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ORIGINAL ARTICLE

Molecular Detection of Mucor Circinelloides in Diabetic Patients

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ABSTRACT

Workload **Aims:** This study was aimed to detect of Mucor circinelloides in diabetic patients and compare between the cultural and molecular methods for rapid and efficiency detection

Materials and Methods: A total of one hundred specimens were collected from patients with diabetes mellitus who attended medical city in Baghdad for a period of four months. Clinical diagnosis were done by physician. The specimens were classified as (Blood, Cutaneous and sputum). According to the gender, the specimens were sixty eight males and thirty two females. Different cultural and molecular detection methods were used for identification of M. circinelloides.

Results: Five isolates of M. circinelloides were identified using different cultural media and PCR method. The molecular weight of M. circinelloides bands using PCR method was ~650 bp. Depending on PCR sequencing method, only one isolate was showed substitution mutation in amino acid is (I).

Conclusions: The efficacy of used different cultural methods and molecular methods to isolate M. circinelloides were similar but, molecular methods are less time consuming.

Keywords: Mucor circinelloides, Diabetic patients, cultural method, PCR methods

INTRODUCTION

Mucormycosis is an emerging infection caused by Mucorales. Paultauf (1885) was the first one who described this fungal infection[1]. mucormycosis comes after candidiasis and aspergillosis as the most common problems invades by fungi of immunocompromised (such as uncontrolled DM) persons throughout the altering in immune defense of them[2–4]. Mucor circinelloides is fungus that belongs to the order Mucorales. It grows as mold in the environment and cause mucormycosis, a potentially fatal infection in immunocompromised patients[5].

Early diagnosis and treatment of mucormycosis is so important to decrease the morbidity and mortality rates of this infection because the studies found that the mortality rate reported in more than half percent of infected patients. Also, the recognition of risk factors is a vital role for earlier diagnosis of mucormycosis.[6]

Diabetes mellitus (DM) is one of the most common endocrine disorder affecting of people worldwide. It is the main cause of some other diseases like kidney failure, strokes, lower limb amputation and heart attacks. According to the World Health Organization (WHO), 1.5 million of people were dead by diabetes in 2019[7,8.]

The genera belonging to Mucoraceae can span from being saprobic to being opportunistic pathogens in both humans and animals[9]. Mucor and Rhizopus genera were the most frequency isolated in comparison with Apophysomyce, Cunninghamella, and Rhizomucor[10–13].

Molecular methods such as Polymerase chain reaction (PCR) and PCR-based sequencing have been used as confirmed and improving detection methods of laboratory diagnosis of patients with mucormycosis, especially M. circinelloides[14–16].

METHODS

One hundred specimens were collected from diabetic patients who attended consultant clinics of medical city in Baghdad for a period of four months. The specimens were distributed as (57 blood, 24 cutaneous and 19 sputum). Sixty eight were males and thirty two were females with age range between 10 and 70 years old. For M. circinelloides identification, the specimens were streaked on plates cultured onto (Sabouraud's dextrose agar (SDA) incubated at 30◦C for seven days, Potato dextrose agar (PDA) at 28◦ C and 40◦ C for five days, blood agar incubated at 30◦C for seven days and Chocolate agar incubated at 35◦C for five days)[17,18].

For M. circinelloides DNA extraction. In brief, twenty mliliters of Yeast potato dextrose medium in sterile universal tube. Then, 20 µl of spore suspension using a micropipette were added to YPD medium and the cultured universal tubes were incubated with shaking at 28°C for 24 hours. After the overnight shaking incubation, Mucor circinelloides mycelial mass were collected and filtered using sterile filter paper and wash with distilled water and freezing dry for DNA extraction according to the protocol of manufacturer using Wizard genomic DNA purification kit (Promega, USA). After extraction of M. circinelloides DNA, the agarose gel electrophoresis was done to prove the presence and integrity of the extracted M. circinelloides DNA by using agaros gel (1%) at 7volt /cm for 1 hour.

The primers that used for amplification of ITS region were selected according to (Iwen, et al., 2007). The primers sequence used for amplification of ITS region of M. circinelloides and their product size are shown in (Table 1)

Table 1: The primers sequence used for amplification of ITS region of M. circinelloides and their product size

Name of primer	Sequence of primer (5'-3')	Size of product
ITS1	(5'-GGA AGT AAA AGT CGT AACAAG G-3')	~650 bp
ITS4	(5'-TCC TCC GCT TAT TGA TATGC-3')	

The PCR reaction was performed for detection of ITS region of M. circinelloides using twenty five microliters volumes including (12.5 µl of GoTaq Green Master Mix 2X (pH 8.5), 5.5 µl of nuclease free water, 400 deoxynucleoside triphosphate mix, 3 mM MgCl2), 2 µl of each primer and 3 µl of M. circinelloides DNA sample. The Amplification of ITS region of M. circinelloides was done using thermal cycler with PCR program consisted of (initial denaturation 95°C for 4.5 min. was joined by 35 cycles of denaturation step at 95°C for 30S, annealing step at 50 °C for 45s, and extension at 72°C for 1 min. and final extension for 5 mins. at 72°C), after the end of PCR run, the gel electrophoresis was done by loading DNA ladder (100 bp) and 5 µl of PCR product were filled on agarose gel (1.5%) and carry out at 7volt /cm for 1 hr., the solution of ethidium bromide (0.6µg/ml) for 20 mins were used for gel staining. The UV transiluminator at 350 wave length was used to visual and photograph of PCR result for M. circinelloides of ~650 bp band. The PCR sequencing was performed depending on PCR product using ITS primers.[19].

RESULTS

According to the mycological and molecular detection methods, the results revealed that five isolates were positive for M. circinelloides, with a percentage (5%).The primary identification of M. circinelloides isolates was depended on macroscopic features of colonies cultured on Sabouraud's dextrose agar , Potato dextrose agar, blood agar and chocolate agar with no growth on PDA at 40° C for five days. Figure (1) shows the microscopic feature of M. circinelloides staining with lacto phenol cotton blue. These results agreed with[19] who found that there are no growth on

PDA medium after six days incubation period and the microscopic examination characterized with globose yellowish brown sporangia carried on long, branched and hyaline sporangiophores. cultural method has been used to identify clinically important species of Mucor.

The PCR detection method of M. circinelloides ITS region showed that the size of product was~650 bp band. Whereas, the Genomic alignment of PCR sequencing results, it was only one isolate has substitution. The substitution was in M. circinelloides (isolate number 4) of amino acid is (I). The most published studies found some difficulties in the identification of this genus at species level[19]. Mucor circinelloides is a saprophytic mold and be a causative agent of mucormycosis, it causes a fatal infection especially in diabetic and chemotherapy patients[5]. Iwen et al. (2007) who described a case of primary cutaneous zygomycosis caused by M. circinelloides in a patient with hyperglycemia; [20]Lazar et al., (2014) who detected of M. circinelloides in immunosuppressed patient with rhinocerebral infection[20]. Arroyo et al. (2016) who isolated a case of M. circinelloides in from blood culture in immunosuppressed patient, Table (2) shows the correlation between positive results and source of specimens[13].

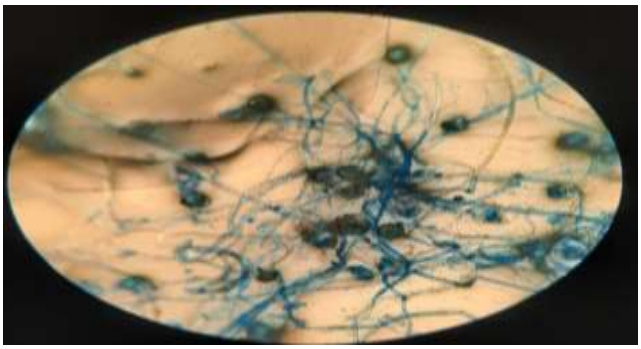

Figure 1: Microscopic feature of M. circinelloides staining with lacto phenol cotton blue (40X)

Table 2: Correlation between positive results and source of specimens.

Results	Type of specimens			Total
	Blood	Cutaneous	Sputum	
Positive results	2 (3.5%)	1 (4.2%)	2 (10.5%)	5 (5%)
Negative results	55 (96.5%)	23 (95.8%)	17(89.5%)	95 (95%)
Total	57 (100%)	24(100%)	19(100%)	100(100%)

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