The influence of He-Ne Laser (632.8 nm) on *Candida albicans* isolated from complete upper dentures: In vitro study

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Abstract:

The main objective of this study is to determine whether the use of He-Ne Laser (632.8 nm wavelength and power 0.5 mW) is an eligible and effective tool to kill or reduce the cell viability of *Candida albicans* isolated from complete upper dentures.

Twenty one swabs were taken from the complete upper dentures. Only six swabs showed positive cultures for *C. albicans*.

The isolate was divided into two groups, group I was not irradiated (control), and group II was irradiated by He-Ne Laser for different periods (10, 15, 20, and 30) min.

After irradiation, the results showed a significant reduction in the viable cell count and colonies diameters especially at exposure periods 10 and 15 min.

Although the low power He-Ne Laser was not eradicating the cell itself, but it affected on the viable cell count and colonies diameters comparing with the control.

Key words: He-Ne Laser, *Candida albicans*, in vitro.

Introduction:

Candida albicans is a common inhabitant of the human oral cavity [1]. Several reports indicated that this organism can be considered as a part of normal human flora [2] and it is an opportunistic yeast [3].

Presence of denture is one of the factors that could enhance the growth of the organism in the oral cavity of human [4]. Mccourtie and Douglas [5] had been shown that *C. albicans* adheres to acrylic surfaces.

Dentures may change the flora of the oral cavity as a result of food debris and plaque that collects between the mucosal surface of the denture and the palate. In addition to that the saliva which is present between the maxillary denture and the mucosa may have a pH lower than usual [6]. Atrophic (Erythematous) candidiasis is most commonly found in patients with ill-fitting dentures or in those who wear their dentures continuously [7].

The He-Ne Laser is electrically pumped gas Laser. Low current (in milliamperes) discharged between the cathode and the anode of the tube of high voltage difference (hundreds of volts) applied between them [8].

Laser can enhance the physiological changes without structural changes, so Low Energy Laser Irradiation (LELI) was developed for using in the medical biology [9].

Frucht-Pery [10] used ArF excimer Laser for *C. albicans* and

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explained that this type of laser may be reduce the yeast in the laboratory significantly. And with some bacteriocidals Tolebzadeh et. al. [11] used CO_2 Laser to treat the limited bacterial infections and they found that the survival of cells decrease with the increase of Laser intensity. Ward et. al. [12] demonstrated that the use of Nd-YAG Laser was able to kill some G^+ and G^- bacteria and 2 types of yeast (*C. albicans*, and *Saccharomyces cerevisiae*). Al-Masraf [13] did a wide study about the effect of He-Ne Laser on *pseudomonas aeruginosa* and she was found that there is a significant differences in the responsiveness of these germs to this type of Laser, thus she also indicated that these germs were resistant to this ray at density of low power while their resistance reduced with the increase of it's power density. Rassam [14] found that He-Ne Laser was the best performing method for elimination of *Staphylococcus aureus* with using (TBO) as photosensitizer.

Materials and Methods:

*** Collection of Samples**

Twenty one swabbed samples were taken. Subjects were eligible if they were edentulous and had a complete acrylic upper denture. Subjects with partial dentures or lower dentures only and those who had used a denture cleanser or antibiotics within the previous two weeks not eligible [15].

All of the tissue bearing surface of the complete maxillary dentures was swabbed for 30 seconds with a dry sterile swab to harvest plaque. Each portion of the surface-bearing side was swabbed three times [16].

All of the swabs were soaked in physiological saline in sterile test tubes.

*** Laboratory Identification**

The swabs were transported to the laboratory to be inoculated on Sabourauds' dextrose agar plates containing pencillin, chloramphenicol, and streptomycin to prevent bacterial growth. The specimens were incubated at 30^{o}C for 4 days [17].

The isolated colonies were identified by the following methods: -

1. Gram-stained film: -

In which *C. albicans* appears as gram positive rounded or oval yeast cells when examined under the light microscope.

2. The morphological appearance of colonies: -

Colonies on Sabourauds' media appear as high convex, soft, cream-colored or off-white, glabrous or membranous and may have a fringe of submerged hyphae with yeast-like odor. The colony became (0.5 mm) in diameter after (18 hr.) and developed into (1.5-2 mm) in (5-7 days) on this medium [18]. Colonies diameters were measured for irradiated and control samples by using of ocular microtome, after putting the opened dishes containing the colonies on the stage of the microscope.

3. Germ tube formation: -

It is one of the most valuable test used in most laboratories for rapid and presumptive identification of *C. albicans*.

This test was performed by inoculating a small portion of the organism's colony (0.5) ml of human serum contained in sterile tubes and incubated at 37^{o}C for 2-3 hours. Then a drop of the sediment containing the organism in the bottom of the tube is placed on a

slide to be examined microscopically for the presence of germ tubes which sometime described as having (hand mirror) shape [19].

The presence of *C. albicans* was considered when the three tests were positive.

*** Laser System**

The Laser was used in this study CW Helium Neon gas Laser (Hamburg-Germany) with a measured output power of (0.5 mW), which emits light in a collimated beam with diameter (4 mm), wavelength (632.8 nm), and power density (3.98 mW/cm^2), which was calculated from this equation [20, 21]: -

Power Density (mW/cm^2) = output power (mW) / area (cm^2).

*** Samples Preparation and Irradiation Procedure**

The yeast was cultured on (9ml) of Sabourauds' dextrose broth in screw cup bottle and incubated at 30ºC for 4 days. After that a series of dilutions were made and then (2ml) of each dilution were added to each of eight sterilized test tubes each of them containing (2ml) of (9%) NaCl. Four of these test tubes were irradiated with He-Ne Laser for different exposure time (10, 15, 20, and 30) min, and the latter four tubes were considered as control.

*** Microbiological Procedures after Irradiation**

For each test tubes (the irradiated test tubes and the control) (0.5ml) was taken and added to sterilized Petri dishes containing SDA. Then, CFUs and colonies diameters were determined after incubation at 30ºC for 4 days.

*** Statistical Analysis**

Statistical analysis was done by two-ways analysis of variance (ANOVA2) to determine the effect of He-Ne Laser on CFUs and colonies diameters of *C. albicans* in different exposure times.

Results:

Laboratory identification revealed that out of twenty one subjects six subjects showed positive cultures only. The prevalence of *C. albicans* isolated from complete upper dentures was found to be (28.6%).

The samples were irradiated by He-Ne Laser for different time periods (10, 15, 20, and 30) min, because of the longest irradiation periods do not appear results better than the shortest periods.

The cell viability is considered the important point for studying the effect of low power of He-Ne Laser on *C. albicans* yeast. Comparing with untreated (control), (CFUs) of irradiated samples peaked at (10 min) irradiation period, and then, declined at (15 min), thereafter when the irradiation period was increased more than that we saw that there was a slightly paralleled increase in (CFUs) of the sample, whereas the control samples (CFUs) increased gradually during the time and peaked at (30 min) of the experiment's period.

(fig, 1) demonstrates the decrease in (CFUs) especially in the periods of irradiation (15-30) min, and there is a significant relationship between (CFUs) of irradiated samples and irradiation periods in count ($P< 0.05$, 0.01).

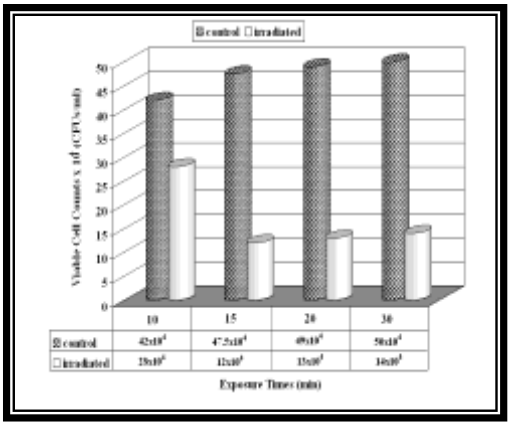

Fig. 1: The effect of He-Ne Laser radiation on CFUs for different irradiation periods

On the other hand, in spite of the irradiation by He-Ne Laser did not kill the cells completely, the survival cells were affected clearly by measuring the colonies diameters of irradiated samples after culturing on solid media and compare with the diameters of non irradiated samples.

(Fig. 2) demonstrates the decrease in colonies diameters especially in shortest period of irradiation (10) min, and there is a significant relationship between colonies diameters of irradiated samples and irradiation periods in count ($P<0.05$, 0.01).

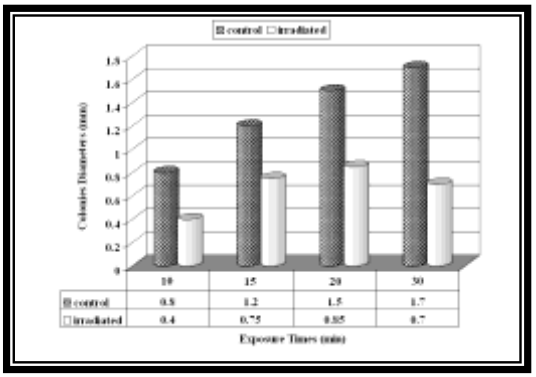

Fig. 2: The effect of He-Ne Laser radiation on colonies diameter for different irradiation periods.

Discussion: -

Our previous results indicated that the prevalence of *C. albicans* isolated from complete upper dentures was (28.6%) and this result comes in disagreement with the finding of Tmilade et. al. [22] who recorded out of 8 patients 5 patients (62.5%) showed positive result to *C. albicans* isolated from removable upper dentures. This disparity may be attributed to differences in the sampling techniques, in the sites of sampling, and in the selection of the subjects (4).

As it is illustrated in (fig. 1), there is a significant decrease ($P<0.05$, 0.01) in (CFUs) especially for the periods of irradiation (15-30) min.

This result shows the effect of He-Ne Laser radiation on the viability of cells which is may be affected on the cell division and do not kill the cell completely, and this is similar to the results of Wilson and Mia [23] and Bown and Lovate [24] on yeast cell. Fedoseyeva et. al. [25] and Karu and Letokhov [26] demonstrated that the irradiation by He-Ne Laser may be cause a decrease in the time of yeast formation. Karu et. al. [27] showed that the irradiation of Hela cells by He-Ne Laser with doses ($10\text{-}10^3$) Joule/cm^2 will stimulate or increase DNA of the cells in S-phase, which also explain the stimulation of cells in long irradiation periods.

When the irradiation period was increased more than that, we saw that there was an increase in viable cell counts, this result may be pointed to the ability of cells to repair the damage that happened by the irradiation with He-Ne Laser in shortest periods by developing a special mechanism to ignore the damage or repair it rapidly, and this may be due to the absorption of cells to the high energy of heat in

the long irradiation periods that enable the cells to restore their viability, so for these reasons the shortest periods were chosen.

As it is shown in (fig. 2), the colonies diameters are well correlated with the irradiation periods, and there is a significant decreasing ($P<0.05$, 0.01) in the colonies diameters of irradiated samples comparing with the control, the same result appeared in the study of Quickenden and Daniels [28] about the effect of the same radiation on *Saccharomyces cerevisiae* yeast.

The reduction of colonies diameters that resulted from irradiated cells may be caused by the effect of He-Ne Laser on growth and proliferation rates of the yeast [29].

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تأثير ليزر الهليوم نيون بالطول الموجي (632.8) على خميرة المبيضات المعزولة من طقم الأسنان العلوي الكامل: دراسة مختبرية في الزجاج

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كلمات مفتاحية: - ليزر الهليوم-نيون, خميرة المبيضات، دراسة مختبرية في الزجاج.

الخلاصة: -

هدف هذه الدراسة هو تحديد ما أذا كان أستخدام الهليوم نيون ليزر ذو الطول موجي (632.8 نانوميتر) وقدرة (0.5 ملي واط) مؤهل و ذو تأثير على قتل او تقليل عدد خلايا خميرة المبيضات المعزولة من طقم الأسنان العلوي الكامل.

تم اخذ (21) مسحة من أشخاص يرتدون طقم أسنان علوي كامل لكن فقط (6) مسحات اظهرت نتيجة زرع موجبة لخميرة المبيضات.

قسمت العزلات إلى مجموعتين، المجموعة الأولى لم يتم تشعيعها (سيطرة)، والمجموعة الثانية شععت لفترات زمنية مختلفة (10، 15، 20، و 30) دقيقة.

بعد التشعيع أظهرت النتائج أن هناك نقصان ملحوظ في عدد الخلايا الحية و قطر المستعمرات خاصة عندما كانت فترة التشعيع 10 و 15 دقيقة.

نستنتج من ذلك، على الرغم من أن الهليوم نيون ليزر لم يقتل الخلية نفسها، لكنه أثر على عدد الخلايا الحية و قطر المستعمرات مقارنة مع عزلات السيطرة.