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Comparative Study on Cytotoxicity Endodontic Irrigants with & Without Silver Nanoparticle

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ABSTRACT

Background: Irrigation is the key part of successful RCT as the impact of irrigation on smear layer has shown a lot of attention in endodontics.
Aim: To determine & assess by comparing the cytotoxicity of EI combined with & without Ag-NP.
Material & method: Samples were assesed in 9 different groups after conducting a certain procedure which includes NS, NaOCl , AgNPs ,CHX, OTC &TCS.
Result: We have found that statistically significant association seen all the groups.
Conclusion: CTC of substances might be somewhat different when tested using various methods.

Keywords: AgNP, CHX, NaOCl,NS,CTC,TCS,EI, smear layer,method,procedure.

INTRODUCTION

RCT was basically done to remove & stop the infection from reoccurance. Irrigation is an essential component in RCT which produces an effective outcomes.[1-4] Various chemical solutions, such as NaOCl, CHX, and saline, are commonly used as EI. [4-7] Thus, it is important for the chemical substances used as irrigants to have beneficial qualities such as antibacterial activity , ability to break down organic tissue, disinfect the RC and promote a positive response in the periapical tissues. [8,9] As an effective irrigating solution(IGS), chlorhexidine gluconate(CHX-GN) has been proposed as a substitute for sodium hypochlorite (NaOCl). [10] By attaching itself to the cytoplasmic membranes of bacteria, this solution works. [11] Moreover, CHX has a beneficial property of a persistent antibacterial impact on the infected canals [12, 13] CHX and NaOCl have been used in particular attempts to treat this deficit. [14,15]

AIM

To evaluate & compare the cytotoxicity(CTC) of endodontic irrigants (EI) combined with & without Ag-NP.

MATERIAL & METHOD

Armamentarium

1. Class 2B hood
2. Pipettes 0.001-1ml , single channel & 0.01-0.3 , multichannel.
3. Plateshaker
4. Benchtop centrifuge
5. 96 Well Plate

Equipment

1. Elisa Reader
2. Incubator

Method

Our study was done in Department of Conservative & Endodontic at KIMDU after ethical approval. Extracted premolar teeth was used to culture PDL cells. Here, PDL was removed from central part of root to prevent contamination. In water–based incubator, culture were incubated for 24 hrs at 37˚C as shown in figure 1.

Group I- NS
GroupII- 3% NaOCl
Group III- 3% NaOCl+Ag-NP
Group IV- 2% CHX
Group V- 2% CHX+Ag-NP
Group VI- OTC (50mg/ml)
Group VII-OTC (50mg/ml+Ag-NP
Group VIII-TCS
Group IX-TCS+Ag-NP

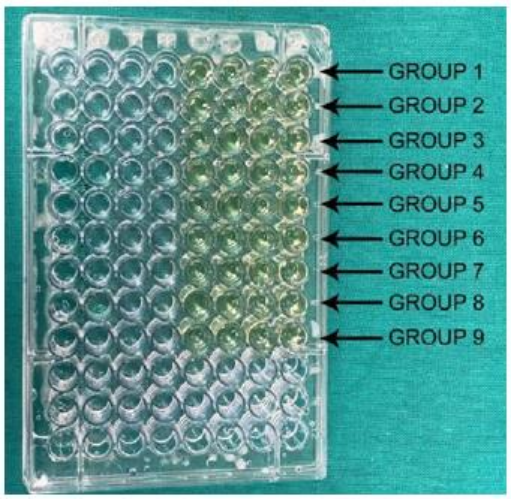

FIGURE. 1- 9 EXPERIMENTAL GROUPS

The CTC of I solution was assessed using Mosman Tetrazolium (MTT) assay at 1, 5 and 15 min of exposure. This solution was filtered, then diluted with DMEM. After dissolving MTT crystal, optical density of irrigant was examined at 530-680nm using Elisa Reader as shown in figure 2.

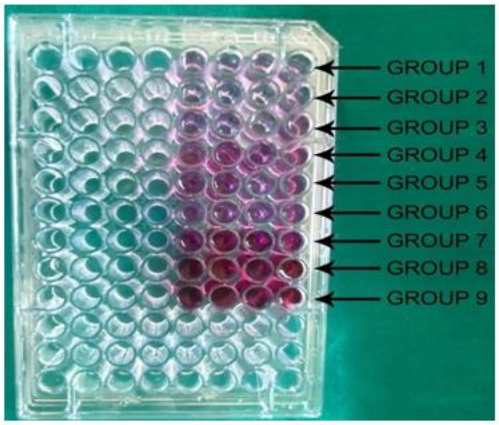

FIGURE. 2- AFTER DISSOLVING THE MTT CRYSTAL

STATISTICAL ANALYSIS

The descriptive data for cell viability were presented for each group as the mean±SD. At various time intervals, cell viability was evaluated across all nine groups using repeated measures ANOVA and then paired comparison using Tukey's Post hoc Test. The data was inputted into Microsoft Excel 2010. The analyses were conducted using version 20 of the SPSS software.

RESULT

	1 min Mean (SD)	5 min Mean (SD)	10 min Mean (SD)
Group 1	**99.5 (1.0)**	**96.25 (1.89)**	**94.5 (1.91)**
Group 2	**16.5 (1.91)**	**14.0 (1.63)**	**10.5 (1.91)**
Group 3	**11.75 (1.7)**	**11.25 (1.89)**	**9.5 (1.29)**
Group 4	**22.5 (1.91)**	**21.5 (1.29)**	**19.0 (1.41)**
Group 5	**20.25 (4.03)**	**16.75 (2.21)**	**16.5 (1.29)**
Group 6	**99.5 (1.91)**	**98.75 (1.7)**	**96.75 (0.95)**
Group 7	**96.75 (2.21)**	**94.25 (2.62)**	**93.0 (2.58)**
Group 8	**97.75 (1.7)**	**95.5 (2.08)**	**91 (1.82)**
Group 9	**92.25 (2.87)**	**89.0 (2.58)**	**87 (3.46)**
One way Anova F test value	**F = 1380.0**	**F = 1720.0**	**F =1750.0**
P value	**p<0.001****	**P<0.001****	**P<0.001****

TABLE 1:OVERALL-COMPARISON WITH EXPERIMENTAL GROUP

Table 1 ,OTC showed the highest cell viability (P<0.05) wheras NaOCl showed lowest.

Without Addition of Ag-NP				
Group	**Comparison Group**	**1 min**	**5 min**	**10 min**
	Group 2	**p<0.001****	**p<0.001****	**p<0.001****
	Group 4	**p<0.001****	**p<0.001****	**p<0.001****

Group 1 vs	**Group 6**	**p =0.941**	**p =0.307**	**p =0.796**
	Group 8	**p =0.997**	**p =0.815**	**p =0.280**
Group 2 vs	**Group 4**	**p =0.023***	**p =0.001***	**p<0.001****
	Group 6	**p<0.001****	**p<0.001****	**p<0.001****
	Group 8	**p<0.001****	**p<0.001****	**p<0.001****
Group 4 vs	**Group 6**	**p<0.001****	**p<0.001****	**p<0.001****
	Group 8	**p<0.001****	**p<0.001****	**p<0.001****
Group 6 vs	**Group 8**	**p =1.000**	**p =0.993**	**p =0.009***

TABLE 2:PAIRWISE COMPARISON WITHOUT ADDITION OF Ag-NP

Table 2 showed significant difference as the average proportion was declined.

Addition of Ag-NP				
Group	**Comparison Group**	**1 min**	**5 min**	**10 min**
Group 1 vs	**Group 3**	**p<0.001****	**p<0.001****	**p<0.001****
	Group 5	**p<0.001****	**p<0.001****	**p<0.001****
	Group 7	**P =0.743**	**P =0.892**	**P =0.974**
	Group 9	**P =0.003***	**P =0.001***	**p<0.001****
Group 3 vs	**Group 5**	**p<0.001****	**p =0.017***	**P=0.001***
	Group 7	**p<0.001****	**p<0.001****	**p<0.001****
	Group 9	**p<0.001****	**p<0.001****	**p<0.001****
Group 5 vs	**Group 7**	**p<0.001****	**p<0.001****	**p<0.001****
	Group 9	**p<0.001****	**p<0.001****	**p<0.001****
Group 7				

vs	Group 9	p =0.169	p =0.026*	P =0.006*

TABLE 3: PAIRWISE COMPARISON WITH ADDITION OF Ag-NP

Table 3 showed that a highly significant difference ($p<0.001$) among 9 groups.

	1 min	5 min	10 min
Group 2 vs Group 3	**p =0.125**	**p =0.612**	**p =0.998**
Group 4 vs Group 5	**p =0.892**	**p =0.057**	**p =0.695**
Group 6 Vs Group 7	**p =0.125**	**p<0.001****	**p =0.607**
Group 8 Vs Group 9	**p<0.001****	**p<0.001****	**p =0.148**

TABLE 4: INTER-GROUP COMPARISON WITH & WITHOUT Ag-NP

Table 4 showed that no difference ($p>0.05$) between both group at all time intervals (1 min, 5 min, 10 min).

DISCUSSION

The use of an irrigant or mixture of irrigants before and after the RC system is instrumented, a procedure known as "chemo-mechanical preparation.[16,17] The effectiveness of hand files, rotary tools, irrigant solutions(IS), and chelating agents in washing, cleaning, cutting, and disinfecting RC supports the quality, dependability, and longevity of new endodontic procedures. Bacterial pathways in the development of periapical diseases have been well characterized in animal models and human studies.[18] There are a wide range of chemical agents that are commonly used in various medical applications. These agents include acids such as tannic and citric acids, as well as NS, H_2O_2 , NaOCl, EDTA, CHX, iodine compounds,

a mixture of acetic vinegar, Tweed 80 detergent, and an isomer of tetracycline. These agents have been used for many years and continue to be utilized in modern medical practices. Various adjuvants are used in the process of disinfecting root canals, such as Electrochemically Activated Solution (EAS), ozonated water, Tetraclean, Photon Activated Disinfection, and plant extracts as Irrigants. It is important to note that rotary and manual devices are responsible for shaping the canal, while irrigation fluids play a role in cleansing the root canal system.[19]

GOALS OF IRRIGATION

By utilizing a cleansing procedure, the irrigation fluid effectively eliminates germs, pulp pieces, and dentinal shavings from the canal. By utilizing irrigation techniques, it is possible to effectively prevent the unwanted extrusion of debris beyond the apex and the occurrence of third packing at the apex. Certain irrigating solutions have the potential to dissolve both dentine and pulp tissue simultaneously. The antimicrobial properties of irrigating solutions are an additional advantage.[20]

CLASSIFICATION OF IRRIGANTS

STOCK [17]

- **A) Chemically Inactive Solution**
  - i. 0.9% Nacl
  - ii. Anaesthetic Solution
- **B) Chemically Active Solution**
  - i. Alkaline Solution
    - a. NaOCl
    - b. Urea
    - c. K (OH)
  - ii. Acidic Solution
    - a. Organic Acid
      - I. Phosphoric Acid (H3PO4)
      - II. Maleic Acid (HO2CCH=CHCO2H)
      - III. Tanic Acid ($C_{76}H_{52}O_{46}$)
    - b. Inorganic Acid
      - I. H2So4
      - II. HCL
  - iii. Oxidizing Agents
    - a. 3% H_2SO_4
    - b. Urea Peroxide ($CH_6N_2O_3$)
    - c. Glyoxide
  - iv. Chealting Agents
    - a. Rc-Prep
    - b. EDTA
  - v. Proteolytic Enzyme
    - a. Streptokinase
    - b. Enzymol
    - c. Papain
  - vi. Other
    - a. Chlorhexidin Gluconate (CHX-GC)
    - b. Glutraldehyde
    - c. Oxidative PoPotential water
    - d. 2% Potentiated Acid
    - e. 1% Pentannedial
    - f. $Ca(OH)_2$
    - g. Bardac-2

The research done by Zambon JJ et al reveals that TCS is a potent antibacterial agent capable of efficiently combating a diverse array of pathogens, such as bacteria, fungi, and viruses. The effectiveness of toothpastes containing TCS in avoiding the buildup of plaque and inflammation of the gums is well acknowledged. TCS is often used as an antiseptic for the skin and other surfaces, and it may also be included into equipment to hinder bacterial infection.[21] Studies have shown that triclosan inhibits the production of fatty acids(FA) in bacteria during the Fab phase of FA biosynthesis. The Fab pathway is a valuable target for antibacterial medicines. Due to the absence of the EACPR enzyme in humans, TCS does not inflict any harm on human cells. A small quantity of the very effective inhibitor triclosan is sufficient to exert a strong antibacterial effect. [21,22] Hence, we conducted a comparative analysis of the CTC of TCS with other commonly used irrigants in this study.[22]

The results of our study shown that human PDL cells exhibited time-dependent cytotoxicity towards NaOCl, CHX, OTC, and TCS solutions.The viability of cells, expressed as a percentage, increased in the following order when exposed to the studied irrigants: NaOCl, CHX, TCS, and OTC. In vitro cytotoxicity testing is only focused on evaluating the toxicity of substances on cells. The susceptibility of periapical tissue to the detrimental impact of pollutants was compared to cell culture.[23].Because materials get diluted with bodily fluids and their concentration fluctuates in vivo, the data obtained from this kind of analysis are unfortunately inadequate for a definitive clinical assessment. [24] Furthermore, it is crucial to take into account the inhibitory impact of dentin on irrigants, considering that the vascular and lymphatic systems, together with phagocytes, all play a role in reducing their effectiveness. [24] In the clinical setting, the CTC of materials diminishes with time at equal doses, as opposed to in vitro conditions.[23,25]

CONCLUSION

OTC and TCS had the least cytotoxicity in comparison to NaOCl and CHX. The presence of silver nanoparticles reduces cell viability. Different methods for assessing the CTC of materials have diverse outcomes. The evidence we obtained only demonstrated cellular toxicity.

The histocompatibility of different irrigants can only be ascertained by the use of organotype RCT models, animal studies, and human clinical trials. Exposure duration, medium composition, and dosage all had a role in the EI-CTC, which was time-dependent. Since our in vitro analysis only estimates CTC at the cellular level, our findings cannot be immediately translated to in vivo examinations. Animal studies should be conducted on RC-I to determine their CTC and in vivo biocompatibility before any human trials are conducted.

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