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Bactericidal effect of Q-switched Nd:YAG laser (1064 nm) on *Streptococcus mutans* from carious teeth ,*in vitro* study

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Abstract: Dental caries (tooth decay) is one of the most prevalent infectious disease and although of multifactorial origin, *Streptococcus mutans* is considered the principal pathogen in its development (i.e. bacterial processes damage hard tooth structure (enamel, dentine and cementum), producing dental cavities (holes in the teeth). The bactericidal properties of the Nd:YAG laser has been researched analyzing its use in caries prevention and bacterial reduction. One hundred twenty five samples were collected from carious teeth and isolated bacteria were diagnosed using microscopic examination, culture, biochemical tests, and Api 20 strep system. The results of this study showed that a noticeable decrease in the viability of Streptococcus *mutans* were obtained using Q-switched Nd:YAG laser (1064 nm), (3 Hz) repetition frequency, (0.796 ,0.955) J/ cm^2 energy density by applying (900,1260) number of pulses. It was concluded that the bactericidal effect of laser irradiation being dependent on the type of bacteria, energy density, number of pulses, and laser dose. Suggesting that the results obtained in this study may be useful in the treatment of dental plaque-related disease.

Introduction

Since Theodore Maiman operated the first ruby laser in July of 1960, biomedical uses of laser have been under investigation (Niemz, 2004; Incei, 2004; Berrocal *et al*, 2005). Dental caries is a multi-factorial oral disease developed by the localized dissolution of the tooth hard tissues ,caused by acids produced by bacteria in the biofilms (dental plaque) which eventually leads to cavities (Sbedil *et al.*, 2008; Jeevarthan *et al*, 2007 and Moat *et al.*, 2002). *S.mutans* is the principal etiological agent of dental caries (Smith *et al.*, 2005; Napimoga *et al.*, 2005) that possesses a variety of mechanisms for survival in the human oral cavity (Chong *et al.*, 2008).

Virulence factors of mutans streptococci promoting their colonization and survival in the biofilm, the dental plaque, are adhesin–like cell surface proteins, acid tolerance, acid production and production of glucosyltransferases, fructosyltransferase, mutacin, intracellular polysaccharides, dextranase, glucan-binding protein, Ag I/II (Lisa Grőnroos, 2000; Kuramitsu, 1993 and Tamesada *et al.*, 2004). In addition, many oral streptococci produces IgA protease which impairs the host defense by cleaving the secretory IgA present, and facilitated the primary colonization of mutans streptococci (L. Grőnroos, 2000). Colonization of enamel surfaces by the cariogenic bacterium *S. mutans* is thought to be initiated by attachment to a saliva-derived conditioning film, the acquired enamel pellicle (Moat *et al.*, 2002; Shimotoyodoma *et al.*, 2002 and Shimotoyodoma *et al.*, 2007) via a sucrose-independent initial adherence to the acquired salivary pellicle (i.e., bacterial surface proteins interact with host or bacterial products adsorbed on the tooth surface), followed by sucrose-

dependent cellular accumulation by aggregation with the same or other species and produce an extracellular polysaccharide matrix (Napimoga *et al*., 2005; Levesque *et al*., 2005; Rentan *et al.*, 2001; Kuramitsu *et al.*, 2001 and Biswas *et al.*, 2005).

Theoretically, the next phase of pathogenesis results from the metabolic activities of these masses of accumulated MS (and possibly of other accumulated microorganism), mutans streptococci are the most prolific producers of lactic acid in these accumulations although other low pH bacteria may also contribute.

Dental caries ultimately issues because the resulting increase in lactic acid concentration cannot be sufficiently buffered to prevent enamel dissolution and cavity formation (Cross *et al*., 2007; Smith, 2003).

Dental caries can potentially be prevented by interfering with transmission of MS, eliminating established MS populations from the oral cavity, increasing the acid resistance of the teeth and control of the carbohydrate composition of the diet (Balakrishnan *et al.*, 2000; Roberson *et al*., 2002).

In view of the growing problem of bacterial resistance to conventional antimicrobials (involves the use of traditional antimicrobial toothpaste in conjunction with the mechanical removal of the biofilm) (Zanin *et al.*, 2005). The use of an alternative approach to which bacteria are unable to gain resistance would be valuable (Wilson, 1993).

Recent research has focused on the bactericidal properties of the Nd:YAG laser , analyzing its use in caries prevention ,other application include diagnosis (transillumination) and bacterial reduction (Jacobs *et al.*, 2003).

This work is a trail in this regard to investigate the effect of laser on the viability of *Streptococcus mutans*, with or without antimicrobial agents in vitro study.

Material and Methods

Samples Collection

A total of one hundred twenty five samples were taken from the oral cavity of patients with ages ranged from (4 years – 69 years), suffering from enamel caries (different stages), fissure and pit caries, dental caries, and root caries, using a sterile cotton swab. The samples were collected during the period from 23/11/2008 – 22/1/2009 at Al-Zewiya health centre in Al-Jadriha and Al-Alwiya centre in Baghdad.

Isolation and identification of *S.mutans*

Patients were examined clinically under the dentist supervision using mouth mirrors and dental explorers to detect the type of caries and determine the decayed, missing, and filled teeth.

The samples were collected from caries lesion of teeth, using a sterile cotton swab; the samples were grown on one of the selective media (mitis salivarius agar (MSA) or mitis salivarius bacitracin agar (MSBA). For primary isolation of *S. mutans*, MSBA was used frequently, the media were incubated anaerobically for 48 hours at 37º C in a candle jar.

The identification of *S.mutans* was based on distinctive colonial morphology on selective and non–selective agar, gram staining, and distinctive cell shape on light microscopy, colony shape on dissecting microscope, specific growth characteristics, catalase test, and sugar fermentation patterns. *S.mutans* isolates can also be identified by the commercial biochemical test system Api 20 strep (BioMérieux, France).

Laser system (Q–switched Neodymium-Ytterium -Aluminum Garnet)

The following parameters were used in irradiation procedure:

- Wavelengths: (1064 nm).
- Laser medium: Nd:YAG.
- Out put energy: (60, 80, 100, 120) mJ.
- Pulse duration: (6 – 10 nsec).
- Aim beam: 5mW diode laser (semiconductor laser).
- Repetition frequency: (3 Hz).
- Light output mode: hand – handle laser piece.
- Number of pulses (180, 540, 900, 1260)
- The energy density (fluence) were :
- 0.477 J/ cm^2 for 60 mJ.
- 0.636 J/ cm^2 for 80 mJ.
- 0.796 J/ cm^2 for 100 mJ.
- 0.955 J/ cm^2 for 120 mJ.

where energy density = E / A, E = is the out put energy of the laser (Joule), A = is the exposed area (cm^2)

Irradiation procedure

A volume of 0.5 ml of each bacterial suspension (dilution 1.5 x 10^8 cell/ml), was transferred to a sterile eppendroff tubes.

These samples were irradiated with Q-switched Nd:YAG laser at a wavelength (1064 nm), the beam diameter was (4 mm), and different energies were applied (60 ,80 , 100 and 120) mJ for a variety number of pulses (180, 540, 900, 1260) and (3Hz) repetition frequency.

A quantity of (0.5 ml) of irradiated bacterial suspension was transferred to a test tube containing 4.5ml of sterilized saline solution (0.9 %W/V), to obtain a dilution of 1/10 (10^{-1}). After homogenization of the solution using the vortex, 0.5 ml from the first test tube was transferred to a second test tube, obtaining a dilution of 1/100 (10^{-2}), this was repeated until a dilution of (10^{-6}) was achieved. Subsequently, 0.1 ml of each dilution was transferred onto a Petri dish containing MSA (at least duplicate). While un-irradiated sample was used as a control for this experiment.

All Petri dishes were incubated an aerobically at 37º C for 48-72 hours. After incubation period, the number of colony forming units per milliliter (CFU/ml) was calculated as the following:

Colony Forming Unit (CFU/ml) = No. of colonies X 1/ dilution factor X 10.

Results

Results of the effect of Q-switched Nd:YAG laser on the viability of *S.mutans*

Figure (1) illustrates the effect of 60 mJ Nd:YAG laser on the viability of *S.mutans* the mean values of inhibition in the viable count were [($120x10^6$), ($80x10^6$), ($62x10^6$), and ($58x10^6$)] CFU/ml, for different number of pulses (180, 540, 900 and 1260) respectively compared with ($173x10^6$) CFU/ml of control.

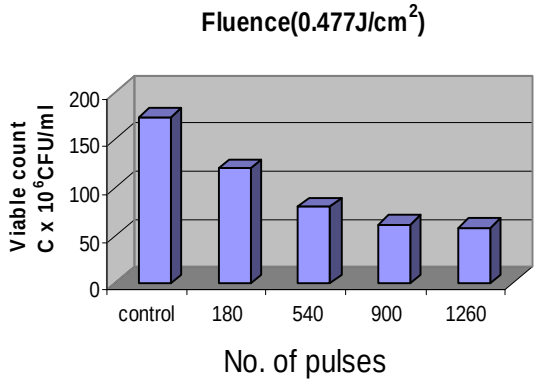

Fig.(1): Effect of Nd:YAG laser on the viability of *S. mutans* at (0.477 J/ cm ^{2}) energy density .

Figure (2) describes the mean values of reduction in the viable count of *S.mutans* for different number of pulses, at (0.636 J/cm^2) energy density, the values were [($59x10^6$), ($47x10^6$), ($35x10^6$) and ($32x10^6$)] CFU/ml respectively compared with ($173x10^6$) CFU/ml of control.

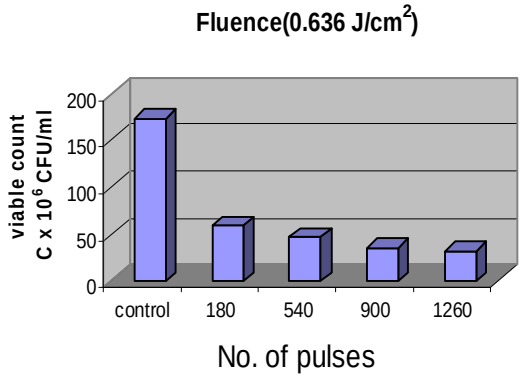

Fig. (2): Effect of Nd:YAG laser on the viability of *S.mutans* at(0.636 J/cm ^{2}) energy density.

Figures (3) & (4) Shows the results of the effect of Q-switched Nd:YAG laser at (0.796 J/cm^2) and (0.955 J/cm^2) for different number of pulses (180, 540, 900,1260).

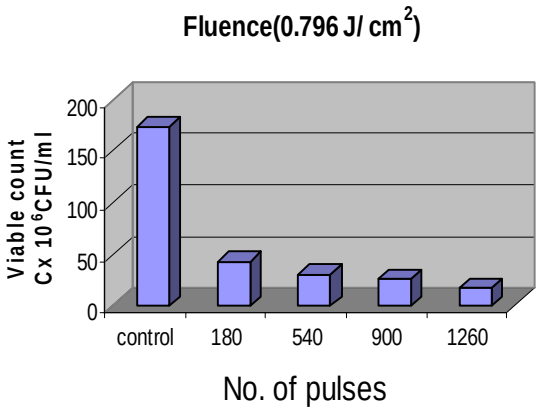

Fig. (3): Effect of Nd:YAG laser on the viability of *S.mutas* at (0.796 J/cm ^{2}) energy density.

The mean values of viability using (100 mJ) output energy were [(42 $x10^6$), (30 $x10^6$), ($26x10^6$) and ($17x10^6$)] CFU/ml. For 120 mJ out put energy the measured values of viability were [($32x10^6$), ($28x10^6$), ($25x10^6$) and ($17x10^6$)] CFU/ml with respect to ($173 x10^6$) CFU/ml of control. The results indicates that the last two energy densities have a significant effect more than that at the first two energy densities and this indicate that the bactericidal effect depend on the energy density .

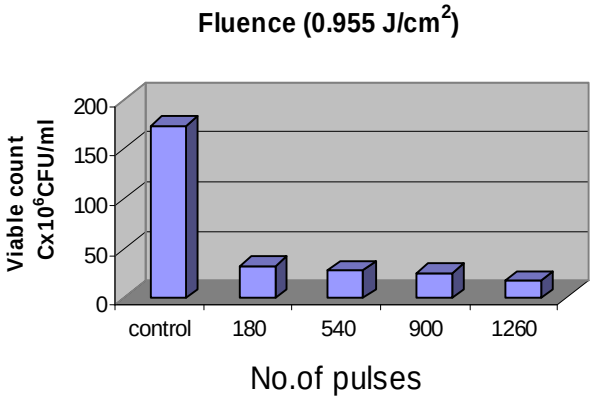

Fig. (4): Effect of the Nd:YAG laser on the viability of *S .mutans* at (0.955 J/cm ^{2}) energy density .

Discussion

The present study examined the susceptibility of *S .mutans* to irradiation with Q – switched Nd: YAG laser (1064 nm), different parameters were used in this study, then appropriate parameters that appeared a noticeable inhibition and killing were chosen for irradiation procedure.

A noticeable killing especially at (100,120) mJ by applying higher number of pulses were observed. These results are confirmed by Jacobs et *al.* (2003) in their study on the action of the Nd: YAG laser in *Lactobacillus* spp. *In vitro* study (Jacobs *et al.*, 2003).

The possible mechanism of inhibition and killing of *S. mutans* using NIR laser is due to photothermal interaction, is a wavelength dependent interaction mechanism (i.e., the NIR light has longer wavelength with low photon energy that induce the vibrational and rotational oscillation within the molecules), then through collisions, this vibrational energy is transferred to kinetic energy of nearby molecules.

An increase in molecular kinetic energy is what appears at a macroscopic level as a temperature rise (heating), therefore, an increase in kinetic energy and velocity of molecular lead to thermal stress (photothermolysis) (Ben, 2007).

Thermal interaction deals with biological effects related to different temperature inside the cells, depending on the type of irradiated cell and laser parameters chosen. Therefore thermal effect of NIR laser irradiation on the bacterial cell result different biological effect such as reduction in enzyme activity, cell immobility, denaturation of protein, increase the permeability, vaporization, and thermal decomposition (i.e., high damage and burst cell) that may occur at high temperature (Niemz, 2004; Ben, 2007) .

These biological effects occur due to matching between the NIR wavelength and the biological acceptor molecules represented by water molecules or other macromolecules such as proteins and pigments.

The main chromophore in the IR is protein. Infrared spectra of protein in the IR are governed by various vibrational modes of the peptide bond (O = C−N−H) (Vogel *et al.*, 2003).

This fact is confirmed by Ramskold *et al.* (1997), in their study on the thermal effect of Nd: YAG laser on the oral bacteria suggesting that the emitted wavelength 1064 nm by Nd:YAG laser is primarily absorbed by dark pigments (Ramskol *et al.*, 1997), these compounds similar to the porphyrins used in light – based therapies, it might be thought that these bacteria would be susceptible to killing by low –power laser light as a result of endogenous photosensitization (Wilson,1993). The oral cavity does contain a number of black-pigmented anaerobes (e.g. *Porphyromonas. gingivalis*).

Also Aoki *et al.*, (2008), explained that the Nd:YAG laser is absorbed selectively by certain pigment, including melanin, hemoglobin and possibly the pigments contained in germs and bacteria , which could make it ideal for killing bacteria (Aoki *et al.*,2008).

Q-switched Nd:YAG laser proved to exhibit a bactericidal effect on the *S.mutans*, this agree with Bergmans *et al* . (2006) on their studies, concluded that Nd:YAG laser irradiation is not an alternative but a possible supplement to existing protocols for canal disinfection (Bergmans *et al.*, 2006). Also Jacobs *et al.* (2003) showed the bactericidal efficiency of Nd:YAG laser together with its ability to increase the acid resistance of enamel in pits and fissures which make it effective in preventing caries (Jacobs *et al.*, 2003) .

The bactericidal effect of Nd:YAG laser was confirmed by Horton *et al* .(1992) they found that subgingival irradiation with light from Nd:YAG laser resulted in significant reductions in viable *Fusobacterium* spp. in perodontitis patients (Horton *et al* ., 1992).

Gutkneck (2008), described the use of Nd:YAG laser in dentistry due to its highly disinfection efficiency.

Conclusions

The results of this study showed that a noticeable inhibition in the viability of S.mutans was obtained using Q-switched Nd:YAG laser (1064 nm), (0,796 ,0.955) J/cm ^{2} energy density using (900, 1260) number of pulses ,and repetition frequency (3Hz). Holds promise for prevention dentistry.

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التأثير القاتل لليزر النديميوم-ياك (1064 نانومتر) على بكتريا المكورات السبحية المعزولة من الأسنان المتسوسة خارج الجسم الحي

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الخلاصة

يعد تسوس الاسنان من اكثر الامراض شيوعا وانتشارا في العالم وينشا من مسببات كثيرة ومتعددة .اذ تشكل بكتريا المكورات المسبحية من اهم هذه المسببات (اي ان الفعالية البكتيرية تسبب تلف التركيب الصلب للسن ابتداءا من طبقة المينا والعاج ثم الاسمنت) مؤديا الى ظهور تجويف في السن . تظهر معظم الدراسات العلمية التاثير القاتل للنيديميوم ياك ليزر موضحا اهمية استخدامها في معالجة تسوس الاسنان ، التشخيص المبكر لتسوس الاسنان، واختزال النمو الميكروبي. في هذة الدراسة جمعت (١٢٥) عينة من الاسنان المتسوسة وتم عزل البكتريا و تشخيصها اعتمادا على الفحص المجهري و الصفات الزرعية والاختبارات الكيموحياتية بضمنها نظام)لاستخدامها في الدراسة وبظروف خارج الجسم الحي . Api 20 strep (تم تشعيع العزلة باستخدام الانيديميوم ياك ليزر (١٠٦٤) نانومتر وتردد (٣) هرتز بتسليط عدة نبضات (١٨٠،٥٤٠ ،٩٠٠،١٢٦٠) وشدة الاشعاع (٠,٤٧٧،٠,٦٣٦، ٠,٧٩٦ ، ٠,٩٥٥ جول /سم ^{٢}) اظهرت النتائج بان حيوية المكورات تقل بزيادة شدة الاشعاع وازمنة التعريض وكان التثبيط بحيوية البكتريا عند استخدام النيديميوم ياك ليزر (٠,٧٩٦، ٠,٩٥٥) جول /سم^{٢} شدة الاشعاع وتردد (٣) هرتز بتسليط عدة نبضات(٩٠٠،١٢٦٠) . كاستنتاج ، اظهرت النتائج بان بكتريا المكورات السبحية مستجيبة للقتل باستخدام ا لنيديميوم ياك ليزر، وتعتمد فعالية القتل على مدة التشعيع و شدتها . يقترح استخدام هذه الطريقة لعلاج الامراض المتعلقة بالصفيحة الجرثومية.