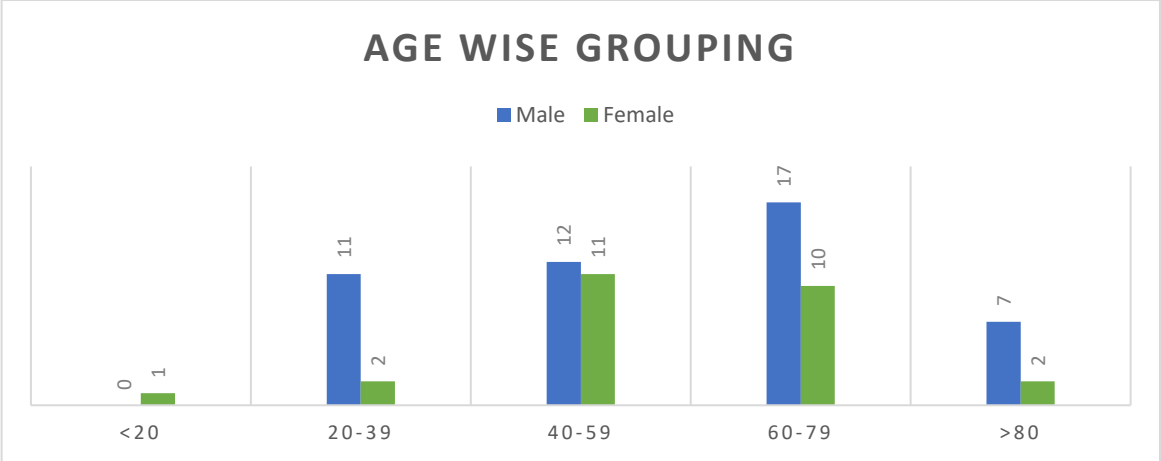

Figure 6: Frequency of age distribution in *Aspergillus* isolates (n=73)

The isolates obtained were distributed as follows: *Aspergillus flavus* constituted 39 (53.4%) isolates, *Aspergillus fumigatus* accounted for 12 (16.4%) isolates, *Aspergillus niger* was represented by 21 (28.8%) isolates, and there was just one (1.1%) isolate of *Aspergillus terreus* (Figure 7).

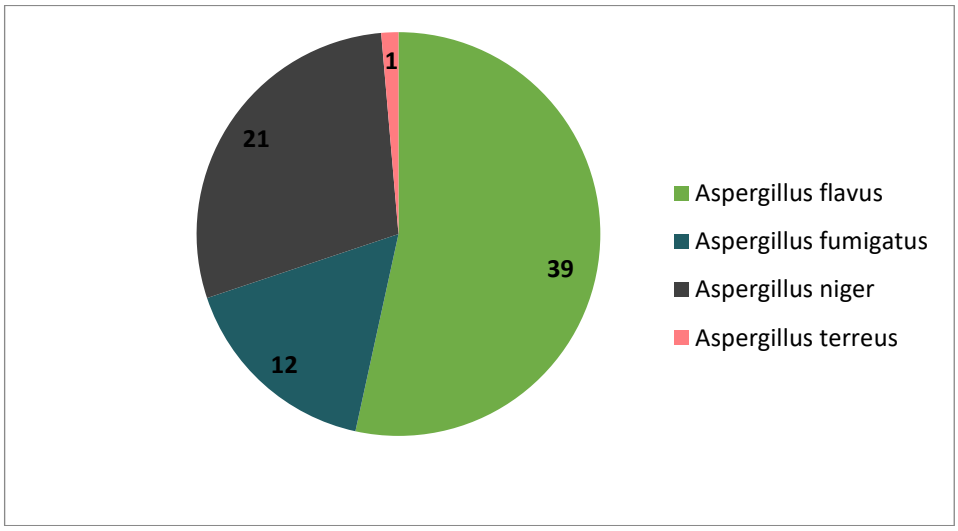

Figure 7: Frequency distribution of *Aspergillus* species (n=73)

The patients displayed a spectrum of different presenting complaints and a wide array of medical backgrounds. Within the cohort of patients examined in this study, chronic obstructive pulmonary disease (COPD) emerged as the most commonly observed comorbid condition (Figure 8). These patients exhibited a variety of symptoms, with dyspnea being the most frequently reported, followed by the combination of dyspnea and hemoptysis (Figure 9).

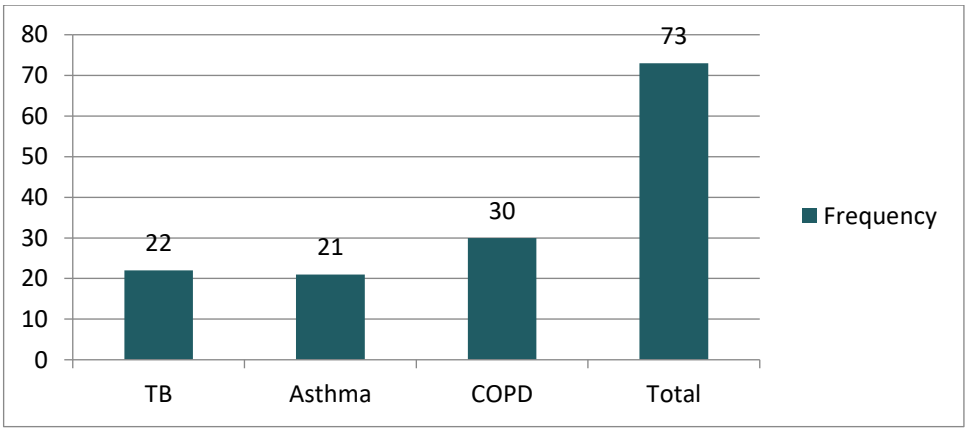

Figure 8: Frequency distribution of comorbid of patients in the study (n=73)

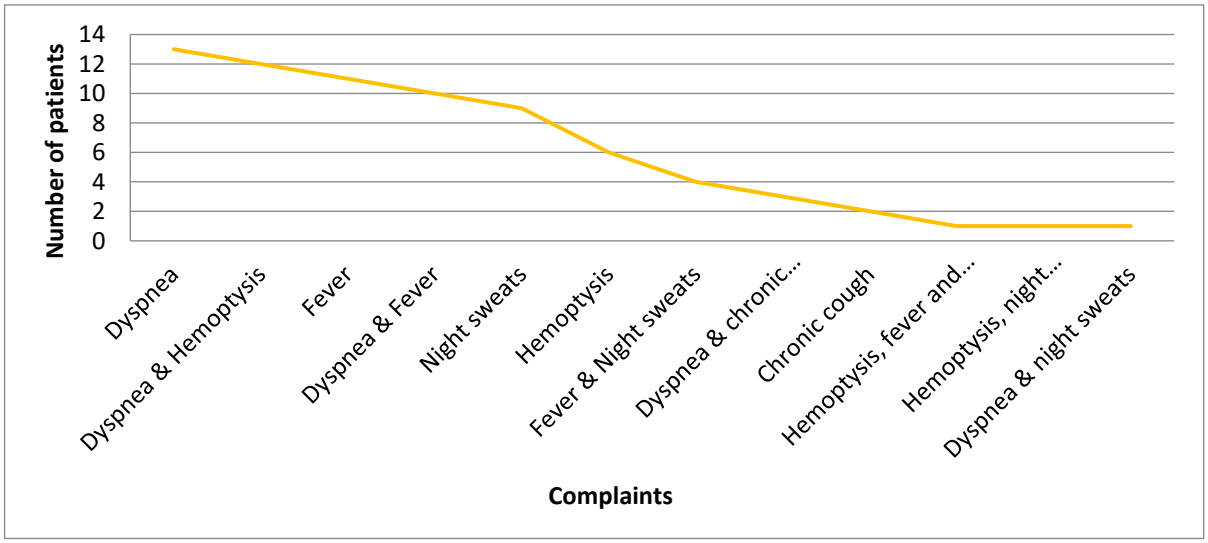

Figure 9: Frequency distribution of presenting complaints of patients (n=73)

Discussion :

This study focuses on the prevalent species of *Aspergillus* endemic in the area and co-morbidities in patients who are suspected to have pulmonary aspergillosis. The sample size used in this research was 73 and *Aspergillus* species were isolated from sputum, tracheal aspirate or bronchoalveolar lavage of patients visiting the Out Patient Department or admitted in the Pulmonology ward of Lahore General Hospital, Lahore. The demographic details of the patients were noted which included gender, age and Medical Record number of the patient, presenting complaints of the patient and significant past clinical history were also noted. In a study conducted by Cheng Lai in China on pulmonary aspergillosis in 2021, similar characteristics were taken into consideration (Chih-Cheng Lai and Weng-Liang Yu, 2021). In the same way, Koehler studied about pulmonary aspergillosis with similar characteristics but in contrast to the current study, all patients included in his study were COVID-19 positive (Koehler et al., 2021)

In the present study, *Aspergillus* species were isolated more among males 47(64%) than females 26(36%). Bitew worked on the prevalence of pulmonary aspergillosis which also described the predominance of male patients (52%) as compared to the female patients (48%) (Adana Bitew and Solomon Bati, 2021). Another meta-analysis on COVID-19 associated pulmonary aspergillosis showed the predominance of male patients (73%) as compared to the female patients (27%) (Salmanton-García et al., 2021). Unlike results of the current study, another research performed by Fischer in 2022 showed unremarkable differences in gender (Fischer et al., 2022).

On the basis of age, five groups were made to simplify the age distribution of aspergillosis and the data presented the fact that most patients infected with *Aspergillus* belong to the older age group. Only fourteen patients in this study fell below the age 40. A retrospective study conducted in China on pulmonary aspergillosis and COVID-19 also showed that pulmonary aspergillosis was more prevalent among people who are above 60 years of age (Wang et al., 2020). Another meta-analysis of the Asian and African countries, conducted by Hosseini in 2020 observed the similar trend of age distribution in patients of pulmonary aspergillosis (Hosseini et al., 2020).

In this study, the most prevalent *Aspergillus* specie to be isolated from the pulmonary specimens was *Aspergillus flavus* followed by *Aspergillus niger. Aspergillus fumigatus* was found to be the third most common and *Aspergillus terreus* was found to be the least common among the four species. In Pakistan, a research was conducted to study azole resistance in Agha Khan Hospital,Karachi by Safia Moin which also showed the similar results (Moin et al., 2020). Unlike the present study, in 2023 , Zhang carried out a research compiling data of two years from a large tertiary care hospital in Shanghai, China on *Aspergillus* species which showed the prevalence of *Aspergillus fumigatus* in that area (Zhu et al., 2023). Another study was conducted at the Agha Khan Hospital, Karachi, Pakistan in the year 2022 by Nosheen who studied the association of COVID-19 and CAPA. *Aspergillus flavus* was found to be the prevalent specie according to this study as well, followed by *Aspergillus niger*.

In the present study, patients presented to the hospital with different complaints. Thirteen (18%) patients presented with only dyspnea whereas twelve (16%) patients presented with dyspnea along with hemoptysis. Eleven (15%) patients presented with fever whereas ten (14%) patients presented with fever along with dyspnea. The results of the presenting complaints in this study seem to be overlapping. Ahmed *et al* (2022) conducted a study in India to evaluate the trends of pulmonary aspergillosis (Ahmed et al., 2022). According to this study, the most common complaint with which the patients presented was dyspnea followed by cough and fever. Lao et al., (2020) also studied the clinical features of pulmonary aspergillosis. According to this study, fifty-eight percent of the patients presented with fever and cough whereas thirty-two percent of the patients presented with dyspnea (Khanam et al., 2023). Liu (2021) also studied the clinical characteristics of patients suspected with pulmonary aspergillosis. According to this study, ninety-three percent of the patients presented with cough (Liu et al., 2021). In a study conducted by Alessandro Russo in 2020, cough was found to be the most common symptom in patients presenting with pulmonary aspergillosis followed by hemoptysis (Russo et al., 2020). Pulmonary aspergillosis can present with a variety of

symptoms and this is why Chronic Pulmonary Aspergillosis (CPA) often remains undiagnosed for years because of the nonspecific symptoms, such as fatigue, cough, weight loss, fever, hemoptysis and dyspnea which can ultimately result in the disease progression (Niu et al., 2020).

Conclusion:

The data obtained from this research can serve as a base for future monitoring of *Aspergillus* species which are clinically significant and endemic in this area. Establishing a connection with comorbidities may play a pivotal role in augmenting our knowledge about the pathophysiology of pulmonary aspergillosis.

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