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MICROBIOLOGICAL AND VISUAL EVALUATION OF CLEANING AND STERILIZATION PROCESSES OF ENDODONTIC INSTRUMENTS

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Abstract: Introduction: Endodontic treatment is not only successful in the correct diagnosis and execution of the technique, but it is especially successful in the dental surgeon's care of the aseptic chain before, during and after patient care. **Goal**: To evaluate the effectiveness of decontamination of cleaning and sterilization processes of endodontic instruments through microbiological and visual evaluation.**Materials and Methods:** This is an experimental study with a quantitative and descriptive approach, where 40 25 mm K#15 endodontic files were analyzed and divided into 4 experimental groups (n=10). The endodontic files underwent different cleaning methods, G1- brushing with neutral detergent, G2- ultrasonic bowl with enzymatic detergent and brushing with neutral detergent, G3- brushing with neutral detergent and sterilization and G4- ultrasonic bowl with enzymatic detergent, associated with brushing with neutral detergent and sterilization. **Results:** After performing the cleaning methods, the files were submitted to microbiological and visual evaluation. Thus, the microbiological analysis showed the turbidity of the files: which G1, G2, G3 and G4 turbid 50%, 70%, 0% and 10% respectively. In the visual analysis the percentage of contaminated files were: G1, G2, G3 and G4 were respectively 30%, 70%, 0% and 10% contaminated. **Conclusion:** It is concluded that the samples that went through the sterilization process, for the most part, are in sterility conditions, especially those that previously underwent brushing with neutral detergent, associated with sterilization.

Keywords: Sterilization, Cleaning, Files, Dentistry.

INTRODUCTION

Biosafety is called a set of technical, administrative and educational conduct and measures that must be implemented by health professionals or the like, to prevent accidents and cross-contamination in hospital environments and outpatient clinics. The prevention of cross-infection is a crucial aspect in dentistry. Professionals working in this area must adopt basic prevention routines during work, as they promote protection of the team, patients and the environment, minimizing the risk of transmission of infectious diseases (PINELLI et al., 2011; PUNJABI et al, 2017).

In addition to the concern to control cross-infection between patients, care must be taken during cleaning of dental instruments, especially in endodontic files (GUADAGNIN et al, 2015). As these are sharp instruments used in remnants of connective tissue that present blood and products of the necrosis process, it cannot be ruled out that there is a possible contamination (PEREIRA et al., 2013).

In the dental environment, the vast majority of instruments used are in turn reused, as in the case of endodontic files that, after a cleaning and sterilization process, are used again. However, the neglect of the dentist in performing an adequate process of cleaning these files can lead to cross infection and this is due to several factors, including the complex design of these files, which are constituted by sharp edges or wires along of its body, whose main function is to perform the filing of the dentin walls, producing dentin scraps (chips), which are lodged along the entire edge, as well as in the channel, which is the groove present between the edges, thus being the locations anatomically of greater difficulty in cleaning. Therefore, demanding from the dentist a proper concern with the cleaning process of these materials (LOPES; SIQUEIRA JÚNIOR, 2015).

Due to the complexity of the design of endodontic files, their cleaning process has aroused the interest of numerous scholars, thus creating and studying numerous

cleaning methods, whether acting in isolation or even in association, being today the most cited in the literature: a mechanical cleaning using different types of brushes and sponges; chemical cleaning through immersion in different concentrations of substances, such as sodium hypochlorite, hydrogen peroxide and enzymatic detergents; and the use of baths in ultrasonic vats (GUADAGNIN et al., 2015; MARTIN; AZEREDO 2014; QUEIROZ et al., 2010).

Therefore, the cleaning methods tested in the literature so far have shown a reduction in the values for the presence of debris in endodontic files, however, none of them until now has been shown to be effective enough to perform an excellent cleaning on these instruments. Thus, there is no consensus in the literature on the best cleaning method for these endodontic files, justifying this research (QUEIROZ et al., 2010; PEREIRA et al., 2013).

It was adopted as the null hypothesis that there would be no difference between the proposed cleaning protocols in light of the microbiological and visual assessment. Thus, this study aimed to analyze the effectiveness of decontamination of cleaning and sterilization processes of endodontic instruments through microbiological and visual evaluation.

METHODOLOGY

KIND OF STUDY

This study is an experimental research with a quantitative and descriptive approach. Experimental research is structured from the definition of the object to be investigated. Variables that influence the object of study can be delimited, establishing control criteria and observing the effects produced by each variable (LOZADA; NUNES, 2018). This type of method can be performed in the field, as well as in a laboratory environment (GERHARDT; SILVEIRA, 2009).

According to Lozada and Nunes (2018), quantitative studies are based on describing results from the analysis of concrete data and objective data, which is a broad method that can be applied in different situations. In this type of study, the data obtained from the descriptive quantitative sample are analyzed and recorded, these data are measured and described in numerical figures, after which the results are evaluated and interpreted using quantitative methods (APPOLINÁRIO, 2011). Descriptive research in general takes the form of data collection, by describing the characteristics of certain populations or phenomena (GIL, 2017).

SAMPLE SELECTION

For this experimental study, 40 K#15, 25 mm type endodontic files were analyzed, where they were collected together with undergraduate students, which had been used only once in patients diagnosed with pulp necrosis, and who did not were subjected to no cleaning process afterwards.

ACQUISITION OF USED FILES AND DIVISION OF GROUPS

After the patients were aware of the research and signed the TCLE, the students who agreed to participate in the research at the Dentistry clinic signaled to the researcher the day and time of the clinical discipline in which the endodontic treatment would be carried out so that the researcher could hand over the file and after at the end of the treatment, carried out by the student, the file was collected, stored and properly identified, without receiving any type of prior cleaning. Then, the researcher, properly dressed with personal protective equipment (PPE), used sterilized forceps to store the endodontic file in a sterilized bottle and identified by a number. This way, the patient who agreed with the research would not be identified during

the assessments. After the acquisition of the transferred files, the files were divided into 4 (four) experimental groups (n=10), classified as G1, G2, G3 and G4.

CLEANING METHODS

Files subjected to mechanical cleaning only

For this step, two different mechanical cleaning processes were analyzed. In a first process, the mechanical cleaning of the file consisted of manual brushing, with a steel brush, associated with neutral detergent. The operator used adequate personal protective equipment, seized the handle of the file, bi-digitally, and then placed the neutral detergent perpendicularly over the file and brushed it with a wire brush. The process was repeated until it was not possible to observe, with the naked eye, the presence of debris on the surface of the file. Therefore, the files were washed in running water, dried naturally and stored in a new sterilized bottle and submitted to microbiological and visual evaluation, immediately after the sterilization process.

The other mechanical cleaning process consisted of cleaning in an ultrasound vat with enzymatic detergent for 3 minutes, and subsequent brushing of the file with a steel brush and neutral detergent, where the file was captured bi-digitally (the operator used personal protective equipment adequate) and brushing was carried out until no debris on the surface of the file was observed with the naked eye. Then, the file was rinsed in running water, stored in a sterilized bottle and submitted to microbiological and visual evaluation.

Files subjected to mechanical cleaning associated with sterilization

Two distinct mechanical cleaning processes associated with sterilization were analyzed. The files were subjected to mechanical cleaning consisting of brushing the file with a steel brush and neutral soap where the file was captured bi-digitally (the operator used adequate personal protective equipment) and brushing was carried out until it was not observed, the naked eye, no debris on the surface of the file. Then, the file was rinsed in running water, stored in a sterilized bottle and submitted to sterilization in an autoclave. After sterilization, the file was submitted to microbiological and visual evaluation.

In another Group, the files were subjected to mechanical cleaning consisting of cleaning in an ultrasound vat with enzymatic detergent for 3 minutes, and subsequent brushing of the file with a steel brush and neutral soap, where the file was seized digitally (the operator used appropriate personal protective equipment) and brushing carried out until no debris on the surface of the file was observed with the naked eye. Then, the file was rinsed in running water, stored in a sterilized bottle and submitted to sterilization in an autoclave. After the sterilization process, the file was submitted to microbiological and visual evaluation.

MICROBIOLOGICAL ASSESSMENT

The microbiological evaluation was performed by inserting the files into new sterile test tubes containing 4ml of BHI culture medium (Brain-herth-infusion, brand KASKI, lot 910161), vortexing them for complete homogenization of the medium, and placed in the bacteriological incubator at 37 degrees celsius together with the rest of the BHI broth and kept in an aerobic environment for 72h.

The files were removed after 72 hours from the greenhouse, where the turbidity or not of the culture medium (BHI) was evaluated. The turbidity shows the microbial growth present in the files, thus characterizing the contamination of the studied files. The files were considered contaminated when the culture medium (BHI) was turbid and

uncontaminated when there was no turbidity in it.

After the cleaning and sterilization process of the aforementioned Groups, the files were placed in new sterile test tubes, containing the BHI culture medium (Brain-herth-infusion, brand KASKI, lot 910161), it was weighed on a scale electronic (exact scales, model 2200A), using 11.11 grams of BHI powder and dissolving it in 300 ml of distilled water. As recommended by the manufacturer of the BHI broth, it was dissolved by heating and with frequent agitation, until there was complete dissolution and the medium became clear.

Before the files were placed in contact with the medium, it was properly wrapped with kraft paper and isolated with an autoclave identification tape, where it was subsequently sterilized in a vertical autoclave (model AV-50) at 121ºC for 15 minutes as also recommended by the manufacturer the middle one. Finally, it was observed that the process of dissolution of the medium was successfully obtained, where it was transparent when leaving the autoclave, and then 4ml of the broth were placed in each sterile test tube, placing the files in each tube. and isolating them with sterile cotton.

The test tubes were further vortexed (model QL-901) for complete homogenization of the medium, and then placed in a bacteriological incubator (ethiktechnology) at 37 degrees celsius along with the rest of the BHI broth and kept in an aerobic environment for 72 hours. After 72 hours, the files were evaluated by a single operator. The turbidity of the culture medium (BHI) showed the microbial growth, characterizing contamination of the studied files. Files were considered contaminated when the culture medium (BHI) was cloudy and uncontaminated when there was no turbidity.

The groups were divided according to the procedures performed, being divided into 4 experimental groups G1, G2, G3 and G4 (figure 13).

Group 1 -- Files brushed with neutral detergent only;

Group 2 - Files placed in an ultrasound vat with enzymatic detergent, associated with brushing with neutral detergent;

Group 3 – Files brushed with neutral detergent, associated with sterilization;

Group 4 – Files placed in an ultrasound vat with enzymatic detergent, associated with brushing with neutral detergent and sterilization.

VISUAL ASSESSMENT

After microbiological evaluation, specimens were cleaned with running water and dried at room temperature. Then, the files were placed on the microscope table and viewed at 10x magnification. This assessment was carried out by only one researcher. Dirty files were considered as those that had some residue of organic matter attached to their spirals.

RESULTS

MICROBIOLOGICAL ASSESSMENT

After 72 hours of incubation of the files in a bacteriological incubator at 37º C under aerobiosis conditions, it was verified the occurrence of turbidity of some files in the groups, G1, G2, and G4, and the non-turbidity of G3, evidencing the non-contamination of the files present in that group. The absolute frequency, that is, the total amount of cloudy tubes present in the study, was given the total of 13 cloudy tubes, showing the contamination of these files. The relative frequency, that is, the total amount of cloudy tubes divided by the total number of files present in the study, was 32.5%.

Of the groups evaluated, the one that obtained the best result in the microbiological evaluation, that is, it did not cloud any sample

containing the BHI culture medium after 72 hours, was G3, where it underwent brushing with neutral detergent associated with sterilization. In G4, the files went through the ultrasonic vat process with enzymatic detergent, associated with brushing with neutral detergent and sterilization. Of the ten files evaluated in this group, only one (10%) clouded the culture medium. In G2, an ultrasound vat with enzymatic detergent was used, associated with brushing with neutral detergent to clean the instruments, a total of seven samples (70%) clouded the BHI culture medium. In G1 the files went through the brushing process with neutral detergent only, where five samples (50%) became cloudy in the BHI culture medium.

VISUAL ASSESSMENT

The files were taken under a microscope and evaluated at 10X magnification, evidenced in some groups of dirt attached to their spirals. G1 had 3 samples (30%) with microbiological residue attached to its spirals. In G2 the same 7 samples (70%) that showed turbidity in the culture medium showed residues. In G3 where the files went through the brushing, neutral detergent and sterilization process, no sample showed residue. While in G4 only the same sample that became turbid in the BHI culture medium showed microbiological residue adhered to the active part of the instrument (figure 1).

DISCUSSION

Cleaning in endodontics is considered to be the previous elimination of debris adhered to instrument surfaces, being a step as important as sterilization. For a favorable prognosis, cleaning endodontic instruments, more specifically endodontic files, must be considered of great importance, as any residues that happen to remain on the instruments will later act as a form of contamination to the endodontic treatment, leading to its failure (GUADAGNIN V. et al., 2015).

Studies show different techniques for an adequate cleaning of endodontic instruments, more precisely of endodontic files, even show more satisfactory results when there is a standardization of the technique to be used (QUEIROZ et al., 2015). However, even if there is a standardization of the cleaning technique for these endodontic instruments, it is not absolutely certain that undergraduate students and graduate professionals will follow this standardization, interfering in the effectiveness of their sterilization.

The endodontic failure rate has been decreasing, but the main agents linked to this failure rate are microorganisms (LUCKMAN et al, 2013). These microorganisms come from both pulp inflammation, as well as those present in instruments used in endodontic treatments, thus characterizing a cross infection (GUADAGNIN et al., 2015). Of the studies that the literature shows regarding cleaning of endodontic instruments, no method was 100% effective (QUEIROZ et al., 2015; PEREIRA et al, 2013).

As experimental groups in this study, four groups of files were evaluated, submitted to different forms of cleaning and sterilization. G3 was the group in which no specimen clouded the BHI culture medium after its removal from the greenhouse. BHI broth promotes the growth and development of microorganisms, so when there is turbidity of this broth, it is confirmed that there are pathogenic microorganisms in the object, when there is no turbidity of this broth there is no microorganism present in the object (MEDEIROS et al., 2019).

After microscopic analysis of the G3 group, the researchers confirmed that there was no contamination of the files, where no residues adhered to the active part of the instrument were observed, coinciding with the

GROUPS	G1	G2	G3	G4
Total files collected	10	10	10	10
Tubes with cloudy aspects	5	7	0	1
Total percentage of contaminated pipes	50%	70%	0%	10%

Table 1: Microbiological results of experimental groups in relative and absolute numbers.

Source: Survey Data(2021).

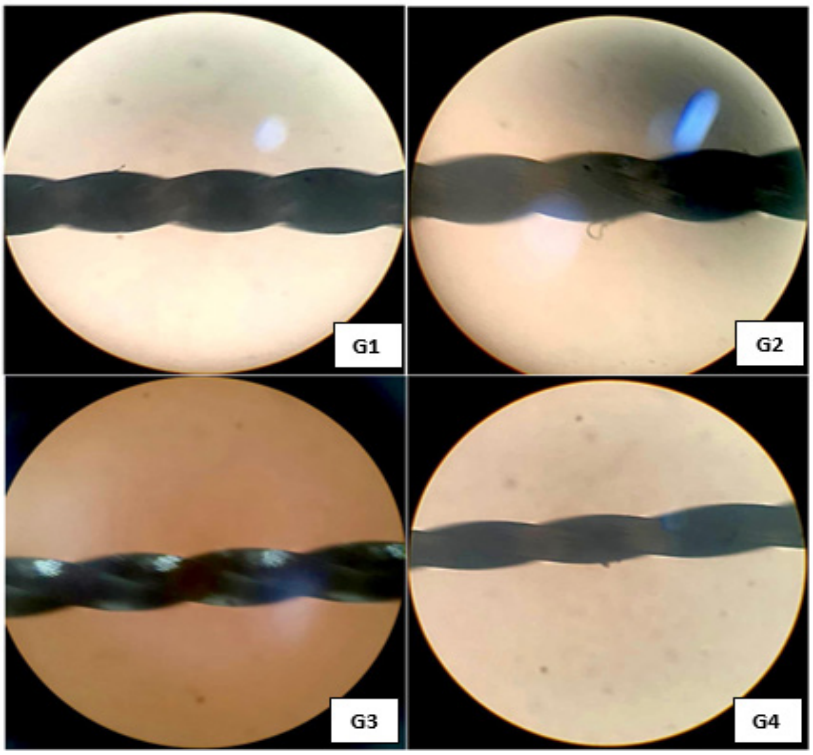

Figure 1: microscopy of files from groups G1, G2, G3 and G4 at 10x magnification.

Source: Survey Data (2021)

GROUPS	G1	G2	G3	G4
Total files collected	10	10	10	10
Files with organic dirt attached to the spirals	3	7	0	1
Total percentage of dirty files	30%	70%	0%	10%

Table 2: Visual evaluation of experimental groups considering absolute and relative numbers, using a microscope with 10x magnification.

microbiological results, where there was no clouding of the endodontic files. Sterilization, above all, is a crucial point in the effectiveness of cleaning endodontic instruments. It promotes almost total cancellation of microorganisms, when well executed and when associated with a good cleaning method (GUADAGNIN V. et al., 2015).

G1 had 50% of its files contaminated after being removed from the bacteriological greenhouse. The same group also evidenced in the visual analysis 30% of their files with organic dirt linked to the spirals, such result may have been taken considering the little magnification of the microscope lens, not evidenced the dirt linked to the spirals. Literature shows that any residues that remain in the instruments will later act as a form of contamination for endodontic treatment, leading to its failure (GUADAGNIN V. et al., 2015; NEELAKANTAN et al, 2019).

Of the groups in question, G2 was the most worrying group in relation to its rates, where 70% of its files were contaminated when observing the turbidity of the BHI culture medium of these files. In this group, the almost total ineffectiveness of the cleaning process using the ultrasonic vat with enzymatic detergent, associated with brushing with neutral detergent, could be noted. It is essential to know the proper technique for cleaning the instrument prior to sterilization, but sterilization is not unnecessary, as it is responsible for eliminating any form of microbial life.

The group where the cleaning process was carried out using the ultrasound vat with enzymatic detergent, associated with brushing with neutral detergent and sterilization, G4, obtained both microbiological and microscopic results, where in both only 10% of the files were contaminated, or that is, only 1 file had microorganisms. Result considered satisfactory when compared to G3. However, it must be taken into account that the success rate of endodontic treatment also depends on the complete sterilization of these materials (GUADAGNIN V. et al., 2015; MARTIN, AZEREDO 2014; QUEIROZ et al., 2010).

Based on the existing literature on the subject, the application of effective cleaning methods for endodontic files must be considered essential, before, during and after their use in patients, these steps being complementary to each other to obtain adequate and suitable instruments of reuse.

CONCLUSION

Analyzing the results obtained and relating them to the proposed objectives of the study, it can be concluded that G3 was the group that presented the best result. Thus, the samples that have gone through the sterilization process are mostly in sterile conditions, especially those which have previously been brushed with neutral detergent, associated with sterilization. However, G2 had the worst results, with cleaning in an ultrasonic vat with enzymatic detergent, associated with brushing with neutral detergent, the least satisfactory cleaning process.

Thus, there is a need to develop more studies that seek more evidence related to the cleaning and sterilization processes of endodontic instruments, given the scarce amount of scientific production related to the topic. It is expected that the results of this research contribute to this development, especially in the reduction of cross-infection in the dental field.

REFERENCES

APPOLINÁRIO, F. **Dicionário de metodologia científica: um guia para a produção do conhecimento científico**. 2. ed. São Paulo: Atlas, 2011.

SILVEIRA, D. T.; CORDOVA, F. P.; BUENO, A. L. M. **Métodos de pesquisa**. Porto Alegre: Editora da UFRGS, 2009. p. 91-96, 2009.

GIL, A. C. **Como elaborar projetos de pesquisa**. 4. ed. São Paulo: Atlas, 2008.

GUADAGNIN, V. et al. **Avaliação microbiológica da eficácia dos métodos de limpeza de limas endodônticas: Revisão de literatura**. Braz. J. Surg. Clin. Res– BJSCR, Paraná, v. 12, n. 1, p. 73-77, 2015. Disponível em: https://www.mastereditora.com.br/periodico/20150902_224015 .p df. Acesso em: 23 jun 2020.

LOPES, H.; SIQUEIRA, J. F. **Endodontia: biologia e técnica**. 4. ed. Rio de Janeiro: Elsevier, 2015.

LOZADA, G.; NUNES, Karina S. **Metodologia Científica**. Porto Alegre: SAGAH, 2018.

LUCKMANN, G.; DORNELES, L. C.; GRANDO, C. P**. Etiologia Dos Insucessos Dos Tratamentos Endodônticos**. Revista Eletrônica De Extensão Da URI, v. 9, n. 16, p. 133-139, 2013. Disponível em: http://www2.reitoria.uri.br/~vivencias/Numero_016/artigos/pdf/Artigo_14.pdf. Acesso em: 24 jun 2020.

MARTIN, G.; AZEREDO, R. A. **Análise do preparo de canais radiculares utilizando-se a diafanização**. Rev. odontol. UNESP, Araraquara, v. 43, n. 2, p. 111-118, 2014. Disponível em:http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1807257720140002001 11&lng=en&nrm=iso. Acesso em: 23 Jun 2020.

MEDEIROS, J. M. E. et al. **Microscopia eletrônica de varredura da presença de resíduos na superfície de corte de instrumentos NITI**. Rev. Odontol. Univ. v. 31, n. 2, p. 116-24, São Paulo, 2019. Disponível em: https://publicacoes.unicid.edu.br/index.php/revistadaodontologia/article/view/823. Acesso em: 23 Jun 2020.

PINELLI, C. et al. **Biossegurança e odontologia: crenças e atitudes de graduandos sobre o controle da infecção cruzada.** Saude soc., São Paulo, v. 20, n. 2, p. 448-461, 2011. Disponível em: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0104-12902011000200016&lng=en&nrm=iso. Acesso em: 24 jun 2020.

PEREIRA, L. B.; OLIVEIRA, M. A. V. C.; BIFFI, J. C. G. **Avaliação da eficácia de métodos de limpeza de limas endodônticas**. Biosci. J., Uberlândia, v. 29, n. 4, p. 1058-1063, 2013. Disponível em: http://www.seer.ufu.br/index.php/biosciencejournal/article/view/18189/12930. Acesso em: 24 jun 2020.

PUNJABI, M.; G DEWAN, R.; KOCHHAR, R. **Comparative evaluation of fracture resistance of root canals obturated with four different obturating systems**. J Conserv D. v. 20, n.6, p. 445-450, 2017.

QUEIROZ, M. L.P et al. **Eficácia de diferentes técnicas na limpeza dos instrumentos endodônticos**. R. Gaúcha de Odont. Porto Alegre, v. 58, n. 3, p. 369- 373, 2010. Disponível em: http: //revodonto.bvsalud.org/scielo.php?script=sci_arttext&pid=S1981 86372010000300015. Acesso em: 24 jun 2020.