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Research Article

The antimicrobial effects of Cold Atmospheric Plasma jet on microorganisms causing dental caries (in vitro study)

Ghada Abdul Salam Ibrahim*

Maha Jamal Abbas**

Eaman Ali AL-Rubaee*

*Ph.D. Student, Pedodontic and Preventive dentistry department, College of Dentistry, University of Baghdad/ Baghdad, Iraq, E-mail: ghadaalbaghdadi15@gmail.com, Phone: 009647711455413

*Professor, PhD in Preventive Dentistry, College of Dentistry, Mustansiriyah University/ Baghdad, Iraq.

*Professor, PhD in clinical Biochemistry, Department of Basic Science, College of Dentistry, University of Baghdad/ Baghdad, Iraq,

Abstract:

Background: The common microorganism in caries process are *Streptococcus mutans* and *Lactobacilli. Candida albicans* have correlation with *Streptococcus mutans* for enhanced dental caries. Cold Atmospheric Plasma (CAP) is called as non-thermal as result of electrons are more heated than heavy particles which are at room climate. CAP jets are ionized native gas outflow, produced underneath typical pressure through microwaves, high frequency or pulsed direct current employing noble gases. There are many application of CAP in dentistry: in periodontology, implantology, prosthodontic, operative dentistry and in cariology. **This study aimed** to measure the sensitivity of the *S. mutans and C. albicans* to CAP jet. **Material and methods**: In this experiment, newly developed Cold atmospheric plasma jet (Iraqi made) using argon gas at a Gas flow rate 10 l/min, 175volt and Frequency 2.45 GHz At room temperature such that the nozzle tip had a maximum distance of 2mm from the agar plate. Cold atmospheric plasma effect was tested at different times on the viable numbers of *Streptococci mutans and Candida albicans***. Result**: Through the irradiation of CAP jet on agar plates the antimicrobial efficacy for *S. mutans and C. albicans* was measured at different time the 3, 6, 9 minutes. Multiple pairwise comparisons using Tukey HSD showed there is significant reduction of colony

forming unit (CFU) for *Streptococci mutans* by plasma at all time compared to control. multiple pairwise comparisons using Tukey HSD showed there is highly significance differences for *C. albicans* was observed for all times in comparison to control ($p<0.01$). The data of the current study was analyzed using statistical package for social science (SPSS version 22). Thus, it can be concluded that Cold Atmospheric plasma (argon gas) jet have anti-microbial efficacy against *Streptococci mutans* and *Candida albicans.* but it effects on *Candida albicans* more than on Streptococci mutans.

Keywords: Antimicrobial effects, Cold Atmospheric Plasma, *Streptococci mutans*, *Candida albicans.*

Introduction

The common microorganism in caries process are *Streptococcus mutans* and *Lactobacilli* [(1)]. *Streptococcus mutans* adhere to the enamel pellicle and to other plaque micorganism. They are acidogenic and acidouric creating the risk for dental caries [(2,3)]. *C.albicans* act as an opportunistic pathogen under certain condition cause a variety of infections, for instance, thrushes in infant and chronic atrophic candidiasis (denture -induced stomatitis) in adult [(4, 5, 6, 7)]. *Candida albicans* have correlation with *Streptococcus mutans* for enhanced dental caries and severe oropharyngeal diseases [(8, 9)]. Studies have confirmed a positive relation between *Candida albicans* and initiation of dental c aries in young adults and children [(10, 11, 12)].

Plasma is most common form of matter (4^{th} class of material). The British physicist Sir William Crookes explored Plasma in 1879, but the Irving Langmuir, an American chemist was determined name "plasma", in the year 1929. Plasma is a group of stripped particles. Formerly the electrons are deprived from molecules and atoms, those particles modify situation and change to plasma [(13)].

Classification of plasmas are classified as "thermal" or "non-thermal" according to temperatures of the ions, electrons, and neutrals [(14)]. Cold Atmospheric Plasma (CAP) is called non-thermal because it has electrons are more heated than heavy particles which are at room climate [(15)]. In last years, cold atmospheric plasma sources (below 40 °C) have been produced to expand plasma treatment to living cell [(14)]. Various methods to produce plasma include plasma needle, Atmospheric Pressure Plasma Jet (APPJ), plasma pencil and Dielectric Barrier Discharge (DBD). Gases be employed to generate CAP are Nitrogen, Argon, Helium, air and Heliox (a mix of helium and oxygen) [(15)]. CAP jets are ionized native gas outflows, produced underneath typical pressure through microwaves, high frequency or pulsed direct current in so-named plasma-jet sources employing noble gases [(16)].

There are many application of CAP in dentistry: in periodontology, implantology, prosthodontic, operative dentistry and in cariology as antimicrobial therapeutic interventions against microorganisms causing dental caries. The antimicrobial effects are depend on the production of reactive oxygen (ROS) and nitrogen species (RNS) involving charged particles, free radicals, electric fields, and electric radiation such as Ultraviolent radiation [(17, 18,,19, 20,21)].

Goree et al, 2006 [22] confirmed that CAP killed *Streptococcus mutans*, a gram-positive cariogenic bacterium. Subsequently, Yang et al. was found that CAP argon plasma brush was very efficient in killing *S. mutans* and *L. acidophilus* [23]. CAP showed antifungal effect on *C. albicans* virulence factors, such as filamentation and adhesion [24].

CAP can irradiate and treat irregular surfaces; making them good for sterilization tooth cavity without need for preparation of tooth. CAP itself is superficial, but the active plasma species it give can freely enter inner the cavities [25]. The aim of this study was to assess the sensitivity of the *S. mutans and C. albicans* to Cold Atmospheric Plasma (CAP) jet.

Material and methods

The Sensitivities of the *Streptococcus mutans and Candida albicans* to Cold atmospheric plasma:

In this experiment, newly developed Cold atmospheric plasma jet (Iraqi made) using argon gas at a Gas flow rate 10 l/min, 175volt and Frequency 2.45 GHz At room temperature such that the nozzle tip had a maximum distance of 2mm from the agar plate. This device was made in Baghdad University, College of Science for Girls, Department of Physics- Medical Physics laboratory for Postgraduate Studies. In this experiment, Cold atmospheric plasma effect was tested at different times (3, 6, 9) minutes on the viable numbers of *Streptococci mutans and Candida albicans*. Pure isolates of the bacteria and Candida, they were preserved in nutrient agar in the refrigerator until required for the study. Pure isolates of *Streptococcus mutans and Candida albicans* can be activated by addition 0.1 ml added to 10 ml of sterilized brain heart infusion broth, then incubated aerobically for 18 hr at 37 ^{o}C before each experiment [26].

In this experiments 4 groups for *Streptococcus mutans and Candida albicans* (one control and others study groups). Standardized suspensions of bacterial and fungal broth that contained 1.5 x 10^8 colony forming units (McFarland Standard 0.5) were prepared. After plating 2 ml of each microbial broth onto the agar, the surface was aired slightly. The apex of the cylinder of CAP jet was placed at 90 angle to the surface of the agar plate and then CAP jet was placed at a length of 2mm from the agar plate. Plasma exposure times were 3, 6, 9 minutes respectively and compared with control before plasma exposure After plasma irradiation, bacteria and Candida was cultured on agar plates. Mitis-Salivarius Bacitracin Agar (MSB Agar), This media for culturing of *Mutans streptococci*. Sabouraud Dextrose Agar (SDA), This selective media for culturing and isolation of *Candida albicans* and then measurements was decided by counting the living cells after plasma exposure by colony forming unit (CFU) and compared with control before plasma irradiation [27].

CFU/ml = No. of colonies x 1/dilution factor x 10

Statistical analysis

Statistical Package for Social Research was used for descriptive analysis, and presentation (SPSS version -22, Chicago, Illionis, USA). Levene test for such a quantitative variable that includes the minimum, maximum, mean, standard deviation (SD), and standard error. Using Tukey's Honestly Significant Difference (Tukey's HSD) for multiple

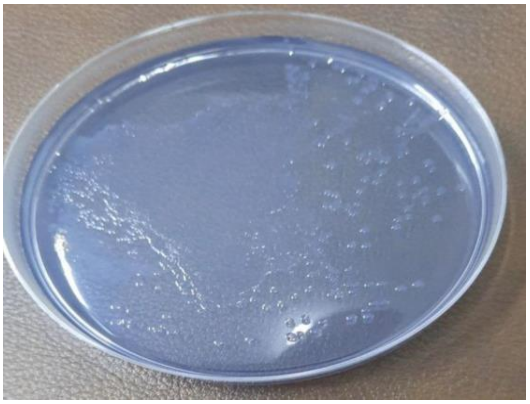
pairwise comparisons among group. Not significant with a P value more than 0.05, significant at a P value lower than 0.05.

Result

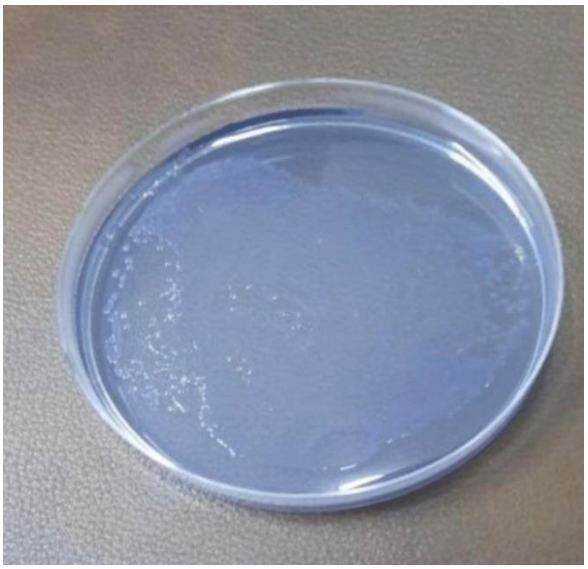
Through irradiation of CAP plasma jet on agar plates, the antimicrobial efficacy for *S. mutans* was measured at different time (3, 6, 9) minutes as shown in figure and table 1 . Multiple pairwise comparisons using Tukey HSD showed there is significant reduction of colony forming unit (CFU) for *Streptococci mutans* by plasma at all time compared to control as shown in table 2. When compared between treatment group there is significant reduction of colony forming unit($p<0.05$).

the antimicrobial effect of *C. albicans* was measured at different time (3, 6, 9) minutes as shown in figure 2 and table 3. Multiple pairwise comparisons using Tukey HSD showed there is highly significance differences was observed for all times in comparison to control before irradiation of plasma ($p<0.01$). When compared between treatment group there is no significant reduction of colony forming unit ($p<0.05$) (table 4).

A



B



C

D

Figure (1): Sensitivities of *Streptococcus mutans exposed* to plasma at different exposure times (A) control (B) After 3 minutes (C) After 6 minutes (D) After 9 minutes

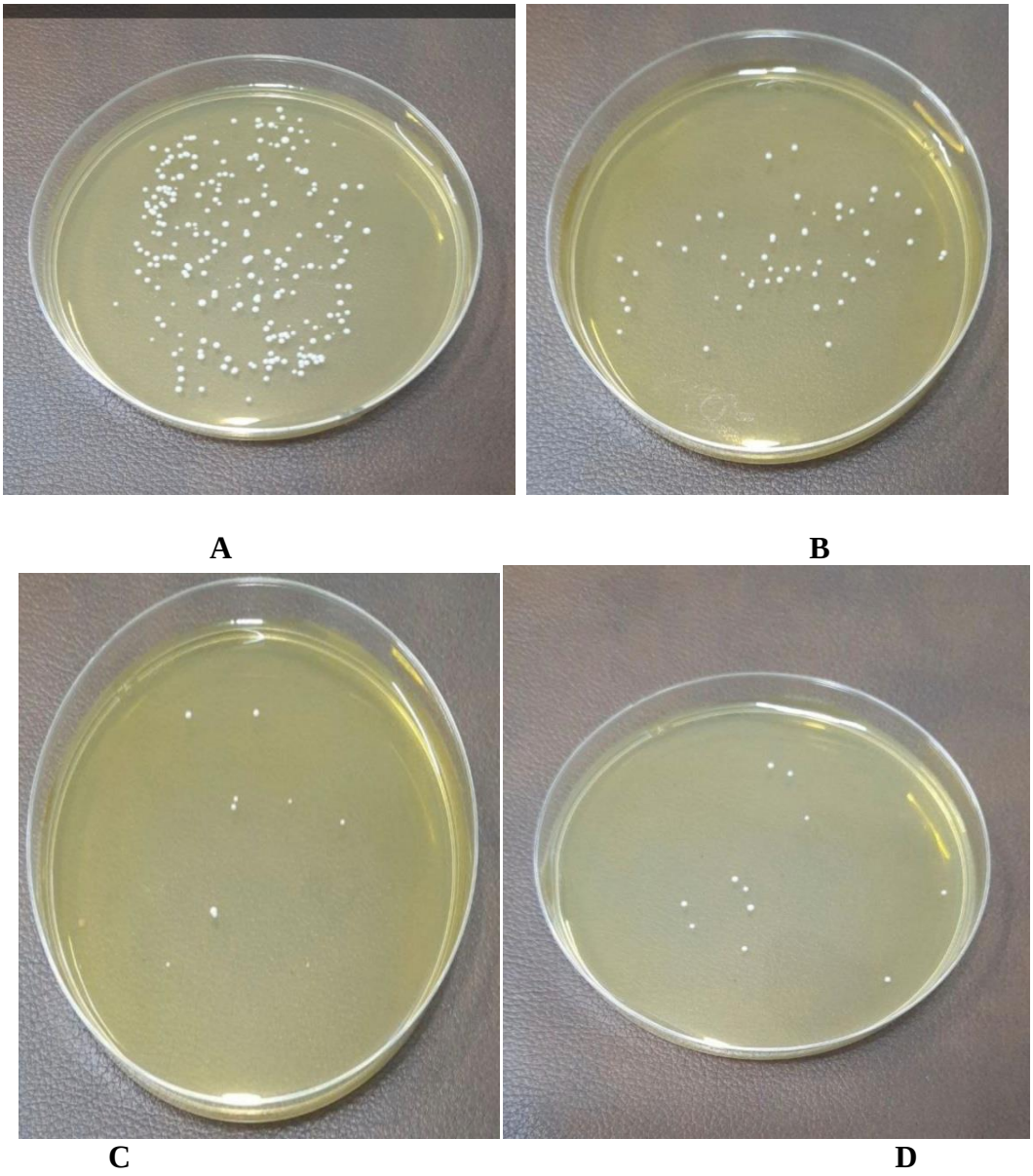


Figure (2): Sensitivities of *Candida albicans exposed* to plasma at different exposure times (A) control (B) After 3 minutes (c) After 6 minutes (D) After 9 minutes

Table (1): Descriptive and statistical test of colony forming unit (CFU) for *Streptococci mutans* exposed to plasma

Statistics	Control	3 mins.	6 mins.	9 mins.	F	P value
Minimum	250.000	100.000	80.000	175.000	41.050	0.000
Maximum	300.000	270.000	225.000	298.000		
Mean	287.700	174.400	156.100	228.200		

±SD	17.783	63.483	52.069	55.226		
±SE	5.623	20.075	16.466	17.464		

Table (2): Multiple pairwise comparisons of colony forming unit (CFU) for *Streptococci mutans* exposed to plasma using Tukey HSD

(I) time	(J) time	Mean Difference (I-J)	p value
Baseline	3 mins.	113.300	**0.001**
	6 mins.	131.600	**0.000**
	9 mins.	59.500	**0.046**
2	6 mins.	18.300	**0.039**
	9 mins.	-53.800	**0.001**
3	9 mins.	-72.100-	**0.000**

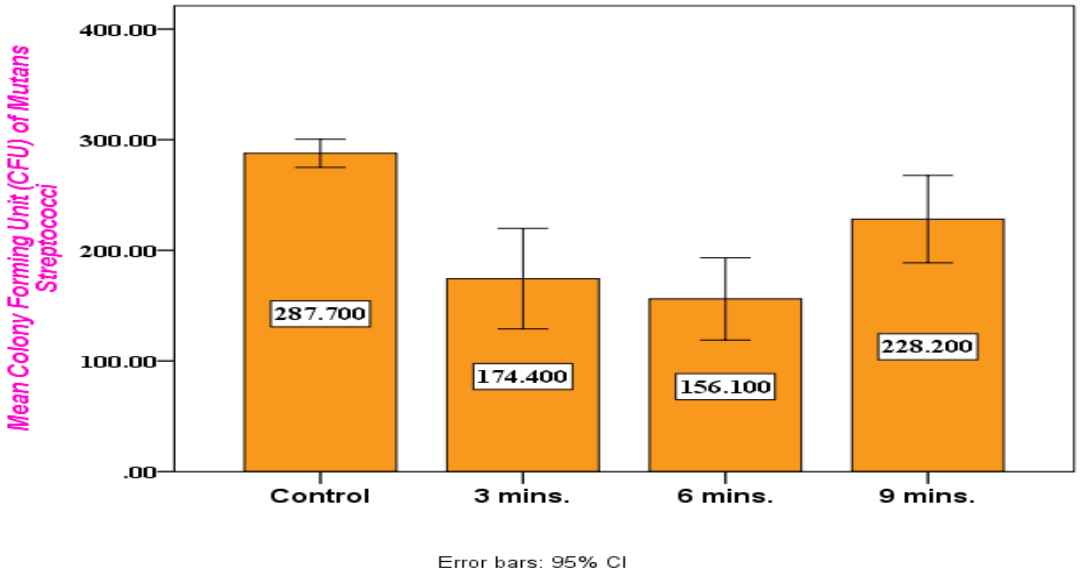


Figure (3): Sensitivities of *Streptococcus mutans exposed* to plasma at different exposure times (A) Control (B) After 3 minutes (C) After 6 minutes (D) After 9 minutes

Table (3): Descriptive and statistical test of colony forming unit (CFU) for *Candida albican*s exposed to plasma

Statistics	Control	3 min	6 min.	9 min.	F	P value
Minimum	50.000	2.000	1.000	1.000	29.702	**0.0000**
Maximum	125.000	47.000	31.000	43.000		**Sig**.
Mean	92.000	24.800	11.100	19.500		
±SD	30.030	17.606	10.908	15.248		
±SE	9.496	5.567	3.449	4.822		

Table (4): Multiple pairwise comparisons of colony forming unit (CFU) for *Candida albican* exposed to plasma using Tukey HSD

(I) time	(J) time	Mean Difference (I-J)	p value
Baseline	3 mins.	67.200	**0.004**
	6 mins.	80.900	**0.001**
	9 mins.	72.500	**0.001**
2	6 mins.	13.700	0.051
	9 mins.	5.300	1.000
3	9 mins.	-8.400	0.742

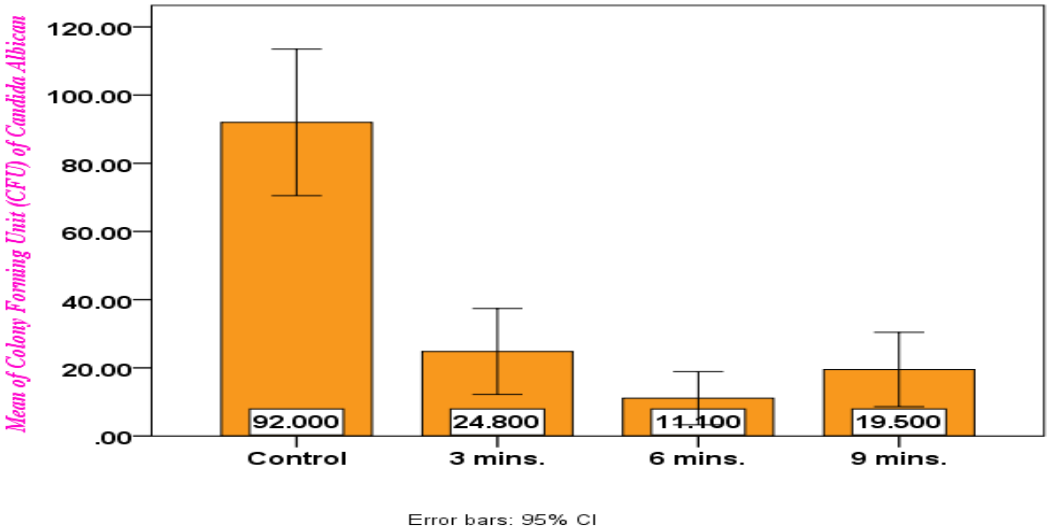


Figure (4): Sensitivities of *Candida albican* exposed to plasma at different exposure times (A) control (B) after 3 minutes (C) after 6 minutes (D) after 9 minutes

Discussion

The reduction in the mean value of the bacterial number (CFU/ml) for *Streptococcus mutans* after treatment with plasma (study groups) was observed when compared with mean value before plasma exposure (control group). A reduction in viable count of bacterial number with a statistically highly significant difference after 3 and 6 minutes and statistically significant difference after 9 minutes when compared with control and an increase in the percentage of bacterial killing was observed with increasing exposure times until 6 minutes then after that there is increase in viable count of bacteria after 9 minutes. A statistically significant and highly significant differences were shown in the bacterial number (CFU/ml) when compared between each group (treatment group). The reduction in the mean value of the *C. albicans* (CFU/ml) after treatment with plasma (study groups) was observed when compared with mean value before plasma (control group). A reduction in viable count of *Candida* number with a statistically highly significant difference at all times when compared with control and an increase in the percentage of *Candida* killing was observed with increasing exposure times until 6 minutes then after that there is increase in viable count of *Candida* after 9 minutes. No significant differences were shown in the *Candida* number (CFU/ml) when

compared between each group. The antimicrobial effects are depend on the production of reactive oxygen (ROS) and nitrogen species (RNS) including charged particles, free radicals, electric fields, and electric radiation such as Ultraviolent radiation. These action guide to antimicrobial activity because of oxidation of DNA and cell membranes. Different parts of the pathogens are attacked by RNS and ROS, the cell wall is etched, and the cell membrane is harm by lipid peroxidation and disruption. The microbial RNA and DNA are weakened by strand breaks, base modification, and oxidative damage. In addition macromolecules, for example proteins may turn into unfolded or altered [17, 20].

The current study agreed with other studies confirmed antimicrobial activity of CAP [٢٨,29]. it disagreed with other study showed argon plasma irradiation effect had no significant decrease of CFU for *S. mutans* between the control and treatment group [27]. A time-dependent rise of the antimicrobial activity was noticed in this study, except when exposure to cold atmospheric plasma for 9 min, there is decrease in the antimicrobial effect. This may because microorganism develop resistant to plasma after 9 minutes.

The susceptibilities of the two tested pathogens to CAP irradiation differ in this study. *S. mutans* showed some resistance to plasma-jet exposure. *Streptococci mutans* is a Gram-positive, non-motile -facultative anaerobe and smaller in comparison with the Candida. Its resistance to CAP jet irradiation may be caused by its small size. Another reason may be the construction of the cell wall of *Streptococci mutans*, that is comprise of strongly cross-linked murein [٢٠].

Conclusion

Cold Atmospheric plasma (argon gas)jet have anti-microbial efficacy against *Streptococci mutans* and *Candida albicans. but it effect on Candida more than on Streptococci mutans*

Conflict of interest: None.

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