

MICROBIOLOGICAL ANALYSIS OF ENDODONTIC FILES USED BY UNDERGRADUATE STUDENTS OF A DENTISTRY COURSE

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Abstract: Goal: The study aimed to analyze the cleaning process and the effectiveness of sterilization of endodontic files used by undergraduate students of the Dentistry course of a college in Alagoas. **Materials and methods:** Endodontic files were collected with students from the 7th, 8th, 9th and 10th periods who were willing to voluntarily participate in the research. To assess the sterility of endodontic files, these instruments were placed in test tubes containing BHI (Brain Heart Infusion) broth and later taken to a bacteriological oven at 37°C for analysis at 24, 48 and 72 hours. Files that caused turbidity in the culture medium were considered infected. **Results:** It was observed that 6.9% of the collected files were contaminated, even after the sterilization process. Among these, 57.1% belonged to academics from the 7th period, 14.3% from the 8th period and 28.6% from the 9th period. The 10th period showed no infected files. **Conclusion:** It is necessary to reinforce the cleaning protocol of endodontic files, as well as to emphasize the importance of its correct execution for endodontic treatment. **Keywords:** Microbiological analysis, Endodontics, Sterilization.

INTRODUCTION

For the success of endodontic treatment, in addition to proper diagnosis, specific planning and the correct execution of the technique, care must be taken with cleaning and sterilization of materials used in patient care (OLIVEIRA et al., 2006). The sterilization process makes it possible to eliminate or destroy all forms of microorganisms present (viruses, bacteria and fungi), and the autoclave is one of the most common and safe means for use in dental instruments (CANAAN et al., 2010).

Prior to sterilization, the instruments must be properly cleaned. The presence of organic matter or residues on the surface of the instruments can interfere or prevent the

action of the sterilizing agent (CHIESA, 2011; FILIPPINI et al., 2004). Several methods are described in the literature for cleaning files, including mechanical cleaning, using different types of brushes and sponges; chemical cleaning, by immersion in different types of substances and concentrations, such as sodium hypochlorite and enzymatic detergents; and the use of the ultrasonic tub (ZIAUDDIN, 2013)

Thus, the present study aimed to verify the cleaning conditions and the effectiveness of the sterilization of endodontic files used in the endodontic clinic by students of four graduation periods, in a dental school.

MATERIAL AND METHODS

The work was submitted to the Research Ethics Committee and approved under CAAE number: 85169917.4.0000.003 and was supported by the institution: "Fundação de Amparo à Pesquisa do Estado de Alagoas" – FAPEAL for a period of 12 months. In accordance with the norms recommended by the National Commission for Ethics in Research on Human Beings (CNS/MS resolution 466/12), the subjects participating in the research authorized their participation by signing the Free and Informed Consent Term (ICF).

The sample consisted of K-File type endodontic files collected after use in the endodontic clinic with students from the 7th, 8th, 9th and 10th graduation periods who agreed to voluntarily participate in the research. After cleaning performed by each participant, each file was individually packaged in a specific surgical grade package with proper identification. After collection, each participant answered a questionnaire in order to identify the type of endodontic procedure performed, the technique and auxiliary materials used for cleaning and disinfection.

The files collected were sterilized and taken for microbiological analysis, whose methodology was based on a study previously carried out by Oliveira et al. (2006). Using aseptic techniques, the endodontic files were removed from the surgical grade with the aid of sterile clinical forceps and placed in a previously identified test tube containing 10 mL of BHI (Brain Heart Infusion) broth. The maneuver of introducing the endodontic file into the test tube was performed close to the Bunsen burner flame and immediately closed, according to aseptic technique.

Then, the tube was taken to a bacteriological oven at 37°C where it remained for 72 hours. The reading was performed at 24, 48 and 72 hours and endodontic files that generated clouding of the culture medium were considered contaminated and those in which the culture medium remained clear were considered sterile. For each stage of collection of endodontic files, the effectiveness and sterility test of the culture medium was carried out to evaluate the real conditions of use.

The data obtained through the questionnaire and microbiological analysis were tabulated and submitted to Fisher's Exact Test and Likelihood Ratio, with statistical significance set at 5% ($p \leq 0.05$).

DISCUSSION

The collection of files was performed from the 7th period, when the endodontic clinic starts at the institution of the present study and there was a greater amount of files collected, justified by the greater demand for endodontic treatment. The 10th period corresponds to the one with the lowest collection due to resistance in the supply of files by the participants.

The files collected were of different calibers, with the largest amount corresponding to caliber #10 (40.6%), widely used during root canal instrumentation because it is often

the initial apical instrument of choice in endodontic treatments.

At the end of the endodontic treatment session, cleaning was performed. Studies address different techniques for proper cleaning (GUADAGNIN et al, 2015; VIEGAS, 2005; PARASHOS et al., 2004) but demonstrate more effective results among students when there is standardization of the technique to be used (PEREIRA et al., 2013). In the institution of the present study, a cleaning and disinfection protocol is recommended, which recommends the immersion of instruments in enzymatic detergent for at least 05 minutes, followed by washing in running water. Place all material in the ultrasonic tank, after the time determined by the tank manufacturer, perform manual cleaning, preferably with a brush with water + neutral detergent.

Despite the existence of the protocol, it is observed that most students do not follow it properly. Regarding the use of ultrasound, the results show its use by only 14.9% of the students. When analyzing the sterility of the files, all that were submitted to ultrasound were sterile.

Ultrasonic baths are effective due to the high energy density generated within the liquid. Its effect is enhanced when there is the inclusion of an enzymatic detergent that reduces the surface tension and ensures a more efficient cleaning action (WALMSLEY et al., 1991). Although the use of ultrasonic devices as well as enzymatic detergents helps in the cleaning process, it is still necessary to be special in the manual cleaning of endodontic instruments before, during and after their use (REISS-ARAÚJO et al., 2008).

After sterilization, the files were submitted to microbiological analysis performed with the BHI culture medium. According to Oliveira et al (2006), the medium is effective for this type of study because it allows the multiplication of a large number of strict and

facultative aerobic microorganisms due to the large nutritional supply. The incubation time of the endodontic files was 72 hours, which according to Filippini et al (2004), is a sufficient period to analyze the presence or absence of turbidity in the culture medium.

In the present study, 6.9% of the analyzed files were found to be contaminated. In the study by Oliveira et al (2006), when evaluating the microbiological conditions of the files used by undergraduate students of six dentistry courses in Rio Grande do Sul, the percentage of 11.66% of files contaminated after sterilization was verified.

In the study by Viegas (2005), sterilization proved to be efficient in the experimental groups, regardless of the cleaning method or its non-use. In the present study, the solutions and methods used with infected files were not statistically significant in relation to sterile files.

Studies show that for the sterilization process to be effective, dental instruments must precede adequate cleaning (CHIESA, 2011). Endodontic files, frequently reused in dental practice, favor the accumulation of residues and make cleaning difficult because they present angles between the spirals and long axis. Any residues that may remain in the instruments will serve as contamination in the treatment, negatively interfering with the patient's prognosis and hindering or preventing the cure of the pathology (REISS-ARAÚJO et al., 2008).

RESULTS

One hundred and one (101) dentistry students participated in the research, making the files available for analysis, with 55.4% of participants enrolled in the 7th period, 31.7% in the 8th, 9.9% in the 9th and 3% in the 10th.

The percentage distribution of the number of files collected in relation to the caliber is shown in graph 1. The largest amount

corresponds, respectively, to calibers #10 (40.6%), #25 (25.7%), #15 (20, 8%).

When verifying the sterility of the files in the culture medium, the present study showed 93.1% of the samples with the clear medium, indicating the sterility of the files, and 6.9% showed turbidity due to bacterial growth.

Of the participating students, 100 (one hundred) were available to answer the questionnaire. It was observed that the files were used in necropulpectomy, biopulpectomy and retreatment procedures, with 66.7% of the infected files being used for necropulpectomy and none of them were used for endodontic retreatment (Table 1).

According to table 1, it is observed that detergent is the substance most used for cleaning files and the use of a brush is more frequent in relation to other materials. The difference between the substances and materials used was not statistically significant. Of the total number of infected files, 83.3% believed that the technique used was correct and 16.7% preferred not to respond.

The solutions used most associated with infection were detergent and 70% alcohol (57.1%); the material that had more infected files was the brush (71.4%), but this proportion is not statistically significant in relation to sterile files, $p > 0.05$, that is, infected files were independent of cleaning procedures.

Table 2 shows the absolute and relative frequency of the information collected in the questionnaires distributed by the periods in which the study was carried out. The type of procedure performed by the students on the day the files were collected showed a difference with statistical significance ($p = 0.001$). Students in the 7th, 8th and 10th period did not undergo retreatment, only the 9th period, representing 19.4% of the total procedures performed.

The solutions, materials and techniques used are independent of the participant's period ($p > 0.05$), that is, the proportions of choice were similar.

Table 3 shows the distribution by period of infected files after sterilization. The 7th period represents 57.4% of the total, but the difference in the amount of infected files between the periods was not statistically significant.

CONCLUSION

Despite the protocol adopted at the college, it is observed that the cleaning method instituted is not followed by most students, especially with regard to the use of the ultrasonic tub. It is necessary, therefore, to reinforce the protocol to be followed in each institution, as well as to emphasize the importance of the sterility of the instruments for the effectiveness of the endodontic treatment.

Questionnaire Questions	Sterile files		Infected limes		Total		P- value ¹
	N	%	n	%	N	%	
What procedure was performed with the files delivered?							
Biopulpectomy	41	43.6	2	33,3	43	43,0	0.556
Necropulpectomy	47	50.D	4	66,7	51	51,0	
Re-treatment	6	6,4	0	0,0	6	6,0	
For cleaning and disinfection of files, which substances are used? ³							
Detergent	67	71.3	4	57.1	71	70,3	0.344
70% alcohol	44	46.8	4	57.1	48	47,5	0.444
Sodium hypochlorite: 2,5%	16	17.0	1	14.3	17	16,8	0.665
For cleaning and disinfection of files, what materials are used? ³							
Gauze	28	29.8	2	28,6	30	29,7	0.656
Sponge	7	7,4	0	0,0	7	6,9	0.595
Brush	69	73.4	5	71.4	74	73,3	0.605
Cuba ultrassônica.	14	14,9	0	0,0	14	14,9	0.722
Do you think you perform the correct file cleaning technique?							
Yes	68	72.3	5	83.3	73	73,0	0.617
No	17	18.1	0	0,0	17	17,0	
I prefer not to answer	9	9,6	1	16,7	10	10,0	
Total	94	100,0	6	100,0	100	100,0	

Table 1 - Absolute and relative frequency of sterility of endodontic files according to questionnaire questions.

1 - Likelihood Ratio Test; 2-Fisher's exact test;* Stylistically significant; 3- The sum of the percentages exceeds 100% due to the possibility of choosing more than one answer.

Source: Research data.

Questionnaire Questions	7° Period	8° Period	9° Period	10° Period	Total	p- value
	n f%1	n i%	n (%)	n (%)	n (%)	
What procedure was performed with the files delivered?						
Biopulpectomy	31 (53,4)	2 (25,0)	10 (32,3)	0(0,0)	43 (43,0)	0,001
Necropulpectomy	27 (46,6)	6 (75,0)	15 (48.4)	3 (100,0)	51 (51,0)	
Re-treatment	0 (0,0)	0(0,0)	6 (19,4)	0(0,0)	6 (6,0)	
For cleaning and disinfection of files, which substances are used? ³						
Detergent	35 (65,5)	7 (77,8)	23 74,2)	3 (100,0)	71 70,3)	0,359
70% alcohol	31 (63,4)	2 (22,2)	13 (41,9)	2 (66,7)	48 (47,5)	0,249
Sodium hypochlorite: 2,5%	10 (17,2)	0(0,0)	7 (22,6)	0(0,0)	17 (16,8)	0,164
For cleaning and disinfection of files, what materials are used? ¹						
Sponge / Gauze	21 (36,2) 6(10,3)	1 (11,1) 0(0,0)	8 (25,8) 1 (3,2)	0 (0,0) 0(0,0)	30 (29,7) 7 (6,9)	0,153 0,327
Brush	30 (65,5)	7(77,8)	26 (83,9)	3 (100,0)	74 73,3)	0,131
Ultrasonic Cuba.	4(6,9)	3 (33,3)	7 (22,6)	1 (33,3)	15 (14,9)	0,061
Do you think you perform the correct file cleaning technique?						
Yes	42 72,4)	6 (75,0)	22 71,0)	3 (100,0)	73 73,0)	0,296
No	12 (20,7)	1 (12,5)	4(12,9)	0(0,0)	17 (17,0)	
I prefer not to answer	4(6,9)	1 (12,5)	5 (16,1)	0 (0,0)	10 (10,0)	
Total	58 (100,0)	8 (100,0)	31 (100 0)	3 (100,0)	100 (100,0)	

Table 2 - Absolute and relative frequency of students per period studied according to questionnaire questions.

1 - Likelihood Ratio Test: * Statistically significant. 3- The sum of the percentages exceeds 100% due to the possibility of choosing more than one answer.

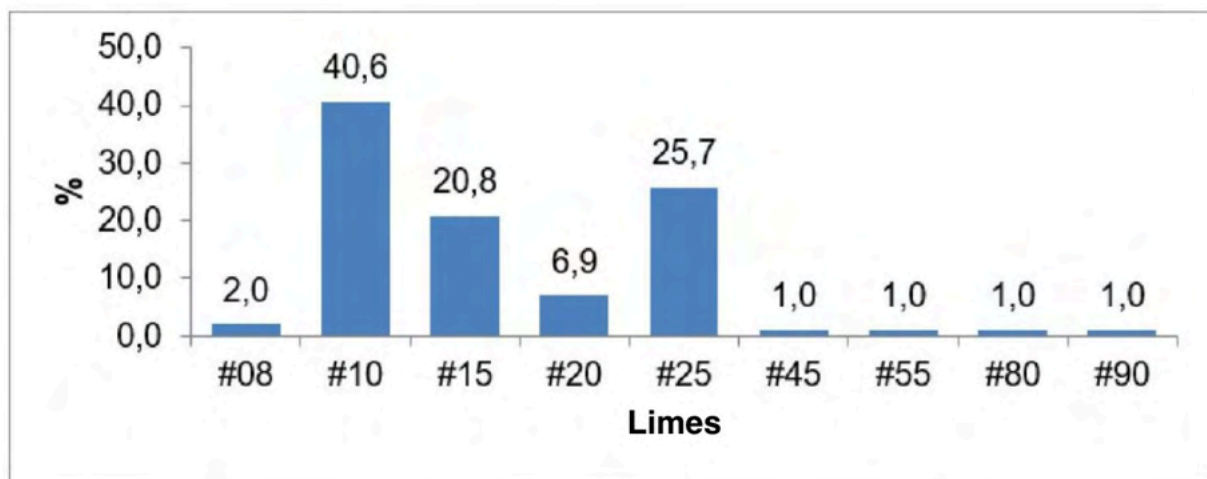
Source: Research data.

	Sterile files		Infected lines		Total		P - value ¹
	n	%	n	%	n	%	
Period							
7 ^a Period	54	57,4	4	57.1	58	57,4	0,885
8 ^b Period	8	8,5	1	14.3	9	8.9	
9 ^c Period	29	30,9	2	28,6	31	30,7	
10 ^d Period	3	3.2	0	0.0	3	3.0	
Total	94	100,0	7	100,0	101	100,0	

Table 3 - Absolute and relative frequency of the sterilization result according to the student's period.

1 - Likelihood Ratio Test: * Statistically significant.

Source: Research data.



Graph1 -Percentage distribution of files used in relation to caliber.

Source: Research data.

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