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Invasive Mucormycosis and the Importance of Molecular Diagnostics

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ABSTRACT

Introduction: Mucormycosis, an infection, with a high death rate, requires understanding its symptoms, diagnosis methods and treatment options due, to its growing occurrence. The research employs a molecular method to evaluate the diagnosis of the mucormycosis.

Materials and Methods: In this study, we obtained 30 samples from patients undergoing diagnosis and conducted DNA extraction. Additionally, DNA extraction was carried out on 30 tissue samples suspected of infection in paraffin blocks. Subsequently, PCR and Real-time PCR were performed using targeted primers, for mucormycosis followed by analysis of the results.

Results: In the research findings among 30 liquid samples 8 tested positive, for mucor using the PCR method. 10 using the Real-time PCR method. Similarly, out of 30 tissue samples, 9 cases showed mucor presence with the PCR method and 11 cases, with the Realtime PCR method.

Conclusion: In this study, real-time PCR and PCR techniques showed promising and faster results in detecting Mucor than the culture approach. The molecular methods provided results that could be of great use for scenarios.

Keywords: Mucormycosis; Diagnosis; Real-time PCR.

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Introduction

Mucormycosis is a fast-spreading infection that poses a severe threat, to individuals with weakened immune systems [1]. In the years there has been a noticeable rise in the number of mucormycosis cases with a focus shifting towards immunodeficiency as a significant factor rather, than diabetes. Mucormycosis refers to infections triggered by mucoral fungi [2]. Many types of mucor fungi can lead to illnesses in humans with Rhizopus and Rhizomucor being the most prevalent ones. Mucorales grow in the normal fungus cultivation environment and are seen as filamentous structure without transverse walls with a perpendicular division [3,4].

Factors that can increase the risk of the disease typically involve conditions, like diabetes, blood malignancies and the use of corticosteroids [5,6]. Mucormycosis has various clinical manifestations. Effective management of mucormycosis often involves timely diagnosis, removal of contributing factors, thorough debridement of affected tissue and administering an appropriate amount of systemic antifungal medication [7,8]. Today researchers are exploring efficient techniques, like DNA detection methods in samples, like serum, BAL and tissue [2,6,9]. Molecular methods, especially Real-Time PCR and PCR, are used in the diagnosis and treatment of many diseases [10-12]. They conducted a study using Real-Time PCR and PCR methods to detect fungi comparing them with culture methods.

Materials and Methods

The research procedure involved acquiring approval, from the ethics committee (IR.TUMS.AMIRALAM.REC.1400.032) to gather 30 tissue samples and 30 fluid samples, from patients referred for diagnosis under the guidance of a physician. To be included in the study, participants needed to meet clinical and pathological criteria for Rhino-Orbital-Cerebral mucormycosis. Patients were excluded from the study if they did not accurately complete their hospital records or did not receive diagnosis and treatment before leaving the hospital for any reason.

To perform real-time PCR and PCR, DNA extraction must be done first. The steps of DNA extraction were performed using GeNet Bio kit and the quality and purity of DNA was evaluated by NanoDrop. Before performing Real-Time RT-PCR and PCR, a specific primer was prepared for mucoral fungi, and the specifications of the primer are shown in Table 1. Real-time PCR was performed in a fnal volume of 20 mL containing: 12mL Ampliqon master mix, 0.3mL of each of F and R primers, 2μL DNA and distilled water. The test was done in 40 cycles using rotor-gene cycler. The temperature and time characteristics are shown in Table 2.

Statistical Analysis

The results were analyzed using statistical software: SPSS Version 22 and the mean and standard deviation were calculated. Paired t-test was used to analyze the difference or relationship between gene expression levels and clinicopathological characteristics. The difference was considered significant at the $P \leq 0.05$ level.

Results

In this study, we looked at a total of 60 samples. 30 tissue samples and 30 liquid samples. To check for the presence of mucor fungus. The microbiology department confirmed all the samples using the culture method, for mucor fungi. Once we collected the samples, we carried out DNA extraction. Checked their purity using the Nanodrop device. Samples that did not have good DNA purity were substituted with new samples. Of course, all DNA extractions were done in duplicate.

DNA samples underwent testing utilizing the PCR method. Initially, all samples were assessed for fungus using a universal primer. Subsequently, a PCR test was conducted using a Mucor primer to detect Mucor fungus. Afterward, DNA samples gathered from fluids and tissues underwent assessment through the Real-time PCR technique. Initially, a general set of primers was utilized for fungus detection followed by a specific primer, for mocure detection. In this investigation among the 30 fluid samples tested 8 individuals showed results for mucor using the PCR method and 10 with the Realtime PCR method. Similarly, among the 30 tissue samples examined 9 individuals tested positive for mucor with the PCR method and 11, with the Realtime PCR method (Figure 1). In Figure 2, an example of agarose gel of the PCR method for mucor is shown. In Figure 3, an example of amplification curves for Real time PCR method is shown.

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Table 1. Mucor primer specifications.

Parameters	*Mucor*
Forward primer	*CTACGACAGTCACATTGGTG*
Length	*20*
Reverse primer	*ATCGGATCTAGAGATCTAGTC*
Length	*21*
product length	*90*
Annealing	*64*

Table 2. Temperature and time conditions of the reaction.

Cycles	*Duration of cycles*	*Temperature*
1	*15 min*	*95°C*
35-40	*15-30 seconds* *60 seconds*	*95°C* *55-60 °C*
1	*Melting Analysis*	*55-95 °C*

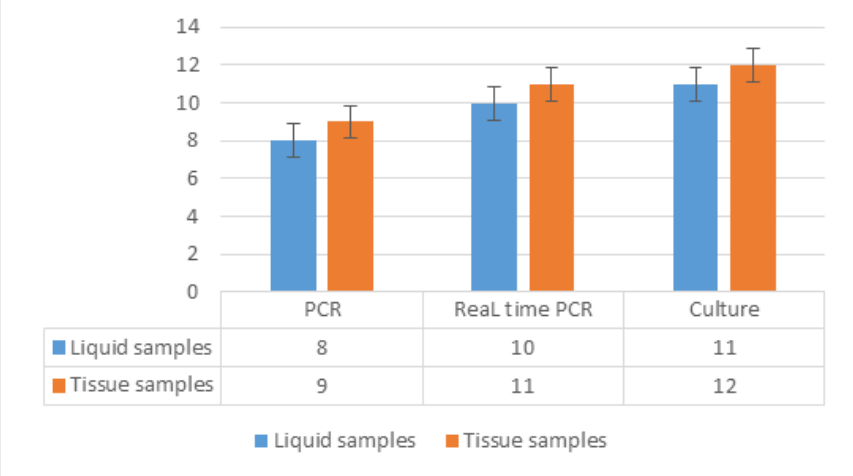

Figure 1. Comparison of the number of positive cases in the performed methods.

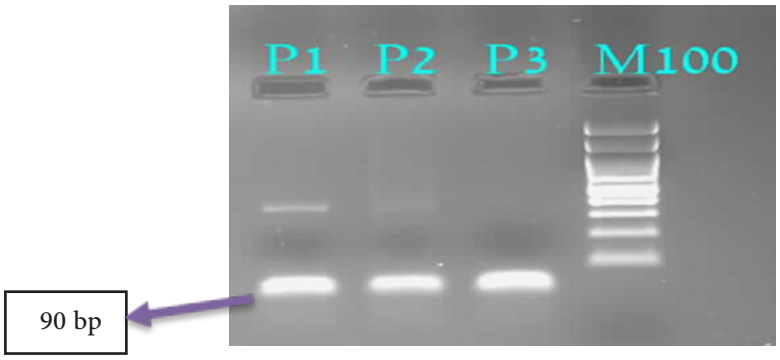

Figure 2. Mucor agarose gel.

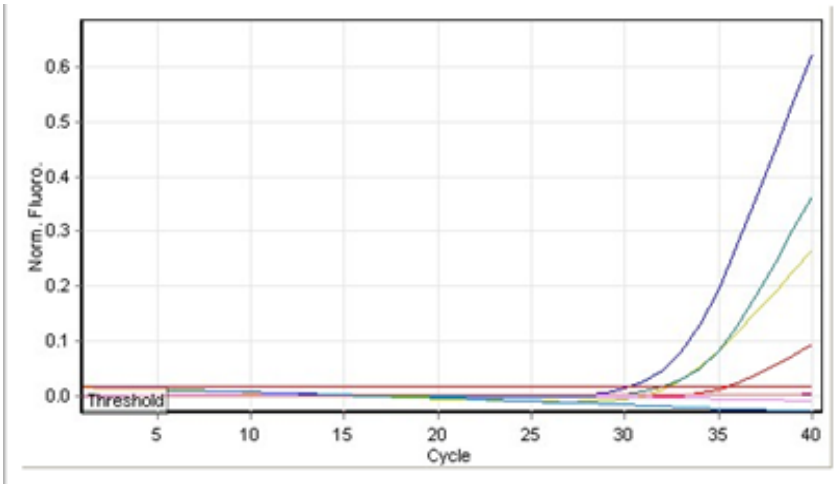

Figure 3. An example of a proliferation curve.

Discussion

In recent years, especially during the COVID-19 pandemic, there have been problems with the increase of treatment-resistant fungal organisms on the one hand and the lack of definitive diagnosis methods and tools on the other hand [5,13]. The concerns are particularly heightened when it comes to two fungi. Aspergillus and Mucor. Timely diagnosis and management of fungal infections remain a pressing issue [9,14]. Advancements in molecular-based and nucleic acid-based techniques have seen progress in recent years with extensive research efforts directed towards identifying fungi like Mucor. PCR and Real-time PCR methods stand out as tools widely utilized for identification and diagnosis purposes in clinical samples [15,16].

The present study was conducted using two methods, PCR and Realtime PCR, and the methods were compared. In this study, out of 30 liquid samples, 8 cases were positive for mucor in the PCR method and 10 cases in the Realtime PCR method. Also, out of 30 tissue samples, 9 cases were positive for mucor in the PCR method and 11 cases in the Realtime PCR method. These differences may be due to the different growth conditions of some fungi or the use of different DNA extraction kits with different sensitivity or different mixes used in the PCR or Real-time PCR process [17]. Springer et al. research used RT PCR to detect mucormycetes. Furthermore, ten patients had both mucormycosis and aspergillosis which were treated separately [18]. It's worth noting that the causes of mucormycosis appear to vary across countries [19]. The data we collected indicate that using techniques to analyze blood serum can play a role, in diagnosing mucormycosis in patients with hematological conditions [18]. According to Millon and colleagues mucormycetes DNA was detectable in around 81% of patients diagnosed with mucormycosis [20]. In a study, the identification of mucor was accomplished through PCR and real-time PCR methods, with success rates. Scherer et al. showed that the sensitivity of molecular diagnostic tests for serum and BAL samples were comparable [21]. The results of Hammond et al. 2011 showed that DNA detection of Mucormycetes by PCR can also be a useful tool to diagnose mucormycosis [22]. All these studies are in line with the recent study and show the importance of molecular diagnosis. However, additional research on molecular diagnostic methods for different causes of mucormycosis should be done so that both mucormycosis agents and its subspecies can be recognized. Also, more studies are recommended for progress and better identification of mucori factors.

Conflict of Interest

There is no conflict of interest to declare.

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