Prevalence Of Alp And Asp Genes In Aspergillus Fumigatus Isolated From Iraqi Patients

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Abstract

Background: Pulmonary aspergillosis is a severe infection in immunocompromised caused by Aspergillus fungi, with *Aspergillus fumigatus* being the most frequently cultured of this genus. Fungal proteases such as serine and a aspartate proteases have diverse importance in fungal pathogenicity.

Materials and Methods: A total of 105 samples were collected from TB patients with symptom of lung infection . These sputum samples were obtained from patients treated in Public health department Chest and Respiratory diseases clinic , from February 2023 to April 2023. All samples information were recorded including age, sex and related diseases, after that PCR was done to detect *alp* and *asp* using specific primers.

Result: Association between culture results of A.fumigatus and patient parameters was done using patients parameters included gender, age, tuberculosis bacilli (T.B) history, diabetes, hypertension and residency, gender were divided into male and female, outcomes were 9 (8.5%) and 5 (4.8%), age groups number and percentage were 0(0.0%), 3(2.9%), 1(0.9%), 2(1.9%), 3(2.0%) and 5(4.7%) in the age groups <=29, 30-39, 40-49, 50-59, 60-69 and >=70 of *A.fumigatus* regarding total number, T.B history, diabetes, hypertension and residency were related to 14 (100%) of *A.fumigatus*, T.B history was recorded in 1(17.1%) of patients, diabetes number and percentage were 7(50.0%), hypertension 8(57.1%), residency was divided into urban 9(64.3%) rural 5(35.7%). **Additionally estimation prevalence of *alp* and asp genes in *A.fumigatus* fungal isolates** has been showed highly prevalence in isolates.

Conclusion: Our data showed increase prevalence of *alp* and asp genes in *Aspergillus fumigatus* in Iraqi patients. This prevalence should be alarming public health.

Key words: *A. fumigatus*, *alp* and *asp* genes.

1-Introduction

There are many different types of Aspergillus spp. in the air, and they can cause Aspergillosis, a fungal infection caused by a saprophytic filamentous fungus (1). *Aspergillus spp*. infections continue to be associated with substantial morbidity and death. (2). The clinical manifestation of Aspergillus that causes lung illness is detect by the fungus' interaction with the host (3). Traditional classifications for Aspergillus-related lung disorders are based on the host's immunologic condition and the presence of other lung disease (1). Allergy, saprophytic colonization, and invasive aspergillosis are the three clinical classifications of aspergillosis (4)

Aspergillosis that effect respiratory tract can manifest itself in a variety of ways, from mild hypersensitivity to rapidly invasion disease.(4). Asthma and cystic fibrosis patients are at susceptibility for allergic Broncho-pulmonary aspergillosis (ABPA) (5). Saprophytic infection is more common in patients with problem in airways such as, bronchiectasis, chronic obstructive pulmonary, disease cystic fibrosis or chronic lung cavities (aspergilloma). Invasive aspergillosis (also known as angio-invasive or Broncho-invasive aspergillosis) is a dangerous infection that affects those who have a compromised immune system (Chabi *et al*., 2015). Wind disperses the spores of this ubiquitous mold in the air, and the major route of infection is inhalation the spores in practically all types of aspergillosis (6). The most important predictor of a successful outcome in immunocompromised persons is early and focused systemic antifungal treatment (2).

Aspergillus fumigatus is a fungus that causes allergic bronchopulmonary aspergillosis (ABPA), a complex lung immunological disorder. Symptoms of bronchiectasis include wheezing, lung opacity, and bronchitis (5);(7). The most well-known manifestation of allergic aspergillosis is ABPA, or acute allergic bronchopulmonary aspergillosis (6). (ABPA) causes pulmonary infiltrates, tenacious mucus plugs harboring *Aspergillus fumigatus* hyphae, increase in total serum IgE and sputum eosinophilia and peripheral blood, in individuals with cystic fibrosis or asthma (8).

Fungal proteases such as serine and a Aspartate proteases have diverse importance in corporate sectors such as the pharmaceutical, detergent, leather, waste, and food industries (9). In the food industry, they are used to make beer, wine, and vinegar. Acidic proteases are used to improve wheat gluten's structural and functional properties (10). In medical sectors, fungal proteases are used as therapeutic agents for the treatment of a variety of diseases such as cancer, HIV, inflammatory diseases, diabetes, and hepatic cancer (11). In the textile and laundry industries, they are used to prepare enzyme-based detergents to remove the tough stains from clothes due to developing excellent washing performance

compared to other microbial enzymes (12). They are also involved in degrading lignocellulosic biomass, and products can be utilized as biofuels for the production of energy at the commercial level (13).

2-Materials and methods:

2-1Sample collection

Samples collected from Respiratory Patients were attending or Chest and Respiratory diseases Consulting Clinic. The patients have lung infection were diagnosis by Physician. These sputum samples were obtained from patients treated in Public health department Chest and Respiratory diseases clinic. All samples were transferred to the laboratory for culturing to isolation and identification fungi after labeling each samples with questionnaire form. PCR was done to confirm diagnosis and to detect alp and asp genes in isolates.

2-2 Molecular study

2-2-1 The Primers Used in this study

The specific primer sequences used in this study 18s rDNA gene is listed in table (1)

Table (1) The specific primer of gene used in this study (14).

Primer name	Sequence 5`-3`	Annealing temp.(C)	Product size(bp)
ITS1	**TCCGTAGGTGAACCTGCGG**	**55**	**600**
ITS4	**TCCTCCGCTTATTGATATGC**		
Pep2A-F	**GGGAGAACTTTGCCTCCATATT**		
Pep2A-R	**CAGTACGCCAGGCACTTATAC**	**55**	**418**
Asp-F	**TGGACATGCATCAACCAA**	**45**	**318**
Asp-R	**GTCAAACTTATAGTCGTG**		
Alp-F	**AGCACCGACTACATCTAC**		
Alp-R	**GAGATGGTGTTGGTGGC**	**63**	**749**

2-2-2 Detection of DNA by electrophoresis

Gel electrophoresis used to identify the product PCR which is used UV transilluminator. DNA detection by compare the size of gene 401 pb by ladder DNA 1500bp.

2-2-3 DNA sequencing

DNA sequencing used sanger, s method that sent to south korea by macrogen company, PCR products were purified and sequenced in both directionusing the BigDye Terminator v3.1 Cycle Sequencing kit on an ABI3730XL Genetic Analyzer (Applied Biosystems, Foster City, CA). The sequence was analyzed in the nucleotide databases using the NCBI's Basic Local Alignment Search Tool Bio ID program to identify the sample. Related sequences of sample or s were obtained from the NCBI's nucleotide database and included in the multiple alignment using the Bio ID program (Tamura et al, 2011).

2-3 Statistical Analysis

Using SPSS V 25 for Windows, data were input and analyzed. Inferential statistics (Chi-Square Test) and descriptive statistics (frequencies, mean standard deviation and accompanying tables and graphs) were applied. A. statistically significant1 P-value ≤ 0

3- Results

3-1 Association between culture results of A.fumigatus and patient parameters

Patients parameters included gender, age, tuberculosis bacilli (T.B) history, diabetes, hypertension and residency were related to *A.fumigatus* fungus outcomes as a number and percentage in order to detect the prevalence or relation of these results to patients recorded positive results, gender were divided into male and female, outcomes were 9 (8.5%) and 5 (4.8%), age groups number and percentage were 0(0.0%), 3(2.9%), 1(0.9%), 2(1.9%), 3(2.0%) and 5(4.7%) in the age groups <=29, 30-39, 40-49, 50-59, 60-69 and >=70 of *A.fumigatus* regarding total number, T.B history, diabetes, hypertension and residency were related to 14 (100%) of *A.fumigatus*, T.B history was recorded in 1(17.1%) of patients, diabetes number and percentage were 7(50.0%), hypertension 8(57.1%), residency was divided into urban 9(64.3%) rural 5(35.7%), table (2).

Table (2). Association between A.fumigatus and patient parameters.

		Culture			
		+ve		-ve	
		Count	%	Count	%
Gender	Male	9	8.5%	56	53.4%
P=0.956	Female	5	4.8%	35	33.3%

Age P=0.941	<=29	0	0.0%	32	30.5%
	30-39	3	2.9%	10	9.5%
	40-49	1	0.9%	13	12.4%
	50-59	2	1.9%	12	11.4%
	60-69	3	2.9%	13	12.3%
	>=70	5	4.7%	11	10.6%
T.B history P=0.399		1	7.1%	13	92.9%
Diabetes P=0.833		7	50.0%	7	50.0%
Hypertension P=0.835		8	57.1%	6	42.9%
Residency P=0.835	Urban	9	64.3%	5	35.7%
	Rural	5	35.7%	9	64.3%

P value < 0.05 was considered significant.

3-2 Estimation prevalence of *alp* gene in *A.fumigatus* fungal isolates

In the current study, the amplification of *alp* gene of fungal species were fractionated on 1.5% agarose gel electrophoresis on 14 isolates of *A.fumigatus*. using PCR technique to indicate presence of *alp* gene. The outcomes revealed that this gene was highly prevalence in isolates, figure (1).

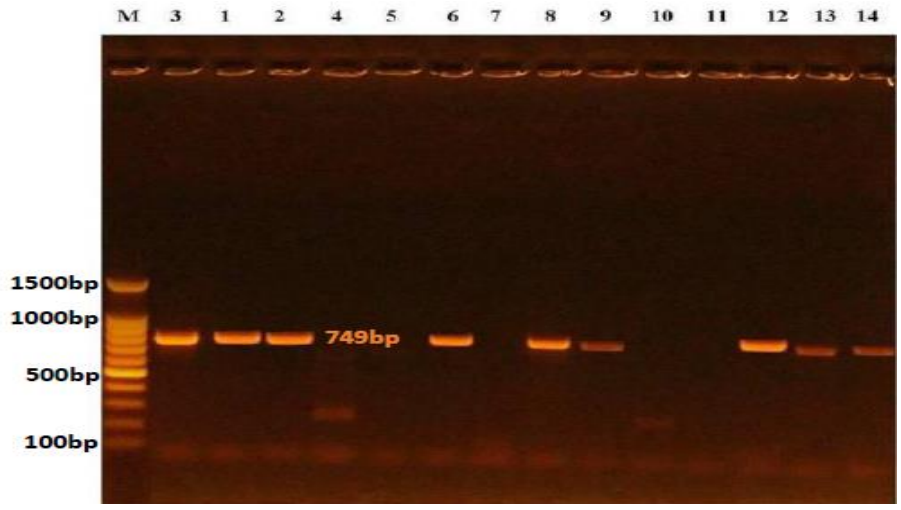

Figure (1): Results of the amplification of *alp* gene of fungal species were fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes 3-14 resemble 749bp PCR products.

3.3 Estimation prevalence of *asp* gene in *A.fumigatus* fungal isolates

In the current study, the amplification of *asp* gene of fungal species were fractionated on 1.5% agarose gel electrophoresis on 14 isolates of *A.fumigatus*. using PCR technique to indicate presence of *asp* gene. The outcomes revealed that this gene was highly prevalence in isolates, figure (1).

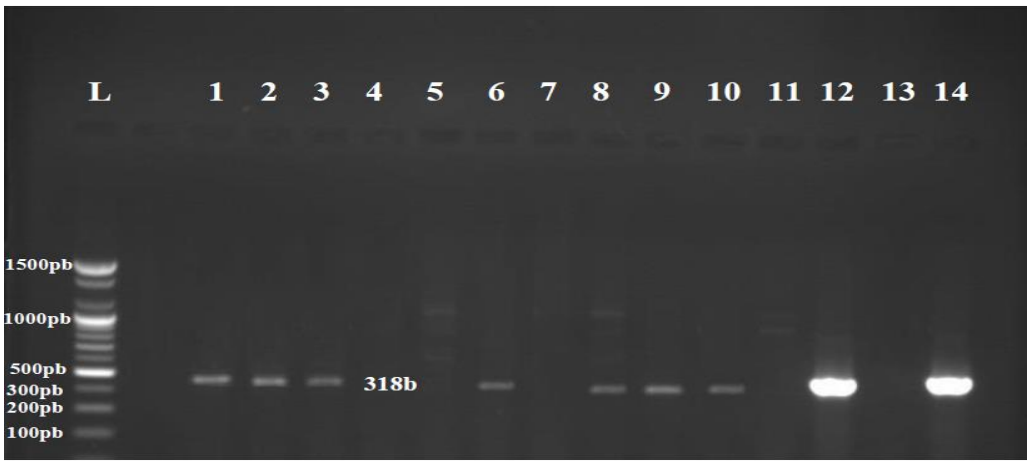

Figure (12): Results of the amplification of *asp* gene of fungal species were fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes 3-14 resemble 318bp PCR products.

4- Discussion

Multiple mechanisms have been described for how sex hormones influence immune function in females and males and effect fungal infection rate. For example, sex hormones regulate immune cell activation and function by binding to estrogen and androgen receptors and interacting with nuclear hormone response elements (HREs) and by modulating the epigenetic landscape directly. Several genes coding for proteins involved in immunity, including gamma-interferon (*IFNG*) and interferon regulatory factor 5 (*IRF5*), possess HREs that, upon activation, drive the production of cytokines and chemokines. IFN-γ is a pleotropic cytokine that is required for protection against a variety of fungal infections and that has been proposed as a potential adjunctive immunotherapy for IFDs. On the contrary, *IRF5* has been shown to relay signals from the dectin-1 receptor during fungal infection towards the production of type I interferons and protection from fungal infection. Whether sex influences IFN-γ and other interferon-related signals in response to fungal infection remains, however, to be assessed (15-17).

In present study gender were divided into male and female, outcomes of A.fumigatus were 9 (8.5%) in male and 5 (4.8%) in female. Our records were in agreement to Gernez et al. (2018) study as data on 85 patients (50 males; 35 females) of these patients. Pneumonias were more commonly attributed to Aspergillus fumigatus (22.4%), the infection were most often associated with A. fumigatus (P Fisher's exact test = .016) (18). Epidemiological studies have implicated differences in sexes with regards to allergic asthma and infections, The effect of host-sex on immune-related cells, proteins and histopathological changes have not been investigated in the context of *A. fumigatus* exposure. Biological sex affects innate and adaptive immune responses, resulting in sex differences in autoimmunity. These sex-specific differences stem from several superimposing elements, including genomic and epigenomic organization, as well as a direct effect of sex steroid (estrogen, progesterone and testosterone) on components of the immune system (19, 20). Schaefer et al. (2020) recorded A. *fumigatus* to be a respiratory fungal pathogen and an allergen. Previous study showed that repeated inhalation of live and dry *A. fumigatus* spores, without any adjuvant, elevated allergic immune response and airway remodeling, sex-specific differences can influence host-pathogen interactions and allergic-asthma related outcomes. Principal Component Analysis (PCA) showed that females exhibited significantly higher levels of immune components than males did. Taken together, data indicate that host-sex is an important factor in shaping the immune response against *A. fumigatus*, and must be considered when modeling disease, in designing diagnostics and therapeutics for *A. fumigatus*-associated diseases or while drafting evidence-based guidelines for safe mold levels (21).

In this study age groups number and percentage were 0(0.0%), 3(2.9%), 1(0.9%), 2(1.9%), 3(2.0%) and 5(4.7%) in the age groups <=29, 30-39, 40-49, 50-59, 60-69 and >=70 of *A.fumigatus* regarding total number. These information were consistence to Düesberg et al. (2020) as the role of fungi in the cystic fibrosis (CF) lung is still not well elucidated. The most common filamentous fungus in CF is *Aspergillus fumigatus* (AF). Prevalence was low in children less than ten years, highest in the middle age and getting lower in higher age (≥ 50 years). Continuous antibiotic lung treatment was significantly associated with AF prevalence in all age groups. Older age up to 49 years and continuous antibiotic use were found to be the main risk factors for AF permanent colonisation. AF might be associated with decrease of lung function if not disguised by chronic PA infection (22). In Hong et al. (2018) study AF, however, is known to be able to cause allergic bronchopulmonary aspergillosis (ABPA) and acute infection of the lung parenchyma (*Aspergillus pneumonia*), which can be severe and difficult to treat. An additional AF entity is the *Aspergillus bronchitis*. Several risk factors for AF colonisation, like continuous antibiotic therapy or chronic lung inflammation especially in older age (23).

While present data were not agreed with Moor et al., (2023) as microbiological data was examined from 100 patients from birth and older, equating to 2455 patient years. *A. fumigatus* was isolated from 66/100 (66%) adult CF patients; (i) F508del/F508del homozygous (82%; 37/45), (ii) F508del/other heterozygous (56%; 25/45), 14 mutations were noted on the second allele, with R560T and R117H collectively accounting for 36% of the second mutations. There was no significant difference ($p = 0.12$) in time to first acquisiton between males and females, whereby males had their first *A. fumigatus* isolate at 118 ± 9.4 months, whereas females had their first *A. fumigatus* isolate at 140 ± 10.8 months. The highest rate of first *A. fumigatus* isolation was from 4 years until 16 years and by the age of 16 years, approximately 85% of *A. fumigatus*-positive patients had recorded their first *A. fumigatus* isolate (24). Pfaller et al. (2022) study found that Aspergillus fumigatus, was more common in younger people than in older people (25). This difference can be attributed to immune status of individuals around the world related to amount of antibiotics misusing during lifetime.

In current study T.B history was recorded in 1(17.1%) of patients, progress of the disease and prolonged treatment with antibiotics or immunosuppressive agents makes tuberculosis patients susceptible to fungal infections. Hosseini et al. (2020) study aimed to determine the prevalence of pulmonary Aspergillus coinfection among patients with pulmonary tuberculosis in Asia and Africa. Study present review of cross-sectional studies was conducted on the prevalence of pulmonary Aspergillus coinfection among patients with pulmonary tuberculosis, the combined Aspergillus coinfection among patients with pulmonary tuberculosis was 15.4% (95% CI: 11.4–20.5), Q = 105.8 and Z = 9.57 in Asia and Africa. The most frequency of *Aspergillus spp.* was related to *A. fumigatus* with a combined prevalence of 57.6%. Most of the studies included in the present review showed a higher *Aspergillus* coinfection in the age group of 40 years and higher. Also, the existence of a correlation between increasing age and Aspergillus coinfection was reported ($p < 0.05$). Aspergillus coinfection was varied between 3.7 and 33.3%. As well as, the combined prevalence of Aspergillus coinfection among patients with pulmonary tuberculosis was 15.4% (26). Xerinda et al. (2014) indicated that pulmonary aspergillosis co-infection includes simultaneous infection of a host's lungs with Aspergillus spp., and Mycobacterium tuberculosis that cause more complications (27). As well, the aged patients over than 50 years, owing to the recurrent TB, the weakening of the immune system, and also the prolonged anti-tubercular therapy, becomes an effective predisposing

factor for the beginning of coinfection by the fungal agents in active pulmonary tuberculosis (28). Eight countries (India followed by, China, Indonesia, the Philippines, Pakistan, Nigeria, Bangladesh and South Africa) account for two thirds of the total TB cases, the 30 high TB burden countries accounted for 87% of new TB cases related to aspergillus infection (29). Consequently, given the high rate of tuberculosis in high TB burden countries, the prevalence of Aspergillus coinfection is high. We showed that the most frequency of Aspergillus spp. was related to A. fumigatus with a combined prevalence of 57.6%. A. fumigatus can cause a variety of pulmonary diseases such as aspergilloma that characterised by saprophytic growth of the fungus in pre-existing tuberculous cavities (30).

Notably, most of the included studies have used phenotypic methods to identify fungi except one case. Therefore, regarding the importance of pulmonary Aspergillus coinfection, the use of molecular methods and serological tests to identify Aspergillus coinfection is needed. On the other hand, mycosis infection if diagnosed early can be treated efficiently, and consequently can prevent from progress to fibrotic stage and avoided pulmonary disability (31).

This difference in reporting Aspergillus coinfection in Pulmonary TB+ patients between different countries predominantly attributed to the geographical location, health policies, climate conditions, and socio-economic condition, phenotypic, duration of anti-tuberculosis consumption by patients, clinical samples types, and molecular methods used for detection of Aspergillus infections in each country (32).

In this study diabetes number and percentage were 7(50.0%), two studies conducted by Osman et al., (2013) included a correlation between DM and fungal Aspergillus. Also, besides Osman and Mathavi et al. (2014) showed a correlation between pulmonary Aspergillus coinfection and smoking. Similarly, Smoking and DM act as co-factors in quickening and increasing the immune-suppression mode (33, 34). The prevalence of mycotic infection in diabetic patients has been confirmed by Khanna et al. (2007) (35), they reported the main factors for prevalence of Aspergillus in pulmonary tuberculosis as following; the existence of resistant strains, the persistence of diseases, and underlying disease including diabetes mellitus (36, 37). In present study hypertension number and percentage were 8(57.1%), Miranda et al., (2015) report a rare case of pulmonary prosthetic valve endocarditis due to *Aspergillus fumigatus*, associated with septic pulmonary embolism and secondary pulmonary hypertension, in a 4-year-old boy with surgically corrected tetralogy of Fallot. The diagnosis and treatment of *Aspergillus* endocarditis remains highly challenging. The best therapeutic option for chronic thromboembolic pulmonary hypertension due to an infectious thromboembolic event is highly debatable and the results are poor.

Agarwal et al., (2009) resented respiratory failure and pulmonary hypertension in the absence of clinical signs of asthma or bronchiectasis, and in a diagnosis of allergic bronchopulmonary aspergilloma [ABPA] was made subsequently on the basis of radiological and laboratory investigations is reported. This case highlights the importance of keeping a high index of suspicion while investigating a patient with pulmonary hypertension in whom the aetiology is not apparent on initial evaluation. Identification of the disease, in its early-stage, can prevent progression to bronchiectasis and fibrotic lung disease, and thus, patients can be saved from the morbidity related to end-stage disease. (38) (39) (40). The aim of Dielievska and Kravchun, (2018) work was to study the prevalence of mycogenic sensitization in arterial hypertension (AH) and its relationship with the disease severity, progression of chronic heart failure (CHF) and contractilic function of myocardium. 44 patients with II and III stages of AH and II - III functional classes of CHF by NYHA (mean age 62.3 ± 1.7 years, 24 males and 20 females) were examined for the presence of mycogenic sensitization. The levels of serum specific IgE antibodies (sIgE) were measured to Candida maltosa, Aspergillus fumigatus, Aspergillus flavus by an ELISA. The presence of mycogenic sensitization was determined at the level of sIgE > 50 KU/l. The aim of Kravchun et al. (2019) work was to study mycogenic sensitization in patients with arterial hypertension (AH) and its relations with the presence of atrial fibrillation. 41 patients with AH participated in the study, concomitant atrial fibrillation was diagnosed in 17 persons. The patients were also analyzed according to the heart rate – 1 group with heart rate 60-90 bpm (N = 18) and 2 group – with heart rate > 90 bpm (N = 23). Fungal sensitization was revealed in 46.3% of the patients with AH. Patients with concomitant atrial fibrillation showed increased sensitization to Candida maltosa, Candida albicans, Aspergillus fumigatus as compared to the patients without atrial fibrillation. The patients with tachycardia in comparison to the patients with normal heart rate were characterized by a prevalence of mycogenic sensitization to Candida maltosa, Candida albicans and Alternaria tenuis as well as by the highest degree of sensitization to Candida maltosa, Candida albicans and Aspergillus fumigatus, since prevalence of sensitization to Candida and Aspergillus species in AH was observed both in patients with tachycardia and atrial fibrillation, the patients with fungal sensitization are recommended to be thoroughly monitored for the development of heart rhythm disturbances (41).

In our study residency results showed was urban 9(64.3%) rural 5(35.7%), Lee et al. (2020) recorded that not only age and sex were available for healthy volunteers, the analysis of factors associated with *Aspergillus fumigatus* positivity was performed among active patients. Variables adjusted in the logistic regression model included age, sex, body mass index rural/urban residency, underlying comorbidities and pulmonary imaging findings. The correlation between serum titres of *A. fumigatus* was analysed, the study suggested significant geographic variation in urban than rural of *Aspergillus fumigatus* infection. The role of *Aspergillus fumigatus* may be a better test of exclusion in areas where the population baseline level is unknown. (42). Schlosser et al., (2016) suggest an excess risk to nearby residents health when compared with the wide range places, but should also consider wider urban and rural areas unaffected by such conditions (43).

Aspergillus fumigatus secretes a range of degradative enzymes that contribute to the ubiquity of the fungus in nature by supporting fungal (44, 45). Many of these biological determinants also play a role in establishing disease in humans and

are associated with virulence and pathogenesis (46-49). How these enzymes directly or indirectly influence bacterial growth has not yet been investigated in detail, however, recent studies have shown that *A. fumigatus* alters the environmental conditions in vitro, by converting a nutrient-poor, nitrate-rich environment into one rich in amino acids. These conditions, known to exist in the cystic fibrosis (CF) airways, may enable *P. aeruginosa* to outcompete *A. fumigatus* by promoting a metabolic-driven increase in bacterial growth (50). Analysis of the culture filtrates produced by *A. fumigatus* identified an abundance of degradative enzymes which are also involved in virulence, including alkaline protease 1, alkaline protease 2, aspergillopepsin-1, and major allergen Asp f 2 (44). The increase in bacterial growth owing to the presence of *A. fumigatus* may affect the ability of host epithelial cells to efficiently internalize incoming pathogens and participate in microbial clearance (51). This may be exacerbated by *A. fumigatus*-mediated inhibition of host cell apoptosis (52).

Aspergillus fumigatus is the most important etiological agent of invasive aspergillosis (IA), especially in immunocompromised individuals (52). The fungus could produce a wide array of secretory enzymes and secondary fungal metabolites with genotoxic and cytotoxic activities as potential virulence factors (53). Gliotoxin as a member of the epipolythiodioxopiperazine (ETP) class of fungal toxins is produced by the non-ribosomal peptide synthetase (NRPS) enzyme encoded by *gliP* in *A. fumigatus* (54). This toxin is able to suppress the host immune system by altering the function of neutrophils and leukotrienes via inhibiting migration, producing superoxide, and inducing apoptosis (55). Different kinds of proteolytic enzymes such as elastase, serine protease, aspartic protease, and metalloproteases are also produced by *A. fumigatus*, *t*hese enzymes have been shown to facilitate tissue invasion by the fungus (56).

Current results were agreed with Sabotič *et al.,* (2012) study as protease VII (OmpT), an aspartic protease, was identified in the culture filtrate and is associated with the inhibition of coagulation and antimicrobial peptide production (57). Additionally Kulshrestha and Gupta, (2023) showed that secreted aspartic proteases are important enzymes for fungal pathogenicity, playing a significant role in infection and survival, showed positivity in approximately all isolates, so there is insight into how SAPs facilitate the transformation of fungal cells into hyphae and engage in biofilm formation, invasion and degradation of host cells and protein, so aspartic proteases could provide an effective treatment option for a wide range of fungal infections (58). Many studies showed that fungal proteases have been widely studied due to their wide diversity, proteases have been isolated from different fungi such as *Aspergillus fumigatus*, and *Neurospora crassa* (59-61). In the same way dos Santos Aguilar and Sato, (2018) study indicated that fungal species such as *Aspergillus* sp., produce serine proteases (62). Aspartate proteases show specificity for aromatics such as phenylalanine, tyrosine, and tryptophan on both sides of the peptide bond (59). Fungi species such as Aspergillus, Penicillium, Rhizopus, and Neurospora produce aspartic proteases (63).

While present information did not compatible to Jaton-Ogay et al. (1994) mentioned that *Aspergillus fumigatus* secretes a serine alkaline protease (ALP) and aspartic protease when the fungus is cultivated in the presence of collagen as sole nitrogen and carbon source. The gene encoding ALP was isolated from all isolates, suggesting that ALP and aspartic protease are not essential for the invasion of the lung tissues by *A. fumigatus.* (64).

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