Genetic Diagnosis of Candida Albicans from Patients with Renal Failure and the Ability to Produce Gliotoxin Mycotoxin

Hussein Ali Abbood Shubbar[1], R. A. Sahib[2]
[1, 2]Department of Biology, Faculty of Science, University of Kufa, Iraq
Email: husseinshuber2020@gmail.com

Abstract

The current study, which is carried out on patients with renal failure in Al-Najaf province, 200 oral samples were collected from the dialysis halls in males and females. In both genders, 75 positive samples were found,females have been shown to have a higher rate of Candida infection than males. Females have 48 positive samples out of 100, while males have 27 positive samples out of 100, the percentage of females in positive samples is 64 %, whereas the rate of males is 36%. a positive diagnosis of samples is made using molecular identification of C.albicans by PCR. The results of HPLC show ability of C.albicans to produce secondary metabolites represented by a similar structure of gliotoxin (GT), 16.33 µg/ml concentration is obtained as the highest concentration in the sample15 grown on Minimum Essential Medium (MEM) and low concentration in a sample 1 cultured on Sabouraud Dextrose Brot (SDB) is 1.568 µg/ml. The secondary metabolite of the extract was examined by FTIR, and it was found that there is a significant homogeneity in the active groups in GT.

1. Introduction

Acute renal failure is a type of kidney disease in which the epithelial cells of the renal proximal tubule in the nephron die due to ischemia or toxic damage , this causes a sudden decrease in glomerular filtration rate due to loss of autoregulation, tubular obstruction, and increased renal vasoconstriction [1].

Candida albicans is a commensal and opportunistic human fungal pathogen whose delicate balance is maintained by the innate immune system and the resident microbiota. C.albicans can colonize the nails, skin, mucosal membranes, and internal organs, among other places on the body [2].

C.albicans is responsible for an unacceptably high number of symptomatic infections each year, ranging from superficial (i.e.,skin and mucous membranes) to invasive (i.e.,internal organs) the majority of these infections occur in whose delicate balance is maintained by the innate immune system and the resident immunocompromised individuals and originate in the gastrointestinal tract. as a result, it is critical to bridge the knowledge gap regarding Candida spp.colonization, commensal lifestyle and transition into a pathogenic state. Surprisingly, Candida. spp appear to perform host-beneficial functions [3].

Candida produced gliotoxin, we added a new dimension to the study of Candida virulence [4]. Gliotoxin (GT) is a mycotoxin that was first isolated from Gliocladium cultures however it has recently been reported that it can also be produced by other fungal species, such as Aspergillus fumigatus, Eurotium chevalieri, Trichoderma virens, Neosartorya pseudofischeri, and some Penicillium and Acremonium species [5]. the gliotoxin cause to reduces the fungus's specific immunity [6].

2. Materials and Methods

Samples collection Candida spp. by cotton swab

Samples were collected from patients with renal failure in Najaf Governorate, 200 samples were collected for the purpose of diagnosing types of yeasts samples collection by using the cotton swab medium transporting and taking a swab from the mouth,the surface of the tongue,the bottom of the tongue,the roof of the mouth and the tonsils isolation and diagnoses of Candida spp. in the patients.

Genetic diagnosis of *C.albicans* diagnosis by PCR

Preparing the primers

The primers used in the diagnostics were received lyophilized and the dilutions were prepared according to the manufacturer's instructions by dissolving the lyophilized primers with double distilled water (ddH2O) solution to give a final concentration of 100 pcm/µl as buffer solution to prepare 10 pcm of the primer, by taking 10 µl of buffer solution. and dilute it with 90 µl ddHO to reach a final volume of 100 µl. Note/ Keep the stock solution at -20° C.

Sample preparation before proceeding with DNA extraction

The isolated samples were cultured in the planning method on medium SDA and incubated at 37 °C for 24 to 48 hours, then transferred to a single colony of yeast grown in SDA medium and grown on for 24 to 48.

DNA extraction and isolation procedures

Wizard DNA the Genomic DNA Purification kit

doi.org/10.31838/hiv22.02.335 Received: 18.05.22, Revised: 19.05.22, Accepted: 10.08.22

(Promega/USA) was used based on the company's supplied

Preparation of the reaction mixture

The reaction mixture was prepared by mixing the components shown sterile small tubes of 0.5 ml with the volume indicated against each substance and these components and volumes were used in the polymerase chain reaction technique for the partial diagnosis of C.albicans Partial diagnosis of C.albicans. used as templates for PCR the Forwared primer (F) C.albicans 5TTT ATC TTG TCA CAC CAG A3 and Reveres primer(R) C.albicans 5GGT CAA AGT TTG AAG ATA TAG GT3 the reaction components from the F and R polymerases were mixed with candida DNA and placed in a PCR device [7].

Agarose Gel Preparation and DNA Loading

The comb made wells were used for loading the DNA samples seven microliters of amplified PCR product were loaded to the agarose gel wells followed by the DNA marker (ladder) to one of the wells. The gel tray was fixed in an electrophoresis chamber and 1 X TBE buffer was added to the chamber until the surface of the gel was covered the electric current was set on 75 volt for 1.5- 2 hr.

Extraction secondary metabolites toxin

Taking one disk from each pure culture of petri dishes containing SDA. Pure isolated of the C.albicans were grown on SDB and MEM broth with 5%serum incubated37°C,for 7days under 5%CO2atmosphcre with 95% humidity and taking pure culture to detect produce toxic secondary metabolites after growth then the pure culture,addition in to the culture chloroform. the mixture was shaken for 15 minutes by a shaker apparatus and after which yested were removed by centerfugation (3000 rpm for 10 min). the mixture was filtered through a filter paper whatman No1. filtrate puts it in separate funnel. (Shah and Larsen., 1991). was dried by reflex condenser filtrate then concentrated to reach about 1ml.

High performance liquid chromatography (HPLC)

HPLC analysis was performed by using Shimadzu instrument type (10AV-LC), (250 mm + 150 mm x 4.6 mm) (Eclipse C8, 5-micron particle size, equipped with binary delivery pump model LC-10A Shimadzu, the eluted peaks were monitored by UV-Vis 10 A-SPD spctrophotometer). Column: uncludar C18-DB, 3um particle size (50x 4.6 mm I.D) column. Mobile phase: methanal_water (43:57, v/v).1%trichloro acetic acid (TCD)the flow rate was set at 1.0 ml/min, with an injection volume of 50µl for purifying patulin and 50 µl for the filter extracts. used for the detection of fungus Gliotoxins [4, 8]. detector UV at wavelength set at 254 nm. Sample injection was 50 ul.

Calculation the concentration of gliotoxin in the sample

Calculation of results amount of gliotoxin in the final solution was deter-mined by using a calibration graph of concentration vs. peak area and expressed as µg/LThe gliotoxin content (C) of the extract C.albicans found by using the equation: Concentration.patulin of sample µg/L= $\frac{\text{area of sample}}{\text{area of standared}}$ ×(concentration. Of standard × dilution factor) where

C.sample: is the concentration of gliotoxin in the final solution (µg/L).

C.stander: is the concentration of standardgliogliotoxin(µg/L).

Dilution factor: the number of times the sample is diluted.by using the equation:

3. Results and Discussion

Molecular identification of C.albicans

Results, a 32 sample diagnosed as C.albicans was chosen to detect the genotype using the PCR device, as well as the use of genetic analysis technology determine the genotypes of C.albicans amplified DNA products were the resulting DNA was agarose gel electrophoresis and it was found to carry weight~354 bp as shown in the Agarose gel plate figure (1). The results agreed with [7] where in his studies specific characteristics and sequences of C.albicans oligonucleotide primers.

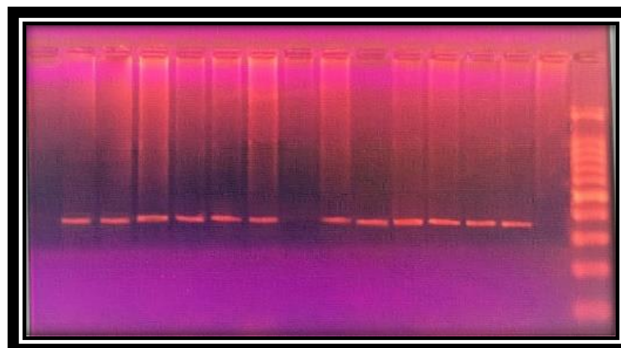

Figure (1) C.albicans DNA on agarose gel electrophoresis size ~354 bp.

Secondary metabolites produce by C. albicans similar to gliotoxin

A-High performance Liquid Chromatography (HPLC)

The HPLC is the most commonly used quantitative method in gliotoxin (GT) research and routine analysis. C.albicans selected to produce structure similar to GT by the HPLC analysis. The HPLC results showed that retention time of major peak of standard GT located at 3.702 minutes, it is identical with the GT produced from a filter extract of C.albicans 3.685 minutes in the same area figure (2). These findings confirmed the presence of structure similar to GT in the samples collected for this study. This study was compatible with a study presented by Shah et al. [4]. The gliotoxin-like material was created. As for the research presented by Shah et al. [4] GT produced by C.albicans in women with vaginitis.The reproduciility of a section of the

samples GT examined in the HPLC device was 99.729% to check the presence of a similar GT in the metabolites of C.albicans.

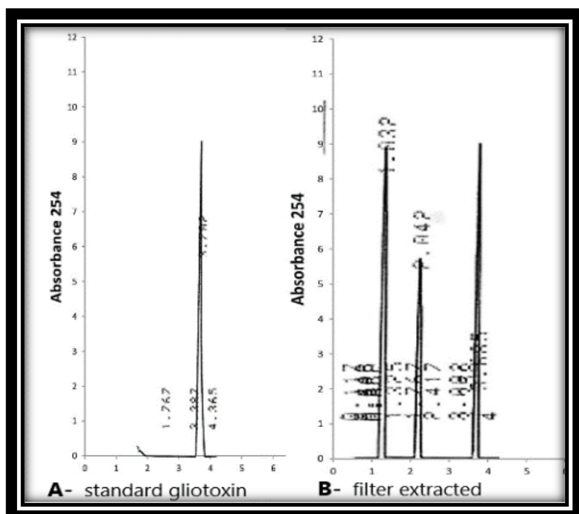

Figure (2) The HPLC results. A-Major peak of the standard GT located at 3.702 minutes; B- Major peak of GT produce by a filter extract of C.albicans 3.685 minutes in the same area.

B-Fourier Transform Intrared (FTIR)

The FTIR assay$(C-O-H)$in wavenumber cm-1 1042.20, the $(C=O)$ in wavenumber cm -1 1637.23, and $(C-O-H)$ wavenumber cm-1 3281.18 were shown in the figure (3). The results were similar to those presented in the study [9, 10].

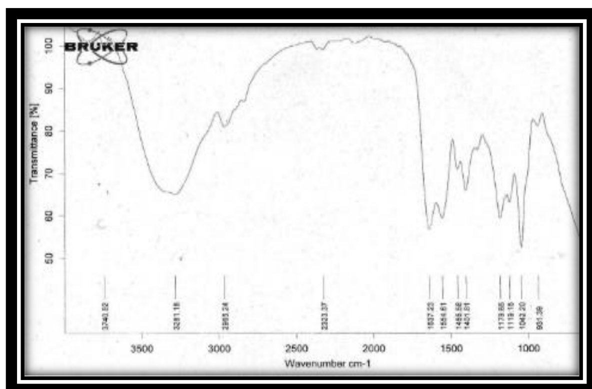

Figure (3) FTIR secondary metabolite produce by C.albicans.similar to gliotoxin.

Concentrations of GT in the C. albicans isolates

The study showed, after culturing the samples on SDB medium and the samples grown in EME medium to obtain concentrations of GT toxin in 15 selected samples of C. albicans by examination in HPLC table (1) shows 16.33 µg/ml concentrations were obtained as the highest concentration in the sample15 grown on medium MEM and low concentration in a sample 1 cultured on SDB was 1.568 µg/ml. The method for determining GT concentrations was compatible with what had done by Bulgari et al. [11] which explained the concentrations of biological samples planned by calculating the area under the peak and comparing it to the area of the calibration curve.

Table (1) Concentration of gliotoxin (GT) from C. albicans.

NO.	Sample	Concentration of GT of samples (µg/mL)	Concentration of standard GT (µg/mL)
1	Sample1	1.568	25
2	Sample2	5.654	
3	Sample3	3.454	
4	Sample4	14.369	
5	Sample5	4.1917	
6	Sample6	4.03	
7	Sample7	16.273	
8	Sample8	16.290	
9	Sample9	4.18	
10	Sample10	15.68	
11	Sample11	16.280	
12	Sample12	5.81	
13	Sample13	15.98	
14	Sample14	15.72	
15	Sample15	16.33	

4. Conclusion

Using genetic analysis methods for diagnosing fungal infections in general C.albicans selected to produce structure similar to Gilotoxin is a secondary metabolite produce by C.albicans which is proved by HPLC and FTIR technique the preferable medium to produce gilotoxin is Minimum Essential Medium (MEM) more than Sabouraud dextrose broth.

References

1. Missoum A. Recent updates on mesenchymal stem cell based therapy for acute renal failure. Current urology. 2019;13(4):189-99. https://doi.org/10.1159/000499272
2. Ward TL, Knights D, Gale CA. Infant fungal communities: current knowledge and research opportunities. BMC medicine. 2017;15(1):1-10. https://doi.org/10.1186/s12916-017-0802-z
3. Romo JA, Kumamoto CA. On commensalism of Candida. Journal of Fungi. 2020;6(1):16. https://doi.org/10.3390/jof6010016
4. Shah DT, Larsen B. Clinical isolates of yeast produce a gliotoxin-like substance. Mycopathologia. 1991;116(3):203-8. https://doi.org/10.1007/BF00436836
5. Scharf DH, Brakhage AA, Mukherjee PK. Gliotoxin–bane or boon? Environmental microbiology. 2016;18(4):1096-109.
6. Fraga-Silva TFdC, Mimura LAN, Leite LdCT, et al. Gliotoxin aggravates experimental autoimmune encephalomyelitis by triggering neuroinflammation. Toxins. 2019;11(8):443. https://doi.org/10.3390/toxins11080443
7. Asadzadeh M, Ahmad S, Al-Sweih N, et al. Rapid and accurate identification of Candida albicans and Candida dubliniensis by real-time PCR and melting curve analysis. Medical Principles and Practice. 2018;27(6):543-8. https://doi.org/10.1159/000493426
8. Gupta A, Gupta S, Siddeek RAT, et al. Demographic and clinicopathological Profile of Gall Bladder Cancer Patients: Study from a tertiary care center of the Sub-Himalayan region in Indo-Gangetic Belt. J Carcinog. 2021;20:6. https://doi.org/10.4103/jcar.jcar_3_21

9. Bhatnagar I, Venkatesan J, Kim S-K. Polymer functionalized single walled carbon nanotubes mediated drug delivery of gliotoxin in cancer cells. Journal of biomedical nanotechnology. 2014;10(1):120-30. https://doi.org/10.1166/jbn.2014.1677

10. Athukuri B, Neerati P. Enhanced oral bioavailability of diltiazem by resveratrol in healthy human subjects: An open-label, two-period, sequential study. J Nat Sci Biol Med. 2020;11(2):100-4. https://doi.org/10.4103/jnsbm.JNSBM_163_19

11. Bulgari D, Fiorini L, Gianoncelli A, et al. Enlightening Gliotoxin Biological System in Agriculturally Relevant Trichoderma spp. Frontiers in Microbiology. 2020;11:200. https://doi.org/10.3389/fmicb.2020.00200