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CONSERVATIVE TREATMENT ALTERNATIVES TO REMOVE STAINS DUE TO DENTAL FLUOROSIS TYPE IV. IN VITRO STUDY

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ABSTRACT

Introduction: Type IV dental fluorosis compromises the aesthetic and psychological expectations of patients. The main cause is excessive intake of fluoride during dental development. Conservative treatments for the removal of some pigmentations are microabrasion and combination with tooth whitening. There are new products on the market, such as Antivet®, which is proposed in this study for comparison with other treatments.

Materials and methods: 12 dental organs were stored in 0.2% thymol at 37°C, which were divided into 4 groups. Group 1 (OP): (n=3) Opalustre®, group 2 (OP+PH): (n=3) treated with Opalustre® adding Whiteness HP Maxx 35%®, group 3 (ANT) (n=3): treated with Antivet® and group 4 (ANT+PH): (n=3) treated with Antivet® and Whiteness HP Maxx 35%®. Microabrasion was performed with Opalustre® for 60 seconds at 10 intervals for a total of 10 minutes of exposure. The abrasion with Antivet® was carried out for 10 minutes, at the end the neutralizing solution of the same treatment was placed for one minute. Whiteness HP Maxx 35%® was applied seven days after the treatments with Opalustre® and Antivet®, for 15 minutes, making 2 more applications for a total of 45 minutes of exposure. The specimens were taken to SEM/EDS (JEOL FE-SEM JSM-7800F) and color measurements were taken with VITA EasyShade V ® spectrophotometer.

Statistical process control: The Kolmogórov-Smirnov test, analysis of variance (ANOVA) was performed with a significance level of .05 in the statistical package IBM SPSS Statistics 23.

Results: in the ANT group, SEM images show the loss of the aprismatic layer and an etched surface in which the periphery of the enamel prisms appeard removed. On the ANT+PH group

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an etched surface was observed around the enamel rods, slight erosion and porosity on the enamels surface. In the mineral content there was a statistically significant difference between the study groups of the minerals C, P, F and Ca ($p < 0.05$) For the color measurement, there was a statistically significant difference in the ΔE value between the OP group compared to the ANT group with a p-value of 0.031, being the highest value in ANT.

Conclusion: The Antivet® treatment showed a more significant favorable color change in the L*, a*, and b* values, as well as an increase in the concentration of Ca and P.

Keywords: Dental fluorosis, EDS, SEM, fluorosis treatment, microabrasion, tooth whitening, CIE L*a*b*.

INTRODUCTION

Dental fluorosis is an enamel dysplasia that causes aesthetic and psychological problems (Kavand et al., 2012). It is characterized by white to brown spots and even defects in the enamel, forming small craters on the surface. (Dean, 1934) It is an irreversible structure condition due to excessive fluoride intake during dental development. (Buzalaf and Levy, 2011) In permanent teeth, it results from the daily intake of water with a high content of fluoride that exceeds the proportion of one part per million (1 ppm). It develops in the first ten years of life during enamel formation. (Betancourt-Lineares et al., 2013).

The process begins in the primary dentition, but the clinical manifestations are more evident in the permanent dentition. (Fejerskov et al., 1990) It is a public health problem in some states of Mexico with high fluoride concentrations in drinking water, such as Aguascalientes, Chihuahua, Coahuila, Durango, Guanajuato, San Luis Potosí, and Sonora.(GALICIA CHACÓN et al., 2011) Dean classified the changes in the enamel, relating the mottled enamel with the amount of fluoride in water through surveys, classifying fluorosis in 5 degrees. (Dean, 1934).

One of the best-known treatments for reducing stains caused by fluorosis is microabrasion, a technique in which hydrochloric acid is applied in different concentrations depending on the commercial presentation, combined with an abrasive to remove the superficial layer of enamel. (Croll, 1989) It results in a color change and shows a highly reflective surface, thus concealing the discoloration that may remain in the tooth enamel. The acid used cannot reach the dentin, so there is no contact between it and the pulp tissue, which is why it is considered a safe and conservative treatment. (Croll, 1994).

Treatment choice depends on the intensity of fluorosis and its correct diagnosis. In light cases, the most conservative methods include enamel microabrasion, tooth whitening, or a combination of these techniques for more severe cases. (Croll and Segura, 1996) Other treatments are resin

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restorations and fixed prostheses with severe fluorosis, resulting in expensive and invasive treatment. (Sherwood, 2010)

The visual method often used for color evaluation is subjective since it can be affected by environmental and personal factors, which reduces its confidence. The use of instruments to measure color gives an objective performance. (Gupta et al., 2005) The instruments used in color measurement are colorimeters, spectrophotometers, spectro radiometers, and digital cameras. Of these alternatives, spectrophotometers are the most accurate and valuable instruments for shade selection. (Johnston and Kao, 1989) The VITA Easyshade® V (VITA Zahnfabrik) is one of the newer spectrophotometers; the color results given are in the shade systems known as VITA classic® or VITA SYSTEM 3D-MASTER® and the CIEL*a* b* shade system.

Three coordinates used to define the color are L*, a* b*, as in the CIE system developed by the International Commission on Illumination. The most significant advantage of this system is the definition of color change between two patterns. (del'Éclaraige, 2001) The L* coordinate defines the color properties from light to dark or white/black, and it goes from 0 to pure black and 100 for pure white. The a* and b* coordinates show the chromatic characteristics, indicating a value of red-green and yellow-blue, respectively. (Colorimetry, 2004)

In dentistry, diverse authors use SEM and EDS to identify the surface appearance and mineral elements in dental enamel (Machoy et al., 2016a; El-Wassefy, 2017). This equipment, due to X-ray's ability to travel through any material without destroying the specimens, is very recommended (Prieto Olavarría and D'Angelo, 2013)

The objective of this study was to evaluate the in vitro effects on the dental structure and the color change after the application of Opalustre® (Ultradent), Opalustre® (Ultradent) with Whiteness® HP Maxx 35% (FGM), Antivet® (MDC), Antivet® (MDC) with Whiteness® HP Maxx 35% (FGM), publish the results and share the information with dentists who need to know which conservative treatment choose to remove type IV fluorosis stains in their professional practice.

MATERIALS AND METHODS

Sample preparation: Twelve teeth with type IV fluorosis were extracted non-traumatic for prosthetic and orthodontic reasons in a period not exceeding three months at the time of the study. They were cleaned to remove any organic debris with a BioSonic® US100R reamer.

For the specimen preparation, the crown was sectioned off the root with abundant irrigation using deionized water. After cleaning tissues and debris on the teeth' surface, specimens were stored in a 0.2% thymol solution at 37°C in a Shel-Lab 1500E incubator until the study began.

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Specimens were separated in the following study groups:

- GROUP A (OP): n=3; treated with hydrochloric acid at 6.6% and silicon carbide particles (Opalustre® Ultradent).
- GROUP B (OP+PH): n=3; treated with hydrochloric acid at 6.6% (Opalustre® Ultradent) and hydrogen peroxide at 35% (Whiteness® HP Maxx 35%, FGM).
- GROUP C (ANT): n=3; treated with 21% hydrochloric acid (Antivet® MDC).
- GROUP D (ANT+PH): n=3; treated with hydrochloric acid at 21% (Antivet® MDC) and hydrogen peroxide at 35% (Whiteness® HP Maxx 35%, FGM).

Spectrophotometer color measurement: All specimens were set on an addition silicone base, then stamped with thick .020" acetate. A 6 mm pit was made in the acetate at the vestibular side corresponding to the size tip of the VITA EasyShade® V spectrophotometer; this was done to always perform the color measurement in the same place. Measurements of the samples were taken to evaluate the color change with the VITA Easyshade V ® spectrophotometer (VITA Zahnfabrik) before and after the treatments.Results were given according to the CIE color system

L*a*b*. Five measurements per sample were made, and an average was obtained. To calculate the color difference between samples, the formula $\Delta E^* = [(\Delta L^*)2 + (\Delta a^*)2 + (\Delta b^*)2]1/2$ was used. A threshold value of ΔE of 3.7 was accepted for a perceptible color difference.

SEM/EDS measurement: A representative specimen from each group was chosen to be measured for mineral content and surface appearance by SEM (FE-SEM JSM-7800F, JEOL). Before being analyzed, the samples were submitted to a drying treatment for 2 hours at 30°C for only one occasion. In the end, they were placed on a base using a double carbon adhesive tape; with the same tape, the area of the stain due to fluorosis was delimited to always be measured in that area. No coating was used on the surface of the samples. The samples were taken to the vacuum chamber of the SEM, operated at 1.00 kV, and then using the AZtecEnergy software, a spectrum of the analysis of the mineral components was taken in an area of 50 µm and quantitative analysis of percentage in weight and molecular mass.

Enamel Microabrasion Technique: The Opalustre® (Ultradent) microabrasion product was applied to the surface with a 1 mm thick layer; ten applications of 60 s each were made following the microabrasion methodology in moderate to severe fluorosis lesions (Celik et al., 2013; Train et al., 1996) using an abrasive rubber cup (OpalCup™ Ultradent) in contra-angle low-speed handpiece at low revolutions, they were rinsed with injectable water for 30 seconds between each application, and at the end, it was performed in the OP and OP+PH groups. After seven days, this treatment was repeated in the OP group.

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The Antivet® (MDC) abrasive solution was placed on the vestibular surface with a cotton swab. When the swab became pigmented, it was changed to another clean swab with the abrasive solution. The procedure was repeated until 10 minutes had been completed. When the process was finished, the excess solution was cleaned using cotton, and the neutralizing solution was placed for 1 minute with a micro brush. In the end, the samples were rinsed with an injectable solution for 30 s. This procedure was carried out in the ANT and ANT+PH groups. After seven days, this treatment was repeated in the ANT group. They were taken to the SEM/EDS and the spectrophotometer for color measurement.

Teeth whitening technique: 35% hydrogen peroxide (Whiteness HP Maxx® 35% FGM DentsCare) was applied following the manufacturer's instructions on the vestibular face of the samples for 15 minutes; they were rinsed with injectable water using a needle for 30 s. Two more applications were made until the exposure time of 45 minutes was completed. This treatment was applied to the OP+PH and ANT+PH groups seven days after applying Opalustre® or Antivet®. Once the treatment was finished, they were taken to the SEM/EDS and the spectrophotometer for color measurement.

RESULTS

Before and after treatments are shown in the four experimental groups (Fig. 1). The clinical changes were given through digital photographs and the superficial appearance using SEM images at 2000X. Some typical characteristics of dental fluorosis are the superficial appearance of irregular and eroded surfaces in all groups. These characteristics were observed before treatment (Fig. 1-1c, 2c, 3c, and 4c).

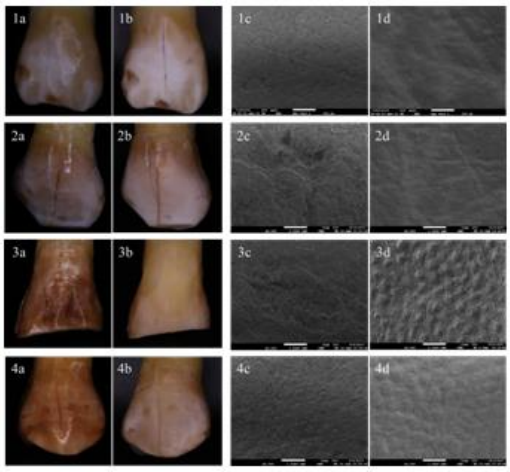

Figure 1: 1) OP, 2) OP+PH, 3) ANT, 4) ANT+PH, a) clinical images before and b) after, c) images of the surface appearance in SEM before and d) at the end of the treatments.

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The OP group (Fig. 1-1d) showed a smooth and polished surface, the OP+PH group (Fig.1-2d) presented an eroded surface with porosities, for the ANT specimen (Fig.1-3d) a type II etching pattern is observed that shows protrusion in the center of the prisms and a marked dissolution of margins in the periphery, of ANT+PH sample (Fig.1-4d) **(protrusion**) it shows erosion in the enamel prisms, in addition to porosity, due to collagen denaturation produced by free radicals of the PH. The most representative graphs are shown, such as P, Ca, and F. The EDS scanned the area distribution of the elements; spectra were obtained from the analysis of the mineral components provided in an area of 50 μm. The initial intake of each group served as a control (Fig. 2). In each spectrum (Fig. 2a), we observe the elements' weight percentage and molecular mass.

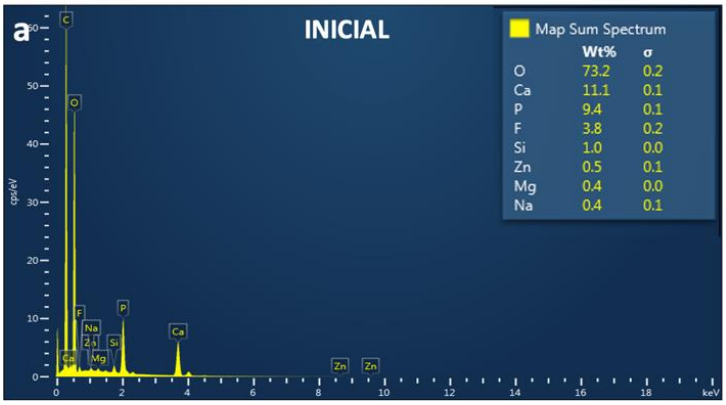

Figure 2: EDS spectrum.

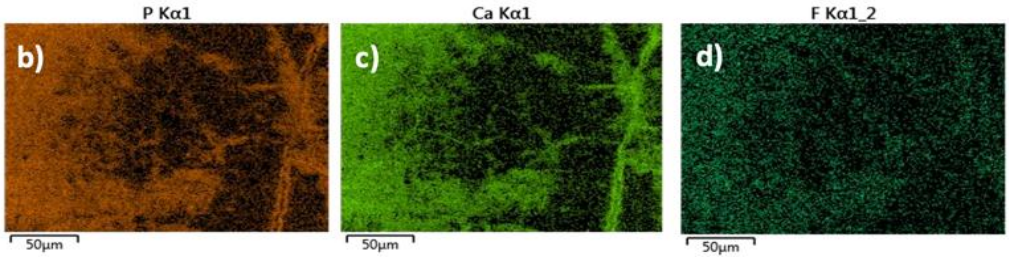

Figure 2: a) EDS of a specimen with flurosis, distribution chart of representative elements. B) P, C) CA and D) F

Through the EDS obtained from the first sample without treatment (Fig. 2), whose average provided us with the mineral content of the specimens with fluorosis, as well as the average results of the mineral content of the experimental groups. It was observed that the OP+PH, ANT, and ANT+PH groups showed significantly higher Calcium and Phosphorus concentrations compared to the Fluorosis and OP groups ($p<0.05$). In addition, the Fluorosis group presented a statistically significant difference in terms of Carbon content between the OP and ANT groups,

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which showed a higher mineral content inverse to what was established in the PH groups ($p<0.05$). Concerning to Fluoride, all the experimental groups showed a decrease in its content, which is statistically significant compared to the Fluorosis control group ($p<0.05$) (Table 1).

Table 1: Descriptive statistics of mineral components of the different study groups.

		Elements									
		O	Ca	P	F	Na	Mg	Si	C	Cl	Zn
Fluorosis	Mean	58.8	14.1	9.3	2.9	0.6	0.3	5.7	15.4	0.6	0.5
	S.D.	20.4	4.2	0.2	1.3	0.2	0.1	6.6			0.0
	Min.	44.3	11.1	9.1	1.9	0.4	0.2	1.0	15.4	0.6	0.5
	Max.	73.2	17.1	9.4	3.8	0.7	0.4	10.3	15.4	0.6	0.5
Final OP	Mean	59.6	11.4	9.3	0.6	0.6	0.1	0.2	18.4	0.2	0.0
	S.D.	0.6	1.5	0.8	0.1	0.1	0.0	0.1	1.2	0.1	0.0
	Min.	59.1	10.3	8.7	0.5	0.5	0.1	0.1	17.5	0.1	0.0
	Max.	60.0	12.4	9.8	0.6	0.6	0.1	0.2	19.2	0.2	0.0
Final OP+PH	Mean	48.2	23.9	13.5	0.3	0.5	0.2	0.2	12.8	0.4	0.3
	S.D.	13.2	9.8	1.7	0.1	0.2	0.0	0.1	2.0	0.1	0.3
	Min.	38.8	17.0	12.3	0.2	0.3	0.2	0.1	11.4	0.3	0.1
	Max.	57.5	30.8	14.7	0.3	0.6	0.2	0.2	14.2	0.4	0.5
Final ANT	Mean	43.5	24.2	12.7	0.4	0.4	0.2	0.3	18.1	0.3	0.2
	S.D.	1.3	0.5	0.2	0.1	0.1	0.0	0.1	0.5	0.1	0.0
	Min.	42.6	23.8	12.5	0.3	0.3	0.2	0.2	17.7	0.2	0.2
	Max.	44.4	24.5	12.8	0.4	0.4	0.2	0.3	18.4	0.4	0.2
Final ANT + PH	Mean	41.0	36.9	15.0	0.2	0.3	0.1	0.1	6.0	0.4	0.2
	S.D.	1.1	0.5	1.1	0.1	0.0	0.0	0.0	0.3	0.0	0.0
	Min.	40.2	36.5	14.2	0.1	0.3	0.1	0.1	5.8	0.4	0.2
	Max.	41.7	37.2	15.7	0.2	0.3	0.1	0.1	6.2	0.4	0.2
P		0.401	0.018	0.006	0.027	0.383	0.101	0.357	0.002	0.074	0.076

Within the color results, the L*, a*, and b* values were needed before and after the treatments, as well as their differences (Table 2) In all groups, color change was shown before (Fig. 1-1a, 2a, 3a,4a), and after the application of treatments (Fig. 1-1b, 2b, 3b,4b), the ΔE results were above the detectable point value of 3.7 and are shown in Table 2.

Table 2: The L* a* b* values before (L1, a1, b1) and after the treatments (L2, a2, b2), the differences (ΔL, Δa, Δb) and the distribution of the ΔE values (all values are above the cutoff value of 3.7)

Groups	L1	L2	ΔL	a1	a2	Δa	b1	b2	Δb	ΔE
OP (n=3)	59.10	77.40	-18.30	8.80	4.50	4.30	43.80	42.10	1.70	18.88
	51.50	65.90	-14.40	8.40	4.10	4.30	44.10	39.70	4.40	15.66
	39.70	67.70	-28.00	15.00	7.00	8.00	38.30	37.20	1.10	29.14
OP+PH (n=3)	59.2	79.1	-19.9	7.8	2.8	5.0	40.3	32.1	8.2	22.1
	15.8	77.2	-61.4	19.6	2.0	17.6	21.6	42.8	-21.2	67.3
	61.3	86.3	-25.0	9.8	3.3	6.5	51.0	28.7	22.3	34.1
ANT (n=3)	14.2	89.5	-75.3	20.1	1.9	18.2	52.2	47.7	4.5	77.6
	62.3	90.4	-28.1	10.0	1.7	8.3	51.5	29.7	21.8	36.5
	36.8	76.1	-39.3	18.0	5.7	12.3	38.6	47.6	-9.0	42.2
ANT+PH (n=3)	27.6	76.0	-48.4	22.3	1.3	21.0	33.4	38.0	-4.6	53.0
	53.1	77.5	-24.4	7.7	0.3	7.4	38.0	32.9	5.1	26.0
	44.5	60.2	-15.7	7.1	4.3	2.8	25.2	20.4	4.8	16.7

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The difference of color perception was determined with the ΔE value, and the Scheffé test was obtained to perform multiple comparisons of the means between the different study groups (Table 3), where statistically significant differences were found between the OP group compared to the OP group. ANT group ($p<0.05$).

Table 3: Multiple comparisons of the ΔE value between groups.

(I) Group		Mean ifference (I-J)	*P*
OP	OP+PH	-19.39	*.237*
OP	ANT	-30.01485*	*.031*
OP	ANT+PH	-10.09	*.741*
OP+PH	ANT	-10.63	*.710*
OP+PH	ANT+PH	9.30	*.785*
ANT	ANT+PH	19.93	*.217*

*. The mean difference is significant at the .05 level.

The graph of the comparison of mean values of ΔE between the different study groups is shown (Fig. 3), where * represents the significant mean difference at .05 level, obtaining a p-value of 0.031 between the OP group compared to the ANT group.

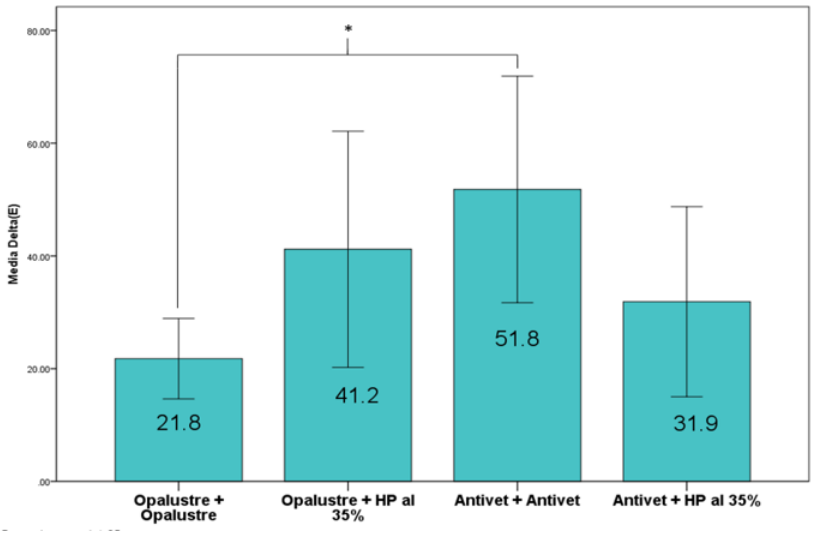

Figure 3: Mean of the ΔE value of the experimental groups.

Correlation graphs were made between variables L* and a* (Fig. 4) and variables L* and b* (Fig. 5). Fig. 4 shows that there is an association between the increase in L* and a decrease in a*

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of the ANT groups followed by OP+PH, as in Fig. 5, which shows the same trend with higher L* and a decrease of the b* value in the same groups.

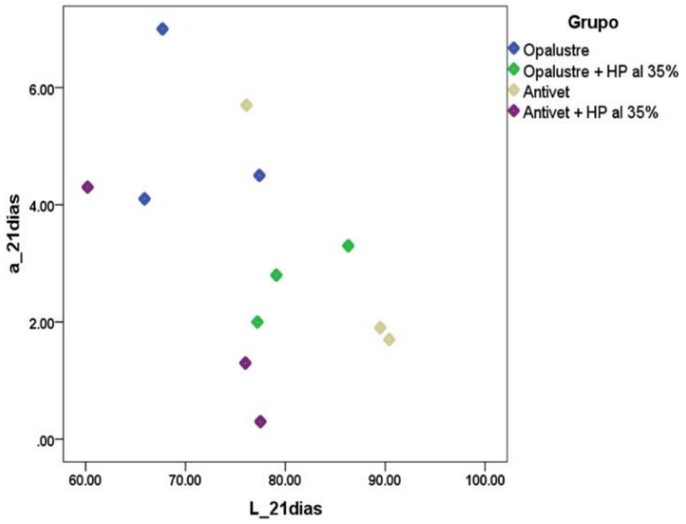

Figure 4: Pearson correlation of L* and a* values.

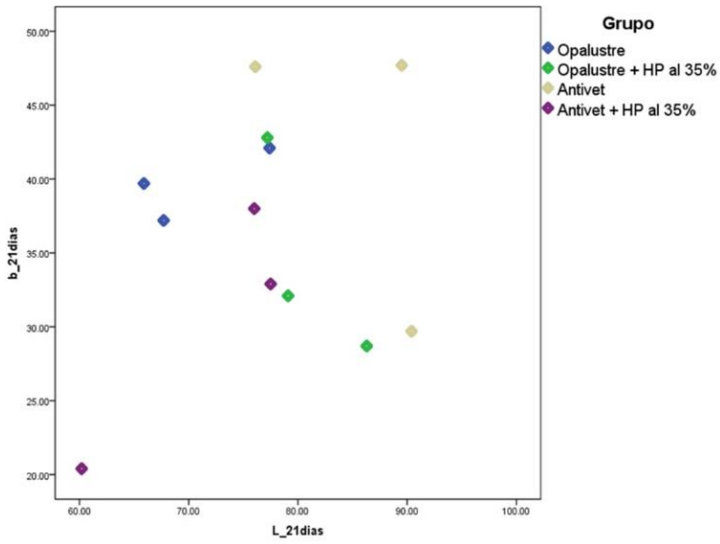

Figure 5: Pearson correlation of L* and b* values.

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DISCUSSION

The intake of excessive amounts of fluoride causes a developmental alteration (Bronckers et al., 2009) that makes the enamel more porous, as the fluorosis samples obtained for this study showed these characteristics.

Microabrasion is a procedure in which the enamel surface is smoothed with acid, and surface discoloration is removed by polishing with a material containing abrasive particles, a previous study described this technique using 18% hydrochloric acid mixed with pumice for the first time (Croll and Cavanaugh, 1986) Providing control over the amount of enamel that had to be removed, removing stains by abrasion and not by the dissolution of the acid. In the present study, microabrasion was the first treatment option for color correction in teeth with stains of uncertain depth. (Croll, 1989, Killian and Croll, 1990)

The same microabrasion methodology mentioned in other studies (Train et al., 1996; Celik et al., 2013) was practiced using a rubber cup and a low-speed piece with soft pressure for 60 s, depending on the severity of fluorosis. This application was performed ten times for moderate and severe lesions during the same session.

We agree that enamel microabrasion with Opalustre® turns out to be effective in removing enamel in superficial cases; however, teeth with moderate and severe fluorosis show more imperfect surfaces. It did not show changes in the removal of brown pigmentation and should be treated with alternative techniques. (Celik et al., 2013)

Enamel microabrasion improves dental appearance by micro reduction of the adamantine surface, so it should be placed before whitening to complement both techniques, which is why we perform the treatments in this order. (Croll, 1994)

Some studies (Ng and Manton, 2007; Ardu et al., 2007, Castro et al., 2014) matched with the results of the OP+PH group, where the brown stains were reduced, there was an improvement in the superficial color, but the stain due to fluorosis was not eliminated, even by combining the microabrasion and tooth whitening techniques.

Using SEM, the structural characteristics of different types of enamel with fluorosis were observed (Thylstrup & Fejerskov, 1979), showing a honeycomb pattern indicating that the rods had broken perpendicular to their long axes. In this study, the enamel also exhibited a highly irregular surface with ridges and valleys.

The OP group showed abrasion and erosion of the surface and the exposure of the underlying enamel prisms. In the ANT group, the enamel surface was eroded by acid, exposing the

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underlying enamel prisms and removal of the periphery of the prisms, much like the images shown in Olin's study. (Olin et al., 1988)

Through the SEM, two types of etching are demonstrated (Kendell, 1989) on the surface after the application of hydrochloric acid, Type 1: etching in the center of the enamel rods and type 2: etching of the peripheries of the enamel rods, coinciding with the images of the ANT and ANT+PH group in which we applied HCl but only in type 2 etching.

In the OP and OP+PH group (before whitening) the prismatic structure was affected, although the prisms had not been eliminated, only flattened on the surface, a concentration was formed in the interprismatic spaces. (Bağlar et al., 2015)

Another study (Zalkind et al., 1996) shows changes in the surface morphology of enamel, dentin, and cementum after applying whitening agents, causing extensive flattening and surface porosity, which we observe in the enamel of OP+PH and ANT+PH group images after PH application.

With the analysis of the mineral content of the human tooth through EDS, characteristic signs of phosphorus, oxygen, sodium, magnesium, and chlorine have been obtained (Machoy et al., 2016b); as long as in the present investigation, they also found of these minerals, carbon, fluorine, calcium, silicon, and zinc.

Coceska determined a statistically significant loss of Na and Mg after applying PH using this same measurement equipment (Coceska et al., 2016). In our study, there was a decrease in both elements, but it was not statistically significant.

In the present study, we saw a statistically significant loss of fluoride in all the experimental groups. An increase in calcium and phosphorus in the OP+PH, and ANT groups, being higher in the ANT+PH group (Ca: 36.9% and P: 15%) compared to the fluorosis control group (Ca: 14.1% and P: 9.3%), for the other experimental groups there was an increase in both elements, this is because the ANT and ANT+PH groups received a neutralizing substance at calcium hydroxide base. Also, the groups that received whitening showed a higher amount of P.

We could not compare these results using EDS since we have not found previous studies of mineral content in samples with type IV fluorosis.

The color was quantified in each group and evaluated using the CIE L* a* b* system, in which L* values range from 0 to 100, with 100 being the brightest, a* value from red to green, and b*, which goes from yellow to blue. The ΔE formula provided by the CIE (de l'Éclaraige, 2001) was used to calculate the color difference. The perceptibility criteria adopted by each author are

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different, so a ΔE value of 3.7 was accepted for a perceptible difference in the color (Johnston & Kao, 1989; Baglar et al., 2015)

After microabrasion, it was demonstrated (Bağlar et al., 2015; Akin et al., 2013; Paic et al., 2008) that positive ΔL* values indicate an increase in brilliance and negative values in Δa* and Δb*, indicating a change to more green and blue tones, respectively, as in this study in the OP group.

In the results of this study, it was found that there is a correlation between the L* values versus the a* and b* values, brightness and saturation (value and chroma) are inversely related (Magne and Belser, 2004), an increase in chroma produces a decrease in brightness.

A previous study (Bağlar et al., 2015) shows similar results to the present study, where the color change before and after (ΔE) of the application of all treatments is above the threshold value of 3.7 in which the change is perceptible.

There are no previous reports of changes in color with 21% hydrochloric acid or its combination with whitening. However, in this study, there was a statistically significant difference between the ΔE of the OP group compared to ANT, obtaining a p-value of 0.031.

CONCLUSIONS

Under the conditions previously described in the study, the following conclusions were obtained:

1. The hypothesis was accepted since Antivet® is more effective than other conservative treatments for the removal of stains due to type IV dental fluorosis.
2. After the application of 21% HCl in the ANT group, the loss of the aprismatic layer and an etched surface in which the periphery of the enamel prisms are removed is shown.
3. After the application of Antivet® with 35% Hydrogen peroxide, an etched surface is shown around the enamel rods, and slight erosion and porosity on the surface of the rods.
4. The analysis of chemical components revealed that the OP+PH, ANT, and ANT+PH groups presented a significantly higher concentration of Ca and P ($p<0.05$), this is attributed to the use of the hydroxide-based neutralizing solution of calcium in these groups.
5. There is a statistically significant difference in the initial ΔE value at 21 days of the experimental groups, showing a p-value of 0.016. A statistically significant difference was found between the OP group compared to the ANT group with a value of 0.031, the value being higher in ANT.

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