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RESEARCH ARTICLE

Antimicrobial Effect of Blue Light Cure and Antibiotics Combination on *Serratia Merasences* Isolated from Apical Root Canal

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Abstract

Objectives: To evaluate the phototoxic influence on the *serratia merasences* potentially by the antimicrobial effect of blue light cure and antibiotics combination. Methods: The growth of *serratia merasences* samples has been found after being exposed to blue light cure with and without the existence of antibiotics (metronidazole, doxycycline) on the brain heart infusion broth for (15, 20, 35, 60, and 70 sec) and then cultured on mitis media to determine its effect. Results: The present study manifested that the bacterial growth was reduced after the exposure to blue light for (60 and 70 sec) in the absence of antibiotics. While, the combination of blue light laser for (35 sec) with the concentration of antibiotics doxycycline and metronidazole (64 Mg/ml, 128 Mg/ml), respectively has yielded 95% growth inhibition. The result revealed the direct antimicrobial influence of light cure and antibiotics metronidazole, doxycycline) combination on the *serratia merrasences* isolations. Conclusions: The influence of an antibacterial synergistic between antibiotics and blue light used has been noticed. The blue light cure in combination with antibiotics (metronidazole, doxycycline) applied to the tooth infection could be a substitution to observe as an extra minimum treatment of antibacterial.

Keyword: *Blue light cure, Antibiotics, Microorganisms.*

Introduction

There are no differences among the topical antimicrobial agents that are mostly used in dentistry and possess a potential bactericidal influence on the oral microorganism. Nevertheless, the majority of agents possess unwanted side influences that which can be reduced via minimizing their level of concentration. The antimicrobial agent's synergic influence may assist their level of concentration to decrease without influencing their microbial effectiveness [1, 3]. The first discovered antimicrobial material was penicillin and this signed the commencement of the "golden age of antibiotics."

Recently, the vigorous shortage of governing in the utilization of antibiotics, large misuse in fields like the feed of livestock, and unimportant prescriptions for the viral infections have resulted the increase of the global rates of the resistance of antibiotic in the microorganisms. The recent antibiotics discovery cannot continue with the resistance speed that evolved via the microorganisms.

Elevation in the resistance of antibiotic universally has propelled the research into the evolution of recent antibacterial strategies [4]. The initial utilization of the photodynamic effectiveness of chemical substances and apparent light in opposition to microorganisms was documented at the earliest time of previous century. H. von Tappeiner et al. stated that the noticed toxic influence in the existence of light wasn't ascribed to the heat. At the beginning of 20th century, he invented the expression "photodynamic reaction" for the light reaction with a nontoxic dye [5, 6].

Beyond the long gap, in 1970's, the Photo Dynamic Therapy, PDT, started to explore for the chosen destroying of malignancies [7]. The familiar characteristics of microorganisms and tumor cells are the effective metabolisms and the more proliferation. Accordingly, when the microorganisms can accumulate various photo-sensitizers, their photodynamic inactivity might be influential [8].

The traditional synergy is performed via combining a pair of chemical antimicrobial agents. Utilization of a chemical photo-sensitizer agent in connection with the destructive light photo-sensitization was revealed to be influential in opposition to bacteria [8, 11]. The ultraviolet, UV, light at a (254 nm) wave length, has been broadly employed to disinfect the surface in the operating places and laboratories, also to disinfect the fruit and water [12, 13].

The (UV) possesses two principal capability advantages: (i) an immediate working, preventing the requirement for a lasting time of exposure; and (ii) its bactericidal effectiveness depends upon the targeting and the resulting a damage to the DNA, so it may be non or less time chosen than the other bactericidal agents [14].

In looking for a highly influential disinfectant agent, the ultraviolet light capability to remove the bacteria of the root canal has been regarded. The UV is broadly utilized to disinfect the laboratory and surgical surfaces, water and the outer fruits surface. In a new elementary investigation [15], it was set that the oral bacteria that usually exist in the infected root canals are more susceptible to the light of UV having (254 nm).

If it were directly exposed, the doses lower than (7mJ/cm2) would be enough to remove the whole bacterial strains examined. Such elementary investigation was designed for exploring the susceptibility of *serratia merasences* bacteria that usually exist in the apical root canals to the cure of (UV) light cure, antibiotics combination an in vitro.

Materials and Methods

Fifty patients were examined as the sample of this study. They were referred to the Laboratory of Microbiology, Department of Microbiology, College of Medicine, and Tikrit University after examining them in dental teaching in College of Dentistry of the same University.

The diagnosis of root canal disease was made through a clinical examination, so patients fulfilling the criteria to be diagnosed as endodontic infection. The evaluation of endodontic criteria was the length of root canal obturation, the density (uniformity) of root canal obturation, and the taper of root canal obturation.

Also, the X-ray of implicated tooth was obtained. A certain score of 0, 1, or 2 was allotted to every factor. Clinical examination and diagnosis in each case were performed by a dentist. The taken samples were conducted under rigorous aseptic circumstances. The implicated tooth was isolated under a dam made of rubber. The area was disinfected with the iodine tincture and the accessible cavity was arranged using a sterile round bur.

By getting the entrance to the pulp, a sterile reamer/file/broach was introduced into the root canal till the apical foramina, and the content of the root canal was determined for culture [16]. The contents of the root canal were inoculated on the brain heart infusion broth, located in an incubator at temperature (37°C) for (18-24 hr). After that, the root canal contents were inoculated on mitis media with tellurite potassium 1% and located in an incubator at temperature (37°C) for (18-24 hr).

The characteristics of colony were noticed in state of every growth, and the microorganism's identification was conducted for the morphology via standard biochemical reactions and Grams staining [17]. To determine the bacterial sensitivity to UV light, each isolate was grown in BHI broth incubated at 37°C for 16 hr, and then it was harvested by centrifugation and re-suspended in equal volume of 0.9% (W/V) normal saline.

One hundred microliter of the saline suspension of organism was transferred into a sterile test tube, and equal volumes of filter sterilized solution of the following substances were added: a) each suspension of *serratia merasences* was exposed to the UV light cure for 15s, 20s, 35s, 60s, and 70s. b) Antibiotics in D.W were added to give the final concentration of 0.5, 1, 2, 4, 8, 16, 32, 64, 128, 256 and 512 Mg/ml for *serratia merasences* and exposed to the UV light cure for 15s, 20s, 35s, 60s, and 70s.

The subculture of these tubes on mitis agar plate was supplemented with tellurite potassium 1% by loopful with sterile loop on agar plates; after the overnight incubation at 37°C, the results were read to the end of visible growth [18].

Results

The growth of bacteria growth was evaluated pursuing the light exposure in combination with various antibiotics concentrations. The current study manifested that the bacterial growth was reduced after the exposure to blue light for 60 and 70 second in the absence of antibiotics. While, the combination of blue light cure 35s and the concentration of antibiotics doxycycline and metronidazole (64 Mg/ml, 128 Mg/ml) respectively yielded 95% growth inhibitions. Also, the present study elucidated that the statistical data strongly implied that the exposure to the UV light cure and treatment with antibiotics led to a significant decrease in the number of bacteria ($p<0.05$) using the T-test. The most effective treatment in killing of *serratia merasences* isolated from the apical root canal was the UV light cure with antibiotics combination than the exposure to the UV light cure only, and the difference between them was statistically important ($p < 0.05$) using the T-test. The decrease in the living bacteria number increased with increasing the time of exposure and this reduction was statistically important ($p < 0.05$) using the T-test. The results depicting the direct antimicrobial effect of the UV light cure and antibiotics (metronidazole, doxycycline) combination on the *serratia merrasences* isolates are given in Tables from (1) to (4).

Table 1: MICs of antibiotics for *serratia merrasences* isolated from the root canal. (No. of isolates = 14)

Concentration of antibiotics (Mg/ml)	D	MET
0.5	14	14
1	14	13
2	12	13
4	12	13
8	11	13
16	9	11
32	7	9
64	5	9
128	3	7
265	0	5
512	0	0
Mean	7.91	9.73
Standard Deviation (SD)	5.26	4.34

D: Doxycyclline, **MET:** Metronidazole

Table 2: The effect of UV light cure on the *serratia merrasences* isolated according to the time of exposure. (No. of total exposed isolates = 14)

Time (Second)	No. of bacteria after exposure to
	UV
15	41
20	41
35	14
60	8
70	5
Mean	11.00
Standard Deviation (SD)	4.24

Table 3: The effect of UV light cure and metronidazole combination on *serratia merasences* isolates under different concentration of metronidazole with various times of exposure. (No. of total exposed isolates =14)

T(sec)	Metronidazole concentrations (Mg /ml) combined with UV light cure (No. of living isolates)										Mean	SD
	1	2	4	8	16	32	64	128	265	512		
15	14	13	13	12	11	10	7	4	0	0	8.4	5.36
20	14	12	11	12	10	9	4	2	0	0	7.4	5.36
35	12	11	9	8	7	7	3	0	0	0	5.7	4.62
60	10	8	7	6	5	5	3	0	0	0	4.4	3.57

70	10	8	6	5	5	4	2	0	0	0	4.00	3.50
Mean	12	10.4	9.2	8.6	7.6	7	3.8	1.2	0.00	0.00		
SD	2.00	2.30	2.86	3.29	2.79	2.55	1.92	1.789	0.00	0.00		

Table 4: The effect of UV light cure and doxycycline combination on *serratia merasences* isolates under different concentration of doxycycline with various times of exposure.(No. of total exposed isolates =14)

T(sec)	**Doxycycline concentrations (Mg /ml) combined with the UV light cure (No. of living isolates)**										**Mean**	**SD**
	1	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**265**	**512**		
15	14	13	11	11	9	7	5	0	0	0	**7**	**5.50**
20	13	12	10	10	9	5	3	0	0	0	**6.2**	**5.20**
35	11	9	7	7	6	5	0	0	0	0	**4.5**	**4.20**
60	10	8	6	5	4	3	0	0	0	0	**3.6**	**3.66**
70	9	7	4	4	3	1	0	0	0	0	**2.8**	**3.22**
mean	**11.4**	**9.8**	**7.6**	**7.4**	**6.2**	**4.2**	**1.6**	**0**	**0**	**0**		
SD	**2.07**	**2.59**	**2.88**	**3.05**	**2.77**	**2.28**	**230**	**0.00**	**0.00**	**0.00**		

Discussion

The UV light has been broadly utilized for disinfecting the surfaces in the laboratories and operating rooms, drinking water and in different same **uses.** In recent time, it has been documented that the oral bacteria usually obtained in the infected root canals are more susceptible to (254-nm) ultraviolet light [15].

The present study evinced that the bacterial growth was reduced after the exposure to the blue light for 60 and 70 second in the absence of antibiotics. While, the combination of blue light cure for 35s and the concentration of antibiotics doxycycline and metronidazole (64 µg/ ml, 128µg/ml), respectively yielded 95% growth inhibition. Completing the treatment of the root canal in one call has recently got admired [19].

Certain documented papers encourage this route by clarifying that a healthy result may be performed even if the canals aren't free from the microorganisms in filled case [20]. Nevertheless, the majority of studies referred to that the rest of the viable bacteria in the root canal are more probably to be connected with the post-treatment disease [21]. While that is an uninterrupted argument, either the encouragers of one call endodontic and those contrasting to its use in the infected root canals are probably agreed with those easy and effective approaches that would make the infected root canals without viable bacteria during one call, it would be advantageous.

The current study exhibited that the statistical data strongly implied that the exposure to the UV light cure and treatment with antibiotics led to a significant decrease in number of bacteria ($p<0.05$) using the T-test. The most effective treatment in killing of *serratia merasences* isolated from apical root canal was the UV light cure with antibiotics combination than the exposure to the UV light cure only, and the difference between them was statistically important ($p < 0.05$) using the T-test. The decrease in the living bacteria number increased with increasing the time of exposure and this decrease was statistically important ($p < 0.05$) using the T-test.

The result manifested the direct antimicrobial effect of the UV light cure and antibiotics metronidazole, doxycycline) combination on the *serratia merrasences* isolates. The (UV) light bactericidal mechanism is depended upon the inducing of (DNA) dysfunction resulted via the cross-linking between the adjacent pyrimidine nucleotide bases (cytosine and thymine) in the same strand of (DNA). That subsequently weakens the hydrogen bonds development with the purine bases on the contrasted strand.

The replication and transcription of DNA are by that means blocked, compromising cellular duties and finally causing the death of cell [22]. To result such damage, the (UV) light should initially be absorbed via the bacterial (DNA). The absorption of the (UV) light via the peaks of the (DNA) at a 254-nm wavelength, which illustrates the influence of the excellent bactericidal of such distinctive wavelength [23, 24]. The similar mechanism may capably be hurtful to the hosted cells. Accordingly, specific primary cautions must be importantly regarded prior to such protocol that may be clinically employed.

The present investigation was made for exploring the capability of the antibacterial influences of the (UV) light against the bacteria usually established in the infected root canals. Such subject was applied at two principle levels: (a) a preliminary susceptibility exploration of the pertinent bacteria to the (UV) light; (b) the (UV) light compared with the antibiotics in the *serratia merasences* resistant strain.

The 'oral bacteria' is usually established in the infected root canals, like F. nucleatum and P. gingivalis, *serratia merasences*; enterococci non-mutans, streptococci and lactobacilli [25]. Such outputs agree with the investigations that obtained a high susceptibility to the (UV) light in a broad extension of bacteria [26]. The susceptibility of bacteria to the other antibacterial agents has been documented to be influenced via the growth and life circumstances of the culture of bacteria [27]. Thus, more experiments must also involve the bacteria obtained from the longer established cultures, in their lately unchanged_phase, in addition to the 'starved cultures'.

It must be fulfilled if the more susceptibility to the (UV), currently documented, is connected to the almost used cultures young age or it's an inherent phenomenon that influences the bacteria from the starved cultures also [26]. If trying the (UV) disinfection of a surface, on which the single bacteria may be exist, like the surfaces that are cautiously cleaned in the operating rooms, an almost low (UV) light dose may be enough. However, of the targeting bacteria on the greatly contaminated surfaces, like the root canal internal surface, the first step must be to decrease the bacteria quantity possibly in great degree via other ways, for example rinsing with antibiotics and instrumentation. Then, only a supplementary stage of that exposing the surface to the (UV) light is influential. Also, it must be observed that a (UV) dose, greatly more than this needed for disinfecting the surfaces of the operating room, may be needed. It will have to substitute for the light absorption via the bacteria in the outer layers of a bacterial multi-layer that may be exist [28].

The investigators revealed that the strains of *Porphyromonas gingivalis, Fusobacterium nucleatum*, *Aggregatibacter actinomycetemcomitans* and *Fusobacterium nucleatum* cultivated in vitro in the planktonic condition were nearly entirely killed beyond the exposure to the "blue light" for a period from (15) (*P. gingivalis*) to (60) (*F. nucleatum*) seconds.

Nevertheless, a similar investigation manifested that the only *P. gingivalis* strains were sensitive to such photo-toxicity whilst in the biofilm condition [29]. The present investigation may work as primary indication that the (UV) light employment to enhance the root canals disinfection may be likely. As the surrounding of root canal isn't alike the culture plate surface, different technical problems would require to be dominated in order to give a homogenous (UV) lightening up to the walls of root canal. Moreover, the environmentally hosted tissues and safety should require to be applied. More investigations to set this approach and to test its safety and performance are presently in advance. It was concluded that there was synergistic effect between photochemical agents.

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