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°C 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°C 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 °C 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°C. 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°C for 15 min. Then, extracted DNA was transferred to a clean tube and stored at -20°C 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 IM 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 immunocompromised 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 Candidal infections among many students who volunteered for the inspection as indicated in (Table 1).

<table>
<thead>
<tr>
<th>Name of Inspected School</th>
<th>Total No. of Students</th>
<th>Male students No.</th>
<th>Female students No.</th>
<th>Total of Suspected Patients / School</th>
<th>% of Candidiasis / Students</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black Gold Primary</td>
<td>362</td>
<td>178</td>
<td>184</td>
<td>195</td>
<td>53.87</td>
</tr>
</tbody>
</table>

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 10231 as 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.

<table>
<thead>
<tr>
<th>Patient's Gender</th>
<th>Infected Students Numbers per grades</th>
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<tr>
<td></td>
<td>1st</td>
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<tr>
<td>Males</td>
<td>7</td>
</tr>
<tr>
<td>Females</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
</tr>
<tr>
<td>Percentages</td>
<td>6.15%</td>
</tr>
</tbody>
</table>

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 of a primary school in 2019. Still, the predominance of invasive Candida albicans increased with student's age.

References