Analysis of Parachloroaniline Formation through Protocols of Associations between Irrigating Solutions During Chemical-Mechanical Preparation
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Abstract—The success of endodontic therapy depends on the biomechanical preparation and hermetic filling of root canals, but does not generate sufficient microbial reduction in most cases. Chlorhexidine has been used in endodontics due to the wide spectrum of action. However, associated with other irrigation solutions can form Parachloroaniline (PCA), a possible carcinogen for humans, according to the IARC (International Agency for Research on Cancer). The aim of this study is to analyze the formation of PCA through protocols that associate irrigation solutions in chemical-mechanical preparation. Fifty lower premolars teeth extracted from humans were selected, then separated into 5 groups (n=10) for 5 different irrigation protocols and at the end were microscopically analyzed. According to the results obtained only the irrigation protocol of group 5 had the formation of PCA in the three thirds of the root.

I. INTRODUCTION
Success in endodontic treatment depends on a sequence of operative acts that should promote correct cleaning of the canals, eliminating all debris and microorganisms present there. According to Leonardo and Leonardo (2017), dental roots have areas of difficult access, allowing more resistant pathogens to be confined and survive during the instrumentation of the channels.

Authors such as Cohen and Hargreaves (2011), state that chemical preparation, characterized by the use of irrigation solutions, complements the mechanical preparation stage of root canals. These solutions are used to increase the effectiveness of instrumentation, remove the smear layer and eliminate microorganisms, preventing them from proliferating and consequently reinfecting the pulp and root canals.

Sodium Hypochlorite (NaOCl) is the most widely used irrigating solution by dentists due to its efficacy as an antibacterial agent and its ability to dissolve necrotic, vital tissues and organic components, making it the first choice in endodontics. Pretel et. al. (2011) report that NaOCl has been used as endodontic irrigator for more than 4 decades, however, even though it is an excellent tissue solvent, when applied at high concentrations, it is toxic and harmful to tissues.

To Camara et. al. (2015), although NaOCl is the closest irrigator to the ideal, it cannot dissolve the inorganic dentin particles and prevent the formation of the smear layer during instrumentation. For this reason, EDTA becomes an auxiliary solution, because it can highlight the bacterial biofilm adhered to the walls of the canals. This association is considered the most effective according to some authors.

Pretel et. al. (2011) states that in addition to Sodium Hypochlorite, Chlorhexidine has become a viable irrigator for use during canal instrumentation and as intracanal medication, due to its specific characteristics of substantiality and its high antibacterial effect.

Some authors, in order to make the most of the chemical properties of irrigating solutions have proposed protocols of their associations, potentiating the desired final effect, that is, the effective chemical decontamination of root canals. According to Zehnder (2006), with this...
association the Sodium Hypochlorite would act in the dissolution of organic components, EDTA as an auxiliary solution would dissolve a smear layer and the Chlorhexidine would perform antimicrobial action.

Cohen and Hargreaves (2011), however, report that studies have shown that this combination can cause a chemical interaction between irrigators, causing a change of color and the formation of a precipitate, neutral and insoluble substance called PCA (Parachloroaniline).

Considering the risks exposed to the use of irrigating solutions, it is of paramount importance that the dentist professional is aware of the use of these solutions and the protocols of association of the same, in order to select the most indicated and use it in the best possible way, thus avoiding that the risks or harmful effects of the therapy chosen to perform the treatment are greater than the harms of the disease.

In view of the above, the present work aims to analyze the formation of Parachloroaniline through protocols of association of irrigator solutions in chemical-mechanical preparation.

II. METHODOLOGY
This study was approved by the research ethics committee of the Lutheran University Center of the Palmas-Tocantins, Brazil. Thus, the analyses were developed in the dentistry laboratories of this university.

Lower 50 premolar teeth with complete rhizogenesis, single channel, rectum, without calcification, with similar sizes and diameters in the cervical, middle and apical thirds were included.

Dental elements were given through a free and Informed Consent Form - left in dental clinics and dental schools in the state of Tocantins.

Cleaning step of specimens:
Root scraping with periodontal curettes (Duflex - SS White, Rio de Janeiro - RJ/Brazil) and prophylaxis with pumice (SS White, Rio de Janeiro- RJ/Brazil) and water were performed using Robson's brush (KG Sorensen, Rio de Janeiro - RJ/Brazil) coupled to a counter-angle with micromotor (Kavo, Joinville - SC/Brazil). The specimens were stored in Timol 0.1% (Formula and Action Pharmacy, São Paulo - SP/Brazil) for a maximum period of three months.

Preparation step of specimens:
The crowns were sectioned at the amelocementary junction with the aid of a carborundum disc (Fava, São Paulo - SP/Brazil) coupled to the straight and micromotor part (Kavo, Joinville - SC/Brazil), standardizing the length of the roots by 15 mm using calibrating endodontic ruler (Dentsply/Maillefer, Ballaigues/Switzerland). As roots were identified with Romans numerals through a marker for projector.

Group distribution step:
After root preparation, these were randomly distributed into five groups with ten specimens each (n=10).

Instrumentation step of the groups:
The odontometry was made visually through a flexofile 10 file (Dentsply Maillefer, Ballaigues/Switzerland) inserted into the channel until it is visualized in the apical foramen, and the obtained value should be 15 mm, and the working length 1 mm before the apical foramen (14 mm).

With the Prodesign S engine and rotational system (Easy, Belo Horizonte/Brazil) the root flue was instrumented, being used the rotational files 30/10 and 25/08 crown direction - apex at 5 mm below the actual length of the tooth (10 mm) for the preparation of the cervical third, and the file 25/01 in 1 mm beyond the actual length of the tooth (16 mm) for the realization of the foraminal patency, and with a file 25/06 was performed the apical stop 1 mm before the actual length of the tooth (14 mm).

Irrigation step of the groups:
• Group 1
Throughout the instrumentation, experimental unit was irrigated with sodium hypochlorite of 2.5% (Formula and Action Pharmacy, São Paulo - SP/Brazil), 10 ml per experimental unit, using the plastic syringe 10 ml (Advantive Nanchang, Shanghai/China) and disposable needle 25 x 0.55 (BD, Curitiba - PR/Brazil) introduced until reaching 2 mm below the working length (12 mm).

The channels, at the end of the preparation, were dried with tip Capillarytips (Ultradent Products, Jordan/USA) coupled to endodontic sucker and with absorbent paper cone (Tanari, Manacapuru - AM/Brazil).

At the end of the instrumentation, irrigation was carried out with 3 ml of EDTA 17% (Formula and Action Pharmacy, São Paulo - SP/Brazil), being 1 ml of EDTA 17% every 1 minute, then the irrigation was performed with 5 ml of sodium hypochlorite 2.5% (Formula and Action Pharmacy, São Paulo - SP/Brazil) and the drying of the channels with tip Capillarytips (Ultradent Products, Jordan/USA) coupled to endodontic sucker and with absorbent paper cone (Tanari, Manacapuru - AM/Brazil).

• Group 2
Throughout the instrumentation, experimental unit was irrigated with chlorhexidine 2% (Formula and Action Pharmacy, São Paulo - SP/Brazil), 10 ml per experimental unit, using the plastic syringe 10 ml (Advantive Nanchang, Shanghai/China) and disposable needle 25 x 0.55 (BD, Curitiba - PR/Brazil), inserted until it reaches 2 mm before working length (12 mm).
The channels, at the end of the preparation, were dried with tip Capillarytips (Ultradent Products, Jordan/ USA) coupled to endodontic sucker and with absorbent paper cone (Tanari, Manacapuru - AM/Brazil).

At the end of the instrumentation, irrigation was carried out with 3 ml of EDTA 17% (Formula and Action Pharmacy, São Paulo - SP/Brazil), being 1 ml of EDTA 17% every 1 minute, then irrigation was performed with 5 ml of sodium hypochlorite 2.5% (Formula and Action Pharmacy, São Paulo - SP/Brazil) and drying of channels with tip Capillarytips (Ultradent Products, Jordan/ USA) coupled to endodontic sucker and with absorbent paper cone (Tanari, Manacapuru - AM/Brazil).

- Group 3

Throughout the instrumentation, experimental unit was irrigated with sodium hypochlorite of 2.5% (Formula and Action Pharmacy, São Paulo - SP/Brazil), 10 ml per experimental unit, using the plastic syringe 10 ml (Advantine Nanchang, Shanghai/China) and disposable needle 25 x 0.55 (BD, Curitiba - PR/Brazil), inserted until it reaches 2mm before working length (12mm).

The channels, at the end of the preparation, were dried with tip Capillarytips (Ultradent Products, Jordan/ USA) coupled to endodontic sucker and with absorbent paper cone (Tanari, Manacapuru - AM/Brazil).

At the end of the instrumentation, irrigation was carried out with 3 ml of EDTA 17% (Formula and Action Pharmacy, São Paulo - SP/Brazil), with 1 ml of EDTA being 17% every 1 minute. Then, it was irrigated with 5ml of saline solution (Formula and Action Pharmacy, São Paulo - SP/Brazil) using the plastic syringe Luer Slip 10 ml (Advantine Nanchang, Shanghai/China) and disposable needle 25 x 0.55 (BD, Curitiba - PR/ Brazil), inserted until it reaches 2mm before working length (12mm). The channels were dried with tip Capillarytips (Ultradent Products, Jordan/ USA) coupled to endodontic sucker and with absorbent paper cone (Tanari, Manacapuru - AM/Brazil).

- Group 4

Throughout the instrumentation, experimental unit was irrigated with 2.5% sodium hypochlorite (Formula and Action Pharmacy, São Paulo - SP/Brazil), 10 ml per experimental unit, using the plastic syringe Luer Slip 10 ml (Advantine Nanchang, Shanghai/China) and disposable needle 25 x 0.55 (BD, Curitiba - PR/Brazil), inserted until it reaches 2mm before working length (12mm). Subsequently, irrigation was performed with 5ml of saline solution (Formula and Action Pharmacy, São Paulo - SP/Brazil) followed by irrigation with 5ml of saline solution (Formula and Action Pharmacy, São Paulo - SP/Brazil) using the plastic syringe Luer Slip 10 ml (Advantine Nanchang, Shanghai/China) and disposable needle 25 x 0.55 (BD, Curitiba - PR/Brazil), inserted until it reaches 2mm before working length (12mm) and again drying the channels with tip Capillarytips (Ultradent Products, Jordan/ USA) coupled to endodontic sucker and with absorbent paper cone (Tanari, Manacapuru - AM/Brazil).

- Group 5

Throughout the instrumentation was irrigated with sodium hypochlorite 2.5% associated with chlorhexidine 2% (Formula and Action Pharmacy, São Paulo - SP/Brazil), 10 ml per experimental unit, using the plastic syringe Luer Slip 10 ml (Advantine Nanchang, Shanghai/China) and disposable needle 25 x 0.55 (BD, Curitiba - PR/Brazil), inserted until it reaches 2mm before working length (12mm). The channels, at the end of the preparation, were dried with tip Capillarytips (Ultradent Products, Jordan/ USA) coupled to endodontic sucker and with absorbent paper cone (Tanari, Manacapuru - AM/Brazil).

At the end of the instrumentation, irrigation was carried out with 3 ml of EDTA 17% (Formula and Action Pharmacy, São Paulo - SP/Brazil), being 1 ml of EDTA 17% every 1 minute. Then, it was erected with 5ml of saline using the plastic syringe Luer Slip 10 ml (Advantine Nanchang, Shanghai/China) and disposable needle 25 x 0.55 (BD, Curitiba - PR/Brazil), inserted until it reaches 2mm before working length (12mm). After this process, irrigation was performed with 5ml of sodium hypochlorite 2.5% (Formula and Action Pharmacy, São Paulo - SP/Brazil) and again the drying of channels with tip
Capillarytips (Ultradent Products, Jordan/USA) coupled to endodontic sucker and with absorbent paper cone (Tanari, Manacapuru - AM/Brazil).

**Specimen Analysis:**
The specimens were sectioned longitudinally with the aid of a Carborundum disc and analyzed under the operative microscope (M 9000 DF Vanconcelos S. A., São Paulo - SP/Brazil) in 12.5x increase, to check if there has been the formation of Parachloroaniline.

### III. RESULTS

Based on the methodology used, of the 50 lower premolar dental elements instrumented, irrigated and analyzed in the cervical, middle and apical thirds using the operative microscope, it was possible to observe a change in color in the three thirds, in all 10 dental elements of group 5 (Fig. 2), where was performed the root canals irrigation with sodium hypochlorite 2.5 directly associated with chlorhexidine 2.

The staining formed almost immediately, when sodium hypochlorite came into contact with chlorhexidine, thus verifying the formation of Parachloroaniline.

![Fig. 2: Formation of Parachloroaniline in the three thirds (Group 5).](image)

**IV. DISCUSSION**

In order to study whether different protocols of association of irrigator solutions could form Parachloroaniline, the lower 50 premolars were separated into 5 groups with 10 specimens each. Only group 5 where sodium hypochlorite had direct contact with chlorhexidine presented a different coloration in the three thirds after chemical-mechanical preparation. This precipitate can arise in several colors and its formation is explained by the acid-base reaction that occurs when there is interaction between the irrigators.

It was observed that in the other experimental groups, the results were negative for Parachloroaniline, because even using protocols of association of sodium hypochlorite with chlorhexidine, both were applied separately and after the root canal was completely dried with endodontic sucker and cones of absorbent papers.

One of the justifications for the formation of Parachloroaniline in group 5 is that sodium hypochlorite when it comes into contact with chlorhexidine causes hydrolysis, transforming it into smaller fragments and forming by-products, including the Parachloroaniline, a solid substance that is separated from the liquid part.

Many authors have been studying the consequences of the formation of Parachloroaniline, which can deposit in the dentinal tubules and obstruct the canals, impair sealing during filling, stain and darken the dental structure, affect the aesthetics of the tooth, besides being toxic to humans and present carcinogenic action.

With the results obtained in several studies, and the divergences found in some results presented, it is still...
necessary to conduct more research involving irrigating solutions and PCA formation, as well as their possible effects on dental structures. With further studies it will be possible to increase the search for reliable scientific references and more safety during the irrigation of root canals using both solutions.

V. CONCLUSION

With the present study, it was possible to observe that irrigation protocols associating the use of sodium hypochlorite 2.5% and chlorhexidine 2% in the same root canal during chemical-mechanical preparation causes the formation of Parachloroaniline, as occurred in the experimental group 5. However, it was visualized in the other groups that this association can be safe, since in the irrigation protocol the solutions are used separately and with the dry channel, avoiding direct contact between the two irrigators. This care absentees or minimizes systemic and/or local risks during or after the therapeutic application of the protocol, thus allowing a successful endodontic treatment and especially without causing harm to the patient.

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