The study of fungal infections and diabetic foot ulcer in Iraqi patients

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Abstract

Fungal infections of the foot are a common and uncomplicated occurrence in the general population. This study aimed to investigate the presence of various fungal species in the diabetic feet of Iraqi patients. A total of one hundred specimens were collected from skin scrapings and nails of the patients and cultured on Sabouraud Dextrose Agar (SDA), then subjected to the isolates were subjected to different microscopic (The potassium hydroxide (KOH) test and the lactophenol cotton blue (LPCB) staining), morphological (cultural Characteristics on medium) and biochemical (hemolysis, catalase and oxidase) for identification of their species. The results of this study indicate that these specimens of DFU patients contain different mold and yeast species. In skin mycosis, Aspergillus flavus was predominant as (18.8%) followed by Candida albicans (12.5%), Aspergillus niger (7.8%), Trichophyton mentagrophytes (7.8%), Trichophyton interdigitale (6.3%), Candida dubliniensis (3.1%), Microsporum canis (1.6%), while, among the 23% of nails scrapings specimens, dermatophytosis were the most isolated than yeast spp. as (50.1%, and 13.9%, respectively). Aspergillus niger was prevalent in nail infections as (27.8%), followed by Trichophyton interdigitale (16.7%), Candida dubliniensis (8.3%), Candida albicans (5.6%), Microsporum canis (2.8%), Trichophyton mentagrophytes (2.8%).

Keywords: *Fungal infection, diabetic food ulcer, PCR, dermatophytosis.*

INTRODUCTION

Fungal infections of the foot are a common and uncomplicated occurrence in the general population. People whose peripheral neurological and/or vascular condition has been damaged as a result of diabetes are more at risk for developing life- and limb-threatening complications as a result of fungal foot infections [1]. Fungi can survive in different temperatures and values of pH settings and may be found in almost every environment [2]. Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia (high blood glucose levels) caused by defects in insulin secretion, insulin action, or both. Long-term damage, dysfunction, and failure of multiple organs, including the blood vessels, heart, nerves, kidneys and eyes, are related with the chronic hyperglycemia of diabetes [3].

One of the consequences of DM is the diabetic food ulcer (DFU), which is expensive and devastating diabetic complication that consist of the lesions of deep tissue related with both peripheral vascular and neuropathy diseases [4]. An urgent need required for novel effective therapy in order to treat the DFU, which considered as a serious public issue, for

decrease the social and economic burden and for decrease the related rates of mortality and morbidity [4], [5]. Mycotic infections are associated with DFUs, and the polymicrobial nature of DFUs raises the probability that this condition will manifest [6]. However, the frequency of fungal colonies in DFUs has only been the subject of a small number of investigations. More over a quarter of DFUs develop fungal infections, but these infections frequently go unnoticed or misdiagnosed in the DFU clinics due to the use of conventional microbiology laboratory procedures [7]. The objectives of the current study are to investigate the presence of various fungal species in the diabetic feet of Iraqi patients.

Methodology

Collection and specimens

A 100 specimens were collected from skin scrapings and nails of the patients with diabetes who visited the diabetic clinic at Al-Ramadi Teaching Hospital in Ramadi city, Al-Anbar state, Iraq between the period from November 2021 to May 2022. This study included patients varied in sexes and ages, whereas their ages raged between (43-37 years). The isolates were subjected to different microscopic, morphological, biochemical and molecular examinations for identification of their species.

Laboratory diagnosis

Morphological examinations

The specimens were culured on the SDA containing-petri dishes. The pathogenic fungal isolate grown on SDA medium has been identified morphologically according to the direct examination using potassium peroxide test (KOH-test) and the cultural characteristics. Lacto phenol Cotton Blue stain (LPCB) was performed according to [8]. Sugar fermentation media and HiCrome agar were utilized for further identification [9].

Biochemical examination

Two biochemical tests were performed, include hemolysis [10] and catalase test [11].

Results

In this study, 100 specimens collected from patients with diabetes who visited the diabetic clinic between November 2021 and May 2022 at Al-Ramadi Teaching Hospital in Ramadi city, Al-Anbar state, Iraq. The specimens were included skin scrapings and nails of the patients, who were diagnosed clinically by a specialist doctor, suffering from Diabetes.

Morphological examinations

The potassium hydroxide (KOH) test

The KOH prep test (also known as a Potassium Hydroxide skin lesion inspection, fungal smear, or skin scraping) is a simple, rapid, accurate, almost painless, and noninvasive process for identifying skin and nail fungal infections. [12] A clinical microbiologist investigated each of the 100 cases of diabetic foot ulcers (DFU) and collected 100 specimens from them (70 skin scrapings and 30 nail scrapings). One hundred samples (100%) were KOH-positive. Only 60% of the 100 positive direct microscopy samples (37% of skin scrapings and 23% of nails) were cultured growth. Direct microscopy had a false-negative rate of 40%, as 30 positive samples gave negative culture findings, and 10 samples were contaminated.

The lactophenol cotton blue (LPCB) staining

The lactophenol cotton blue (LPCB) wet mount preparation is the most widely used method of staining and observing fungi and is simple to prepare. The preparation has three components: Lactic acid preserves the fungal structure and clears the tissue while phenol acts as a disinfectant which will kill any live organisms; and cotton blue, which stains the chitin in the fungal cell walls, imparts blue

coloration to the fungal spores and hyphae [13].

Culturing on Sabouraud Dextrose Agar (SDA)

All specimens in this study were culture on Sabouraud Dextrose Agar (SDA). The results were represented in the following figures (1 to 7).

Figure 1: Morphological appearance (a) C. albicans strain. (b) C. Dubliniensis.

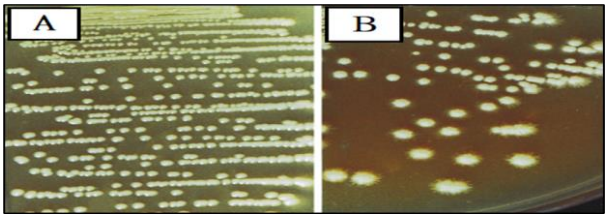


Figure 2: Candida albicans colonies cultured on: a) SDA, (b) HiCrome agar

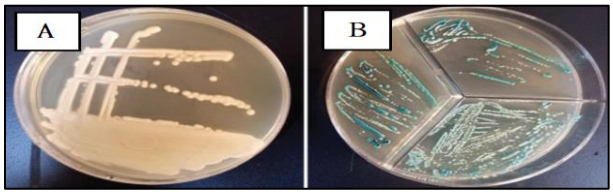


Figure 3: A and B: Aspergillus niger grows on SDA, C: microscopic field (40x) of A. niger

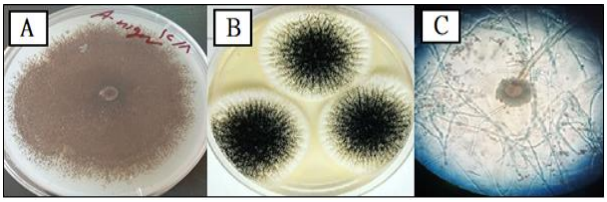


Figure 4: A) Aspergillus flavus grows on SDA, B) Microscopic field (40x) of A. flavus

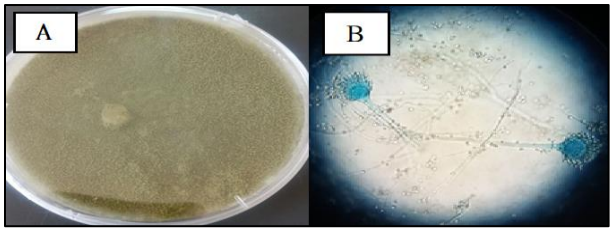


Figure 5: A) Microsporum canis on SDA, B) Microscopic field (40x) of M. canis

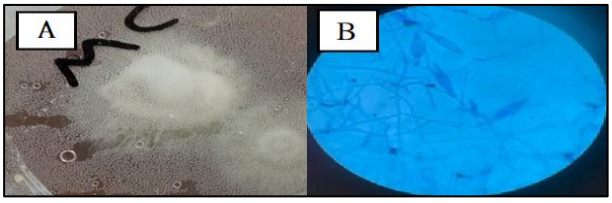


Figure 6: A) Trichophyton rubrum on SDA, B) Microscopic field (40x) of T. rubrum

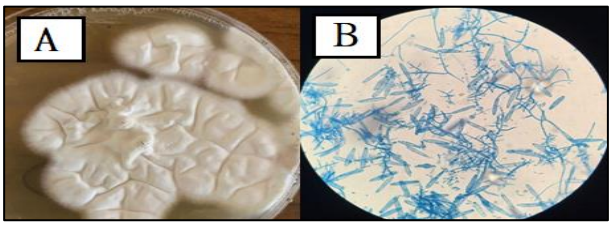
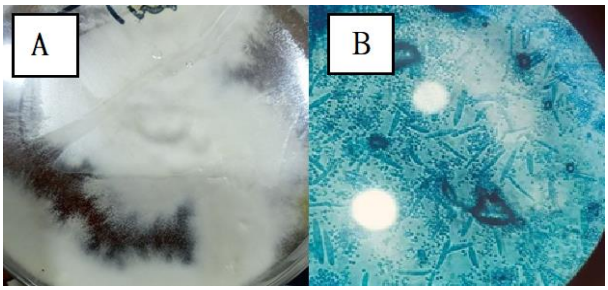


Figure 7: A) Trichophyton mentagrophytes on SDA and B) Microscopic field (40x) of Trichophyton mentagrophytes



Biochemical examinations

In this study, three biochemical tests used to identify fungal species, they were Hemolysis Test and Catalase. In pathogens, the production of a hemolytic protein capable of lysing erythrocytes has also been proposed as a survival mechanism for fungi [14]. Catalase is present in Aspergillus species. It catalyzes the breakdown of H2O2 into O2 and H2O. Conidial and mycelial catalases provide defense against H2O2 for the fungus [15], [16]

The distribution of data based on the sex and age

As presented in Table 4 and 5 below, most common age group involved was 53-62yrs

years in 46 (46%) cases. Of the 100 patients enrolled in this study, (54%) were males, and (46%) were females.

Table 3: Distribution of specimens according to Gender and Age Categories

		Culture Results				Total	
		Growth		No growth			
		Count	Percent	Count	Percent	**Count**	**Percent**
Age Categories	43-52yrs	22	22.0%	7	7.0%	**29**	**29.0%**
	53-62yrs	31	31.0%	15	15.0%	**46**	**46.0%**
	63-73yrs	17	17.0%	8	8.0%	**25**	**25.0%**
Total		70	70.0%	30	30.0%	**100**	**100.0%**
Gender	Male	38	38.0%	16	16.0%	**54**	**54.0%**
	Female	32	32.0%	14	14.0%	**46**	**46.0%**
Total		**70**	**70.0%**	**30**	**30.0%**	**100**	**100.0%**

The incidence of DFU according to culture was not statistically significant with smoking, as the (P=0.3>0.05).

Table 4 Distribution of specimens according to Smoking

			Culture Results		Total
			No growth	Growth	
Smoker	No	N	21	55	76
		%	27.6%	72.4%	100.0%
	Yes	N	9	15	24
		%	37.5%	62.5%	100.0%
Total		**N**	**30**	**70**	100
		%	**30.0%**	**70.0%**	100.0%
P-value					0.358

The incidence of DFU according to culture was not statistically significant with rising DM duration, as the (P=0.3>0.05).

Table 5: The proportion of diabetic foot ulcers with the duration of diabetes

			Culture Results		Total
			No growth	Growth	
DMD Categories	5-9yrs	N	7	22	29
		%	23.3%	31.4%	29.0%
	10-14yrs	N	1	10	11
		%	3.3%	14.3%	11.0%

	15-19yrs	N	14	26	40
		%	46.7%	37.1%	40.0%
	20-24yrs	N	5	6	11
		%	16.7%	8.6%	11.0%
	25-30yrs	N	3	6	9
		%	10.0%	8.6%	9.0%
Total		**N**	**30**	**70**	100
		%	**100.0%**	**100.0%**	100.0%
Pearson Chi-Square					4.539
Asymptotic Significance (2-sided)					0.338

The incidence of DFU according to culture was not statistically significant with HbA1c level, as the (P=0.9>0.05).

Table 6: The proportion of diabetic foot ulcers with the HbA1c

			Culture Results		Total
			No growth	Growth	
HbA1c %	11	N	8	18	26
		%	26.7%	25.7%	26.0%
	12	N	10	26	36
		%	33.3%	37.1%	36.0%
	13	N	12	26	38
		%	40.0%	37.1%	38.0%
Total		**N**	**30**	**70**	100
		%	**100.0%**	**100.0%**	100.0%
Pearson Chi-Square					0.137
Asymptotic Significance (2-sided)					0.934

The distribution of fungal pathogens among samples of patients with DFUs

A 15 (15%) of the sixty microorganisms isolated from the specimens gathered for this investigation were yeast species, while 45 (45%) were dermatophyte species. Most infections in this study were skin mycosis (37%).

Table 7: The spectrum of fungal pathogens isolated from the specimens

Etiological agents		Specimens						Total	
		Nail			Skin				
		N	%	% Total	N	%	% Total	**N**	%
Yeast	*Candida albicans*	2	5.6%	2.0%	8	12.5%	8.0%	**10**	10%
	Candida dubliniensis	3	8.3%	3.0%	2	3.1%	2.0%	**5**	5%
Mold	*Aspergillus flavus*	0	0.0%	0.0%	12	18.8%	12.0%	**12**	12%
	Aspergillus niger	10	27.8%	10.0%	5	7.8%	5.0%	**15**	15%
	Microsporum canis	1	2.8%	1.0%	1	1.6%	1.0%	**2**	2%
	Trichophyton interdigitale	6	16.7%	6.0%	4	6.3%	4.0%	**10**	10%
	Trichophyton mentagrophytes	1	2.8%	1.0%	5	7.8%	5.0%	**6**	6%
Contamination		3	8.3%	3.0%	7	10.9%	7.0%	**10**	10%
No growth		10	27.8%	10.0%	20	31.3%	20.0%	**30**	30%
Total		36	100%	36%	64	100%	64%	100	100%

Discussion

Diabetes mellitus (DM), also referred to as diabetes is a collective of metabolic disorders of multiple etiologies that result in chronic diseases marked by hyperglycemia due to inadequate action and/or production of insulin, a hormone responsible for regulating the levels of glucose in the bloodstream [17]. Diabetic-foot ulcers (DFUs) are one of the most significant consequences in diabetic individuals (>75%), causing up to 20% of diabetes-related hospitalizations [18], and approximately 15% of DFU result in amputations, which account for 80% of nontraumatic lower-extremity limb amputation and are correlated with 5-year mortality rates between 43% and 55%, more than prostate or breast cancer and Hodgkin's disease [19]–[21]. The foot lesions are susceptible to subsequent bacterial, fungal, and viral diseases, which are frequently chronic and treatment-resistant [22].

The majority of the DFU mycobiome consists of a balance of pathogenic and normal flora yeasts. [23] In diabetic foot wounds, the development of fungi mycobiome is related with delayed healing with poor wound outcomes. In the meantime, they may develop mixed biofilms with bacteria and worsen the severity of the wounds; [24], also, the clinical manifestations of mycological diseases are poor and ambiguous, leading to a prolonged diagnosis that exacerbates the problem [25]. Hyperglycemia, vascular dysfunction, neuropathy, and different immunological abnormalities are major factors responsible for the infection of Candida, Dermatophytes, and Aspergillus spp. by patients with DM. [26]. Foot complications are common in the elderly diabetic [27], because the prevalence of DFU rises with increasing age [28]–[32]

However, fungal species are opportunistic pathogens that depend on strain virulence and the status of the host, regardless of age and/or gender of the patient, because of environmental exposure to these ubiquitous organisms [33]. Although, smoking patients who suffer from diabetes are more susceptible to fungal infection than nonsmoker patients because smoking has adverse effects on the immune system, respiratory tract, and soft tissues. [34]. In DFU with poor control, the

incidence of fungal infection was much higher, necessitating special focus and therapy. [35] In addition, patients are unable to detect infections since they cannot feel cuts and irritations. [36] The fungal infection is challenging to identify and a significant source of morbidity and mortality in DM patients. [37].

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