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Molecular Diagnosis of Invasive Candida albicans strains prevalent among a primary school Children

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Abstract

This study has been undertaken to investigate the prevalence of the invasive *Candida albicans* fungus in oral swabs collected from primary school children and to confirm fungus pathogenicity by molecular methodology. Accordingly, the total of (362) students in the age range of (6-12) years from both sexes was orally examined for the incidence of *Candida albicans* from December 2019 to February 2020. Oral swabs were directly cultured on Sabouraud agar medium, and positive cultures were examined microbiologically. Results of Gram's staining revealed the isolation of the invasive *Candida albicans* confirmed by the germ tube test. This fungus was significantly ($P<0.05$) prevalent in most of the school students, where males were more affected (63.59%) than female students (36.41%) across all age groups. Moreover, the molecular analysis of the isolated fungal strains confirmed the invasiveness of the isolates.

Keywords: Molecular diagnosis, *Candida albicans*, school children, RT-PCR.

I. Introduction

The malaise of thrush has been known to occur in people for over 2000 years, where Candida species presented as both commensals and opportunistic pathogens in the oral cavity [1-3]. For decades, it has captivated the clinicians to investigate its pathogenicity and to improvise newer therapeutic regimens based on the updated molecular research [2-4]. On the other hand, *Candida albicans* fungal strains were found as a commensal in the oral cavity of about (46%) of one-year-old infants and (39%) of 1–6-year-old children [5]. The turning of *Candida albicans* into a pathogen is usually determined by the host immune system where candida infections might range from non-life threatening superficial mucocutaneous disorders to an invasive disseminated disease involving multiple organs [6-8]. In recent years, a remarkable increase in Candidiasis has been observed, as reflected by the increased incidence of *Candida albicans* infections among children frequently [7&8]. The probable explanations included changes in the practice of medicine like the introduction of broad-spectrum antibiotics, immunosuppressive agents, and morbid conditions such as diabetes and severe malnutrition in children [9-11]. On the other hand, oral Candidiasis became a significant source of morbidity, as it can cause chronic pain or discomfort upon eating, limiting nutrition intake in children of different ages [10 &11].

Therefore, this research was suggested to evaluate the predominance of invasive *Candida albicans* fungus in oral swabs collected from primary school children and to confirm the fungal pathogenicity by molecular means.

II. Methods

A. Study Population

The total of (362) primary school children were the subject of this study living in different districts in Kirkuk city/Iraq in the age range of (7-12) years from both sexes.

This study conducted during the year of 2019, according to the procedure cited in Ref.[12]

B. Sample Collection

Oral samples obtained by swabbing the oral mucosa (palatal mucosa and tongue dorsal) of all patients with sterile swabs. Then, samples were directly cultured on Sabouraud's dextrose agar medium (Himedia/India) supplemented according to the procedure mentioned in [14&15]. Plates were incubated at 37℃ for 24-72 hours.

C.Microbiological analysis of the isolated strains

1. Gram's Staining:

Single colonies picked with a loop and were stained according to the manufacturing company Kit protocol (Himedia/India). Slides were examined the magnification of (100X) objective of a light microscope and were photographed.

2. Germ tube test:

This procedure is followed according to [16] for identifying *Candida albicans*. The isolated, purified strains were incubated in human serum at 37℃ for 2 hours, and the ability to produce short, slender, tube-like structures (germ tubes) were recorded as a pathogenic strain.

D. The molecular analysis of the isolated strain invasiveness

1.*DNA Extraction*

All isolates were subcultured twice on Sabouraud's dextrose agar and were incubated at 37 ℃ for 48–72 h before molecular analysis. Crude DNA extracts were prepared by a simple boiling method according to [17]. A loopful colony was suspended in 2 ml of Sabouraud's dextrose broth medium and was incubated overnight at 37℃. One milliliter of this suspension was transferred to a sterile Eppendorf tube, centrifuged at 13,000 rpm for 10 min. A volume of 200 ml of sterile distilled water was then added to the pellet and boiled at 100℃ for 15 min. Then, extracted DNA was transferred to a clean tube and stored at -20℃ before RT-PCR.

2. *Identification of Invasive Candida albicans Isolates Genotype by RT-PCR Amplification of 310 bp genotype*

Two primer pairs used to span the site of the transposable intron in the 25S rRNA were CA-INT-L (50-ATAAGG GAA GTC GGC AAA ATA CCG TAA-30) and CA-INT-R (50-CCT TGG CTG TGG TTT CGC TAG ATA GTA GAT-30) as described by [17]. Amplification reactions were performed in 50 µl final volume containing 3.5 mM MgCl2, 200 lM dNTP mix, 1 U Taq DNA polymerase (Sigma, USA), 100 pmol (each) of the primers and 4 µl DNA template. Then, the Genotype of the isolates were investigated by restriction endonuclease analysis by HaeIII and MspI (Thermo Scientific/ France), according to Karahan *et al*. [18]. DNA extracts of *Candida albicans* isolates were digested with HaeIII and MspI restriction endonucleases along with the extracted DNA of the standard invasive *Candida albicans* ATCC 10231 (WDCM 00054 Vitroids™ /Sigma-Aldrich/Germany) and run on RT-PCR (BioRad/USA). The products were characterized by

electrophoresis on 2% agarose gels and visualized in U.V. transilluminator (BioRad/USA) after ethidium bromide staining.

E. Statistical analysis

Data from the study were analyzed using a T-test of the SPSS program Ver.10 for Windows, where a *P value of <0.05* was considered indicative of a statistically significant difference.

III. Results & Discussion

Candida albicans is a dimorphic, opportunistic pathogen that causes infections in immuno-compromised individuals where the virulence of this organism is a function of a multiplicity of factors working jointly to overwhelm the host defenses [17]. Recent findings showed an increase in the global burden of fungal infection, especially for *Candida albicans,* where the proportion represented by children is mainly unknown [6,7&12]. Besides, most of the local studies had neglected the incidence of the pathogenic *Candida albicans* in the oral cavity of small children [22-26].

Thereby, this study was conducted to investigate the prevalence of invasive *Candida albicans* in the oral cavity of school children and to identify the isolated strains by real-time polymerase reaction molecular mean.

In accordance, the total of (362) primary school children were included in this study, and the results of the oral examination showed a high prevalence of Candidial infections among many students who volunteered for the inspection as indicated in (Table 1).

Table (1): The prevalence of Candidial infections among students in a primary school in Kirkuk city inspected in this study.

Name of Inspected School	Total No. of Students	Male students No.	Female students No.	Total of Suspected Patients / School	% of Candidiasis / Students
Black Gold Primary	362	178	184	195	53.87

Results of the table above showed that about (195) students out of (362) students examined from the particular primary schools had displayed suspected Candidiasis with an incidence rate of (53.87%) among all ages and in both sexes. The same results were obtained by [12&24], where they declared a percentage of (55.33% and 47.3%) of Candidiasis among preschool and school children in different parts of the world, respectively.

Subsequently, the suspected Candidiasis oral samples were microbiologically analyzed after the cultivation on selective medium for the identification of the fungal strain and the outcomes of the Gram staining of the wet oral samples and germ tube test had revealed the isolation of Gram's positive budding yeast cells that was almost bearing a resemblance to *Candida albicans* as shown in (Figure 1).

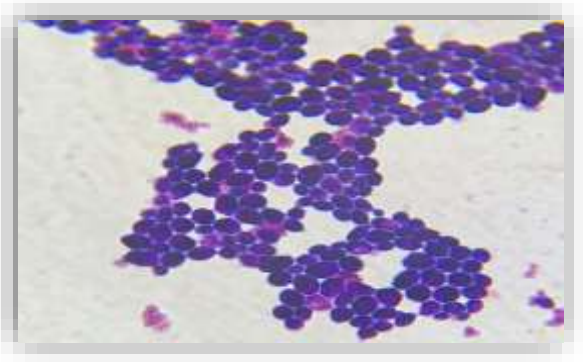

Figure (1): A light microscope photograph under (100X) magnification showing Gram's positive budding yeast cells of suspected *Candida albicans* strain isolated from a ten years old male student during the study.

As a consequence, molecular analysis was conducted for the identification of invasive *Candida albicans* isolates against the genotype sequence of the standard virulent strain of *Candida albicans* ATCC 10231as described by [19].

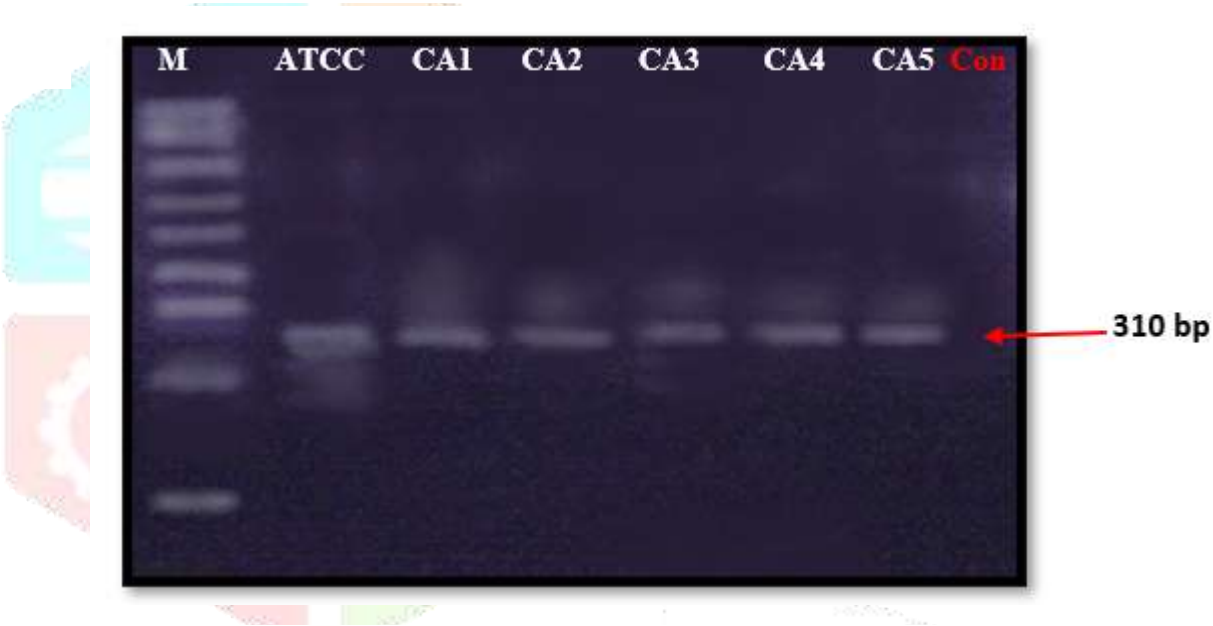

Figure (2): Electrophoretic pattern of RT-PCR amplified DNA products. Lane M, 100-bp marker; Lane 1, *C. albicans* ATCC 10231 (310 bp): Lanes (CA1-5), the tested *C.albicans* isolates of the study: Lane Con, negative control, no DNA template (distilled water).

Other Studies [17-19] had identified virulent strains of *Candida albicans* strains at different genotypic base pairs probably because this fungus has significant phenotypic and genetic diversity [20&21]. It contains a diploid genome with 14.4 megabases that are arranged as eight chromosomes [21]. The heterozygosity and heterozygous of the genome are thought to be related to *C. albicans* virulence [24]. Also, it is now well documented that the genomic instability of *C. albicans* plays a significant role in its pathogenesis.

Since the genetic variability of *C. albicans* can modulate the candida behavior at the host defense mechanism [21]. Thus, the predominance of the invasive strains was analyzed according to the study levels of the infected school children and results were declared in (Table 2)

Table (2): Distribution of invasive Candida albicans concerning student's grades in the Primary school being examined during the study period.

Patient's Gender	Infected Students Numbers per grades					
	1st	2nd	3rd	4th	5th	6th
Males	7	10	21	22	26	38
Females	5	7	11	12	16	20
Total	12	17	32	34	42	58
Percentages	6.15%	10.30%	16.41%	17.44%	21.54%	29.74%

From the results of the table above, it could be concluded that mouth thrush predominated all ages of students recording a percentage of (63.59%) in male students and (36.41%) in female students respectively. These results agree with the results obtained previously by [21, 15&27], where they exposed that, mouth thrush was very prevalent among preschool and School children due to increased oral cavities due to teeth loss at these ages. However, in this study, it was noticed that the percentage of Candidiasis was significantly higher in males than female students, probably due to poor oral hygiene practices by boys. Moreover, it could be noticed from the table above that the predominance in Candida albicans incidence increased with the age of the students where students of 11-12 years old were more affected by Candidiasis compared to the younger ones probably due to a relationship between personal hygiene with nutrition. The same results obtained by [28&29], where they concluded that girls had better hygiene practices and eating routines than the boys. Also, the majority of the health problems affecting school children are preventable by the promotion of hygienic practices and healthy eating habits.

Conclusions

The Maintenance of a healthy mouth environment, a balanced microbial ecology is very crucial to children's health. Besides, invasive *Candida albicans* was significantly high ($P<0.05$) among male students than females of a primary school in 2019. Still, the predominance of invasive *Candida albicans* increased with student's age.

References

[1]. Höfling JF, Anibal PC, Obando-Pereda GA, Peixoto IA, Furletti VF, Foglio MA, Gonçalves RB. Antimicrobial potential of some plant extracts against Candida species. Brazilian Journal of Biology. 2010 Nov;70(4):1065-8.

[2]. Owotade FJ, Patel M. Virulence of oral Candida isolated from HIV-positive women with oral Candidiasis and asymptomatic carriers. Oral surgery, oral medicine, oral pathology and oral radiology. 2014 Oct 1;118(4):455-60..

[3]. Barnett JA. A history of research on yeasts 12: medical yeasts part 1, Candida albicans. Yeast. 2008 Jun;25(6):385-417.

[4]. Rautemaa R, Ramage G. Oral candidosis–clinical challenges of a biofilm disease. Critical reviews in microbiology. 2011 Nov 1;37(4):328-36.

[5]. Williams D, Lewis M. Pathogenesis and treatment of oral candidosis. Journal of oral microbiology. 2011 Jan 1;3(1):5771.

[6]. Yilmaz AE, Gorpelioglu C, Sarifakioglu E, Dogan DG, Bilici M, Celik NU. Prevalence of oral mucosal lesions from birth to two years. Nigerian Journal of Clinical Practice. 2011;14(3):349-53.

[7]. Lund RG, da Silva Nascente P, Etges A, Ribeiro GA, Rosalen PL, Del Pino FA. Occurrence, isolation and differentiation of Candida spp. and prevalence of variables associated to chronic atrophic Candidiasis. Mycoses. 2010 May;53(3):232-8.
[8]. Lalla RV, Patton LL, Dongari-Bagtzoglou A. Oral candidiasis: pathogenesis, clinical presentation, diagnosis and treatment strategies. Journal of the California Dental Association. 2013 Apr;41(4):263-8.
[9]. Martins N, Ferreira IC, Barros L, Silva S, Henriques M. Candidiasis: predisposing factors, prevention, diagnosis and alternative treatment. Mycopathologia. 2014 Jun 1;177(5-6):223-40.
[10]. Tawfeeq AA, Taher S.A. Epidemiological study evaluating the impact of front door duct slot of a combined domestic sewer–rainwater drainage system on children health in Kirkuk, 2017. Karbala International Journal of Modern Science. 2018 Dec 1;4(4):369-76.
[11]. World Health Organization. Water quality: Guidelines, standards and health: Assessment of risk and risk management for water-related infectious disease. WHO; 2001.
[12]. Tawfeeq AA. Prospective Cohort Study Defining *Candida albicans* Infections In Some Primary Schools In South of Kirkuk/Iraq 2019. Proceeding of The 6th International Scientific Conference of Genetic and Environment Baghdad, Iraq.150-158. http://agerciraq.wix.com/agerc
[13]. Waldman A, Gilhar A, Duek L, Berdicevsky I. Incidence of Candida in psoriasis–a study on the fungal flora of psoriatic patients. Mycoses. 2001 Apr;44(3-4):77-81.
[14]. Segal E, Elad D. Fungal vaccines and immunotherapy. Journal de Mycologie Médicale. 2006 Sep 1;16(3):134-51.
[15]. Rozkiewicz D, Daniluk T, Zaremba ML, Cylwik-Rokicka D, Stokowska W, Pawińska M, Dabrowska E, Marczuk-Kolada G, Waszkiel D. Oral Candida albicans carriage in healthy preschool and school children. Advances in medical sciences. 2006;51:187-90.
[16]. Nassir, Noor Ismaeel. "Incidence of C. albicans isolates from oral and vaginal candidiasis, study of their susceptibility and cross-resistance to some antifungal agents". 2010.Thesis. College of Medicine / Al-Qadisiya University/ Iraq.
[17]. Bellemain E, Carlsen T, Brochmann C, Coissac E, Taberlet P, Kauserud H. ITS as an environmental DNA barcode for fungi: an in silico approach reveals potential PCR biases. BMC microbiology. 2010 Dec 1;10(1):189.
[18]. Karahan ZC, Akar N. Subtypes of genotype A Candida albicans isolates determined by restriction endonuclease and sequence analyses. Microbiol Res. 2005;160:361–6.
[19]. Budzyńska A, Sadowska B, Więckowska-Szakiel M, Różalska B. Enzymatic profile, adhesive and invasive properties of Candida albicans under the influence of selected plant essential oils. Acta Biochimica Polonica. 2014 Mar 19;61(1).
[20]. El-Naggar MY, Al-Basri HM, Karam El-Din AZ. Molecular diagnosis of Candida albicans using real-time polymerase chain reaction of a CaYST1 gene. Journal of Taibah University for Science. 2010 Jan 1;3(1):8-13.
[21]. Dadar M, Tiwari R, Karthik K, Chakraborty S, Shahali Y, Dhama K. Candida albicans-Biology, molecular characterization, pathogenicity, and advances in diagnosis and control–An update. Microbial pathogenesis. 2018 Apr 1;117:128-38.
[22]. Habeeb, R.A., Al-Saadi, A.M. and Jasim, N.O. "Isolation and identification of some types of Candida spp." J. Babylon University/ Pure &Applied Science. 2015. Vol 23(3):955-964.

[23]. Ashraf, J. M. "Role of Candida albicans Fungi in foundation some protozoa and bacteria". Tikrit Journal of pure science. (2010) Vol.15(1):14-19.
[24]. Ali, C. I., Mahmood, A. R., Jafar, N. A., & Khorsheed, S. "Prevalence of enteropathogenic diarrhea in Children up to 2 years in Kirkuk province". Tikrit Medical Journal. 2009. Vol. 15(2):124-131.
[25]. Haydar, M. A., Al-Hamadani, A., H. and Al-Muhana, A., M. "Genotyping and antifungal susceptibility profile of *Candida albicans* isolated from neonatal thrush infections in Iraq. Al-Qadisiya Medical Journal. 2017 Vol.9(15):240-249.
[26]. Hameed, A.R., Ahmed, L. and Ali, S.M. "The Prevalence of Candida spp. Among Children with Diarrhea in Baqubah-Iraq". International Journal of Advanced Research in Engineering & Technology. 2018. Vol.1(3):34-43.
[27]. Moreira, D., Spolidorio, D. M. P., Rodrigues, J. A. de O., Boriollo, M. F. G., Pereira, C. V., Rosa, E. A. R., Hofling, J. F. "Candida spp. biotypes in the oral cavity of school children from different socioeconomic categories in Piracicaba - S.P., Brazil". Pesqui Odontol Bras. 2001. Vol.15(3):187-195.
[28]. Sarkar M. Personal hygiene among primary school children living in a slum of Kolkata, India. Journal of preventive medicine and hygiene. 2013 Sep;54(3):153.
[29]. Deb S, Dutta S, Dasgupta A, Misra R. Relationship of personal hygiene with nutrition and morbidity profile: A study among primary school children in South Kolkata. Indian journal of community medicine: official publication of Indian Association of Preventive & Social Medicine. 2010 Apr;35(2):280.