The effect of argon laser and chlorhexidine on *klebsiella pneumoniae* isolated from root canal content.

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Abstract

The present study undertaken to identify klebsiella pneumoniae from root canal samples of clinically symptomatic nonvital teeth having periapical on first opening of root canal of 27 such intact teeth and to identify the effect of argon laser and chlorhexidine on isolated *Klebsiella pneumoniae*. Out of 27 samples, positive cultures were 30 samples and the isolated bacteria included *Staphylococcus aureus*, *klebseilla pneumonias*, *streptococcus mutans*, *Pseudomonas aeroginosa and*, *E.coli*. The result it showed that the minimal inhibitory concentrations of chlorhexidine disinfectant(mouth wash)for *Klebseilla pneumoniae* were ranged from 0.014-0.11µg\ml Also it is found that *Klebsiella pneumoniae* completely killed after exposure to argon laser for 10-16 minutes

Introduction:

Periapical pathos's is considered as an endogenous infection caused by oral microflora .Bacterial infection of dental pulp results in pulpal destruction and subsequently stimulates an inflammatory cell response and destruction of bone in the perapex (1). The relationship between bacterial infection of dental pulp and periapical lesion formation has been elegantly demonstrated in the classic studies of Kakehashi <u>et al</u>⁽²⁾. Many investigators have attempted to isolate and identify various microorganisms from root canals and periapical lesions^(3,4,5,6). Convincing evidence has accumulated that infections of root canals are polymicrobial infections. Further .Hashioka correlated the clinical symptoms with microorganisms isolated from root canals of symptomatic non vital teeth⁽⁷⁾.Over the years ,changes in the type of bacteria isolated and their antibiotic sensitivity have been identified and pattern of resistance to various antibiotics have been developed⁽⁸⁾. 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⁽⁹⁾. This laser is used in many applications such as: Forensic Medicine, Entertainment, General Surgery, Ophthalmic Surgery, Holography, Optical "pumping" source⁽⁹⁾.

Argon has approximately the same solubility In water as oxygen gas and is 2.5 times more soluble in water than nitrogen gas. Argon is colorless, odorless, tasteless and nontoxic in both its liquid and gaseous forms. Argon is inert under most conditions and forms no confirmed stable compounds at room temperature ⁽¹⁰⁾.

.Although argon is a noble gasIt has been found to have the capability of forming some compounds. For example, the creation of argon hydrofluoride (HArF), a metastable compound of argon with fluorine hydrogen, was reported by researchers at the University of Helsinki in 2000⁽¹⁰⁾. Although the neutral ground-state chemical compounds of argon are presently limited to HArF, argon can form clathrates with water when atoms are trapped in a lattice of the water molecules.⁽¹¹⁾ Also argon-containing ions and excited state complexes, such as ArH+ and ArF, respectively, are known to exist. Theoretical calculations have shown several argon compounds that should be stable but for which no synthesis routes are currently known Argon laser has killing effect on Klebsilla sp. and Pseudomonas sp⁽¹²⁾. Chlorhexidine is an antimicrobial agent used to control oral diseases ,it consists of molecules with both hydrophilic and hydrophobic properties ⁽¹³⁾. It has a broad antimicrobial spectrum and is generally more effective against gram-positive than against gram –negative bacteria ⁽¹⁴⁾.

Aim of study :

This study was carried out to evaluate the effect of argon laser and chlorhexidin on *klebseilla pneumonias* isolated from root canal content.

Material and Method:

Twenty seven male and female patients with age group of 19-54 years attended Dental Hospital in Tikrit university were included in this study. Each patient have at least one intact non vital tooth, presenting with one or more of the following symptoms:- Spontaneous pain, Pain on percussion, Swelling. An X-ray of the involved tooth was taken and teeth showing periapical pathos's were included in the study. The sample were taken under strict aseptic conditions. The involved tooth was isolated under a rubber dam. The field was disinfected with tincture of iodine and the access cavity was prepared with a sterile round bur.

On gaining access to the pulp, a sterile reamer/file/broach was inserted into the root canal up to the apical foramina and root canal content were obtained for culture. Next reamer or file containing root canal contents was placed in a sterile test tube, sealed and transferred to the laboratory. Root canal contents were inoculated on Blood agar and MaConkey agar then incubated at 37 °C for 18 -24hrs. Colony characteristics were noted in case of any growth and identification of microorganisms was done according to the morphology using gram staining and by biochemical reactions^(15,16,17,18,19,20,21).Determination of minimal inhibitory concentrations of chlorhexidine double dilutions of chlorhexidine of different concentrations ranged between 0.014 -7.2 µg |ml in MacConkey agar plates were prepared .A fresh inoculums of the tested bacteria in MacConkey broth and incubated at 37 °C overnight . All plates were inoculated with broth culture of bacteria and incubated at 37 °C for 24 hours . Each agar plates containing Klebsiella pneumoniae are exposed to argon laser light with output 150 mW, 515 nm at distance 15cm from lens for 2 to 16 minutes ,was utilized against klebsiella pneumoniae isolated in the present study ⁽²²⁾. The results were read to the end of visible growth .

Results:

The distribution of pathogenic bacteria among patients showed that *Klebsiella pneumoniae* was the most common pathogen isolated from female than male as shown in tables (1,2). The MICs (Minimal Inhibitory Concentrations)of chlorhexidine for *klebsilla pneumonia* isolated from root canal content ranged between 0.014 – 0.11 µg /ml (Table 3). Table (4),revealed that all *klebsiella pneumoniae* isolated from root canal content were killed after exposure to argon laser light for 10 minutes . The decrease in number of living bacteria increased with increasing the time of exposure to laser light .

Sex	No .of isolated klebsilla pneumonia
Female	10
Male	5
Total	15

Table(2): Types of bacteria isolated from root canal content.	
Types of bacteria	No. of isolates
klebsiella pneumoniae	15
Staphylococcus aureus	7
Streptococcus mutans	3
Escherichia coli	6
Pseudomonas aeruginosa	4

Table (3) :- minimal inhibitory concentrations of chlorhexidine (mouth wash) for *klebsiella pneumoniae* isolated from root canal

content .(No. of isolates $= 15$)		
Concentrations of	No of bacteria	
chlorhexidine µg/ml	%	
0.014	8	
0.028	5	
0.056	3	
0.11	0	
0.22	0	
0.045	0	
0.9	0	
1.8	0	
3.6	0	
7.2	0	

 Table (4):- The effect of laser exposure on klebsiella pneumoniae according to time.

(No. of total exposed isolates =15).

Time /min	No. of viable bacteria (%)
2	11
4	7
6	5
8	3
10	0
12	0
14	0
16	0

Discussion:

The present study has revealed 100% positive cultures of aerobic microorganisms . Two isolates were found per tooth, which confirms the polymicrobial nature of root canal infections and is comparable to the work of Oguntebi <u>et al</u>⁽²³⁾ where 2 isolates per tooth are reported.</sup>Aerobic isolations, the finding are comparable to other workers but the proportion of klebsiella pneumoniae isolation is much more than that reported by other workers $^{(8,24)}$. In the present study, the highest incidence of dental caries was found among females than males among 27 patients examined .It was noticed a significant difference between females and males . Nibras et al (25), found that oral hygiene practices were higher for girls. Al - Barhawe ⁽²⁶⁾ and Abdul-Rahman ⁽²⁷⁾, found that severe gingivitis was recorded in females more than in males. The minimal inhibitory concentrations (MICs) of chlorhexidine (CHX) for klebsilla pneumonia isolated from root canal content were studied. The MICs of CHX for isolated bacteria ranged between 0.014-0.11µg\ml. The result were in agreement with that reported by Delany et al.⁽²⁸⁾, who found that .The MICs of CHX used for klebsilla pneumonia were 0.11µg\ml .CHX is able to bind to different anionically charged elements within the oral cavity that is teeth and mucous membrane which leads to progressive damage to the membrane. The leakage of low molecular weight components decreases the reflect on the coagulation and precipitation of the cytoplasm by the formation of phosphated complex and this bacterial stage is irreversible .The difference in effect of CHX on the inner membranes suggests some degree of specificity of the action of CHX on membranes⁽²⁹⁾.Argon laser with output 150 mW , 515 nm at distance 15cm, was utilized against klebsiella pneumoniae isolated in the present study⁽²²⁾.Our results showed a decrease in total number of klebsilla pneumonia after exposure to argon ion laser for 2,4,6,8 minutes and killed all isolates completely after exposure to argon laser light for 10 minutes. Charvalose <u>et al</u> (12) found that *klebsilla* pneumonia killed after exposure to argon laser, this near that argon laser have sterilizing characteristics. Al-Mosawy⁽²²⁾found that *Staphylococcus aureus* killed after exposure to argon laser for 5-35 minutes. Al-Obaidy ⁽³⁰⁾ reported that the helium\ neon gas laser have been used widely for killing bacteria related to wound infection⁽³⁰⁾. Al-Shakarji⁽³¹⁾ and Jasim⁽³²⁾ found that strains of Pseudomonas aeroginosa and Staphylococcus aureus, respectively become sensitive to some antimicrobial agents after photochemical exposure. The decrease in number of living bacteria increased with increasing the time of exposure to laser light.

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تأثير الاركون ليزر والكلوروهكسيدين على الكيبسيلا نيمونيا المعزولة من مادة قنوات الجذور

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الملخص

اخذت هذه الدراسة للتعرف وعزل الكليبسيلا نيمونيا من عينات قنوات الجذور للاسنان الميتة التي فيها اعراض سريرية وجراحة في ذروة الجذر في الزيارة الاولى لحشوة قناة الجذور لـ٢٧ سن وكذلك لمعرفة تاثير الاركون ليزر والكلوروهكسيدين على الكليبسيلا نيمونيا الماخوذة من ٢٧ عينة. تم عزل عدة انواع من البكتريا في الاوساط الزرعية وتتضمن هذه البكتريا المعزولة

Stsphyllococcus aureus, streptococcus mutans, klebsiella pneumoniae, pseudomonas aeroginosa , Escherichia coli اظهرت النتائج ام اقل تركيز مثبط للمطهر الكلوروهكسيدين (الغسول الفموي) للكليبسيلا نيمونيا يتراوح بين 0.014 –0.11 ملغم / مل. كذلك وجد ان الكليبسيلا نيمونيا قتلت تماما بعد تعرضها الى الاركون ليزر لمدة ١٠ – ١٦ دقيقة.