

## The Influence of Nystatin and Argon Laser Combination on Candida Albicans and Actinomycetes Isolated from Oral Cavity of Children.

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### Key words

Candida albicans ,  
actinomycetes,  
nystatin , argon laser.

### Abstract

The present study has been undertaken to illustrate the effect of argon laser and nystatin on isolated candida albican and Actinomycetes from oral cavity of children. Out of 20 samples, positive cultures were 26 bacterial samples and 12 fungus samples, the isolated bacteria included Staphylococcus aureus ,klebseilla pneumonias ,and streptococcus viridans .The isolated fungi included candida albican and Actinomycetes. The present study shows that determination of minimal inhibitory concentrations of nystatin ranged from 1-12 i.u./ml in sabroud agar plates were prepared and cultured with tested fungi ,incubated at 25 °C for 24-48 hrs.The candida albican and Actinomycetes fungi exposed to argon ion (Ar<sup>+</sup>) laser after focal with a lens of (15) cm after focal point for periods of time (1,1.5,2,4,6,8,10,12,14,16)min, and shows the effect of combination between nystatin and laser light.The result showed that the minimal inhibitory concentrations of nystatin for candida albican were 1-12 i.u./ml but the minimal inhibitory concentrations of nystatin for Actinomycetes were 3-12 i.u./ml .Also it is found that candida albican completely killed after exposure to argon laser for 12-16 minutes. While Actinomycetes completely killed after exposure to argon laser for 10-16 minutes.The combination between laser and nystatin were more effective,all candida albican isolated from oral cavity were killed after exposure to (argon laser light , nystatin combination) for 10 minutes / 7 i.u./ml, while all Actinomycetes killed after exposure to (argon laser light ,nystatin combination) for 6 minutes/5 i.u./ml .The widely uses of laser in oral and dental treatment encourages to further investigations . Recent advances has shown the save and destructive results by using laser open the hopes to introduce laser strongly in treatment of oral and dental diseases.

### Introduction

The occurrence of Candida spp. can be observed in the oral cavity; they are commensal yeasts which are part of normal oral microbe. Infections caused by these organisms are associated with

factors such as: decreased immunity, endocrine disorders, soft tissue lesions, poor oral hygiene, long-term therapy with antibiotics, hormone disturbances and others. The variety of clinical manifestations of oral candidiasis reflects the diversity of the countless

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predetermining conditions<sup>(1)</sup>. The oral yeast tends to increase as a result of an increment in the number of retentive sites such as prostheses, orthodontic appliances and pacifiers<sup>(2)</sup>. Moreover, other factors such as socioeconomic conditions seem to interfere in the colonization by *Candida*<sup>(3,4)</sup>. Koga-Ito *et al.*<sup>(5)</sup> reported an increased isolation of *Candida* spp. in mouth breather children in relation to control ones, indicating the environmental influence in candidal colonization. After birth, infants are exposed to continuous person-to-person and environmental contacts with microbes, and the development of the indigenous microflora begins on the surfaces of the human body. In a developing ecosystem, microbial colonization may easily occur because of the still inadequate host response. Adhesion is the initial event in the colonization of microorganism. In the mouth, only mucosal surfaces are available during the first months of life. After teeth emergence, the number of attachment sites and potential niches increases significantly. Microorganisms adhere not only to oral surfaces but also to each other, forming multigene communities where specific partner relationships influence their composition and stability<sup>(6)</sup>. Superficial infections of skin and mucosal membrane by *Candida* causing local inflammation and discomfort are however common in many human populations. While clearly attributable to the presence of the of the genus *Candida*, candidiasis describes a number of different disease syndromes that often differ in their causes and outcomes. Commonly referred to as a yeast infection, it is also technically known as candidosis, moniliasis, and oidiomycosis<sup>(7)</sup>. Actinomycosis is a subacute, chronic, cellulitic invasion of the soft tissues that causes the formation of external sinus tracts that discharge sulfur granules. Actinomycosis is caused by various bacterial species of the actinomycete group. Usually, the disease is caused by *Actinomyces israelii*, an anaerobic gram-positive organism that enters the tissue through a break in the mucosa<sup>(8)</sup>. The *Actinomyces* genus of bacteria includes other species that normally inhabit the oral cavity but are

seldom pathogenic. Actinomycosis begins as an inflammatory soft tissue mass, which can enlarge into an abscess like swelling, with penetration of the overlying skin and the development of recognizable draining fistulae<sup>(8)</sup>. The Argon laser was invented in 1964 by William Bridges at Hughes Aircraft and is one of a family of Ion lasers that use a noble gas as the active medium<sup>(9)</sup>. This laser is used in many applications such as: Forensic Medicine, Entertainment, General Surgery, Ophthalmic Surgery, Holography, Optical "pumping" source<sup>(9)</sup>.

The polyene antibiotics, including nystatin, pimaricin, amphotericin, and candicidin, comprise a family of very valuable antifungal polypeptide compounds, and they are typically produced by soil actinomycetes<sup>(10)</sup>. Nystatin suspension has been used since the 1950s<sup>(11)</sup>. It is well tolerated and remains the most frequently prescribed agent for thrush. Gastrointestinal absorption of nystatin is insignificant. Most orally administered nystatin is passed unchanged in the stool. In patients with renal insufficiency receiving oral therapy with conventional dosage forms, significant plasma concentrations of nystatin may occasionally occur. Nystatin acts by binding to sterols in the cell membrane of susceptible *Candida* species with a resultant change in membrane permeability allowing leakage of intracellular components. Nystatin exhibits no appreciable activity against bacteria, protozoa, or viruses<sup>(12)</sup>. This study aimed to evaluate the influence of argon laser and nystatin suspension on *Candida albicans* and *Actinomyces* isolated from oral cavity of children.

## Materials and Method

Twenty male and female patients with age group of 3-6 years attended Dental Hospital in Tikrit university were included in this study. The samples collected by swabbing from oral cavity of children. Each swab cultured on sabroud dextrose agar then incubated at 25 °C for 24-48 hrs. Determination of minimal inhibitory concentrations of nystatin ranged from 1-12 i.u./ml in sabroud agar

plates were prepared and cultured with tested fungi ,incubated at 25 °C for 24-48 hrs. According to the procedure of Wilson and Partten <sup>(13)</sup> each isolate was grown in sabroud dextrose broth incubated at 24C<sup>o</sup> for 24-48 hrs. ,then it was harvested by centrifugation and resuspended in equal volume of 0.9 % (W\V) normal saline. One hundred microliter of the saline suspension of organism was transferred into sterile test tube and equal volumes of filter sterilized solution of the following substances were added:

A- Each suspension of candida albican and Actinomycetes exposed to laser light with output 150 mW , 515 nm at distance 15cm fromfor 1,1.5,2,4,6,8,10,12,14 and 16minutes.

B- Antifungal(nystatin) in D.W was added to give final concentration of 1,2,3,4,5,6,7,8,9,10,11 and 12 i.u.\ml for candida albican and Actinomycetes ;and exposed to laser light for 1 ,1.5,2,4, 6,8,10,12,14 and 16 minutes.

C- Subculture of these tube on sabroud dextrose agar plates by loopful with sterile loop on sabroud dextrose plates; after 24-48 hrs., incubation at 25C<sup>o</sup>, the result were read to the end of visible growth <sup>(13)</sup>.

## Results

The distribution of pathogenic fungi among children showed that candida albican was the most common pathogen isolated from male than female ,while Actinomycetes was the most common pathogen isolated from female than male as shown in tables (1,2).The MICs (Minimal Inhibitory Concentrations )of nystatin for candida albican isolated from oral cavity of children ranged between 1-12 i.u. /ml ,while the MICs(Minimal Inhibitory Concentrations )of nystatin for Actinomycetes isolated from oral cavity of children ranged between 3-12 i.u. /ml ( Table 3) . Table (4),revealed that all candida albican isolated from oral cavity were killed after exposure to argon laser light for 14 minutes ,while all Actinomycetes killed after exposure to argon laser light for 12 minutes. Table (5),revealed that all candida albican isolated from oral cavity were killed after

exposure to argon laser light , nystatin combination for 10 minutes/7 i.u./ml,while all Actinomycetes killed after exposure to argon laser light ,nystatin combination for 6 minutes/5 i.u./ml .The decrease in number of living fungi increased with increasing the time of exposure to laser light .

## Discussion

The most common fungal infections in infants and children are mucocutaneous candidiasis, pityriasis versicolor, tinea corporis, tinea pedis and tinea capitis <sup>(14)</sup>. Actinomyces species are prevalent in the oral cavity. The bacteria are isolated from the interdental sulci, periodontal membranes, tonsillar crypts, and saliva<sup>(15)</sup>. Poor oral hygiene and dental caries appear to be the primary predisposing conditions for the development of actinomycosis. In addition, the presence of associated bacteria appears to be fundamental to the development of clinical infection.The present study has revealed 100% positive cultures of aerobic microorganisms .In this study ,the highest incidence of candidiasis was found in males than females,while the highest incidence of actinomycetes was found in females than males among 20 patients examined .It was noticed a significant difference between females and males. These results agrees with results of Nibras et al <sup>(16)</sup>, who found that oral hygiene practices were higher for girls. Also, Al – Barhawe <sup>(17)</sup> and Abdul-Rahman <sup>(18)</sup>, found that severe gingivitis was recorded in females more than in males. Najjar <sup>(8)</sup>,found that the ratio of male/female approximately 3:1 for actinomycetes. The minimal inhibitory concentrations (MICs) of nystatin for canida albicans and actinomycetes isolated from oral cavity of children were studied. The MICs of nystatin for isolated fungi ranged between (1-12 i.u./ml for candida albicans,and 3-12 i.u./ml for actinomycetes). The result were in agreement with that reported by Holbrook et al.<sup>(19)</sup>,who found that the MICs of nystatin used for candida albicans were 12 i.u.\ml. Nystatin was the first widely used antifungal antibiotic, and is especially effective against the Candida

species of fungi. It kills the Candida yeast by interfering with its cell membranes, allowing the contents of the toxic cells to leak out into gut. Incidentally, it is these poisonous chemicals that are the cause of the famous ‘Candida die-off’ or ‘Herxheimer reaction’<sup>(20)</sup>. Argon laser with output 150 mW, 515 nm at distance 15cm, was utilized against candida albicans and actinomycetes isolated in the present study. Our results showed a decrease in total number of candida albicans after exposure to argon ion laser for 1,1.5,2,4,6,8,10,12 minutes and killed all isolates completely after exposure to argon laser light for 14 minutes, while a decrease in total number of actinomycetes after exposure to argon ion laser for 1,1.5,2,4,6,8,10 minutes and killed all isolates completely after exposure to argon laser light for 12 minutes.

Also, the total number of isolated fungi decrease after exposure to argon laser combined with nystatin more than used each of them alone. Our study agrees with Henry et al<sup>(21)</sup>, who found that low exposure time of argon laser are effective against candida albicans and several types of fungi. Fungi absorbed laser light after exposure to this light, it will be converted the light energy to thermal energy. Laser light enter into fungal cells and increased the microbial cell wall temperature leading to rupture microbial cell wall and exit the lived component to outside. The continuous exposure to laser light increased from kill microorganism<sup>(22)</sup>. The decrease in number of living fungi increased with increasing the time of exposure to laser light.

Table (1) :- Sex distribution of isolated candida albicans and Actinomycetes.

sex	No. of isolated candida albicans	No. of isolated actinomycetes
Female	3	1
Male	5	3
Total	8	4

Table(2):- Types of fungi isolated from oral cavity of children.

Types of fungi	No. of isolates
Candida albicans	8
Actinomycetes	4

Table (3) :- minimal inhibitory concentrations of nystatin for candida albicans and Actinomycetes isolated from oral cavity of children .

MICs of Nystatin i.u./ml	No. of isolated candida albicans	No. of isolated actinomycetes
1	6	4
2	5	4
3	5	3
4	4	2
5	3	2
6	3	1
7	2	1
8	1	0
9	1	0
10	0	0
11	0	0
12	0	0

Table (4):- The influence of argon laser exposure on candida albicans and Actinomycetes according to time.

Time /min	No. of viable candida albicans	No. of viable actinomycetes
1	7	4
1.5	5	3
2	3	3
4	2	2
6	2	2
8	1	1
10	1	1
12	1	0
14	0	0
16	0	0

Table (5):- The influence of combination between nysatin and argon laser exposure a on candida albicans and Actinomycetes according to time.

Nystatin(i.u./ml)/ Laser(min)	No. of viable candida albicans	No. of viable actinomycetes
1/1	6	3
2/1.5	4	3
3/2	2	2
4/4	2	1
5/6	1	0
6/8	1	0
7/10	0	0
8/12	0	0
9/14	0	0
10/16	0	0

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