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HISTOLOGIC COMPARISON OF CONVENTIONAL AND MICROWAVE ASSISTED TECHNIQUES OF DECALCIFICATION OF TEETH

Oral Pathology

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ABSTRACT

The study of hard and soft tissues of teeth is of utmost importance for the histologists to study the development of teeth and their anomolies, pathologists for diagnosing the calcified tumors and recently of great help in forensic science. Decalcification of the hard tissues of the teeth and bone is necessary to study them. It is a process of complete removal of calcium salts from the tissues like teeth, bone and other calcified tissues. Microwave irradiation in domestic oven has been used for decalcification with notable time reduction and preservation of tissue architecture. The present study was done to evaluate the efficiency of different decalcifying agents and to compare conventional and microwave method of decalcification in preserving the soft tissue architecture.

KEYWORDS

Teeth, decalcification, acids and chelating agent.

INTRODUCTION

Decalcification of calcified tissues plays an important part in histological techniques.[1] It is a process of complete removal of calcium salt from mineralized tissues like teeth and bone and other calcified tissues making them suitable for section cutting and further histologic evaluation. Examination of demineralized sections of dental tissues and bone is necessary to study various pathological and developmental process.[2] Human dentition and bone has been the subject of intense histological investigations for many years. Dental pulp evaluation is often a part of research protocols followed in assessment of pulpal biological response to new restorative materials.

Hence the present study was undertaken to compare the efficiency of nitric acid, formic acid, hydrochloric acid + formic acid and ethylene diamine tetra acetic acid as decalcifying agents in conventional method of decalcification and also to compare the efficacy of nitric acid in microwave assisted decalcification in preserving the morphological architecture of the soft tissue.

In this study an attempt has been made to evaluate the action of different decalcifying agents on teeth with respect to preservation of soft and hard tissue architecture.

MATERIALS AND METHODS

The present study was conducted at Sri Sai college of Dental Surgery, Vikarabad. It comprised of 50 permanent teeth which were procured during routine dental extractions done in our college in the Department of Oral and Maxillofacial Surgery, washed and preserved in 10% formalin till decalcification was started.

Ethical clearance for undergoing the study was obtained from the Institutional Ethical Clearance Committee.

Decalcifying solutions used were (5% EDTA, 5% Nitric acid, 8% Hcl+8% Formic acid and 10% Formic acid)

The freshly extracted teeth which included incisors, canine, premolars and molars were collected. An access cavity was made on the occlusal aspect and 1/3rd of the apical end of the root was cut in order to facilitate pulp fixation. Teeth were then fixed in 10% formalin for a minimum of 2-4 days.[2,3] 10 teeth were placed in each of the decalcifying solution i.e. 5% nitric acid, 10% formic acid, 8% Hcl+ 8% formic acid, and 5% EDTA at pH 7.4 using conventional method of decalcification.

A Samsung 183 ST microwave with a maximum output of 850 W was used to decalcify another 10 teeth in 5% nitric acid.

Data analysis was carried out using SPSS software version 16. The level of significance was accepted at *P* values <0.001(Table 3). For statistical analysis ANOVA with post-hoc Tukey's test was used.

RESULTS

Conventional: Number of days taken for decalcification was found to be more with EDTA followed by formic acid, Hcl+formic acid and nitric acid. Hard tissue staining and clarity of dentinal tubules were found to be good in sections obtained using EDTA (figure 1) followed by nitric acid (figure 2), Hcl+formic acid (figure 3) and formic acid (figure 4).

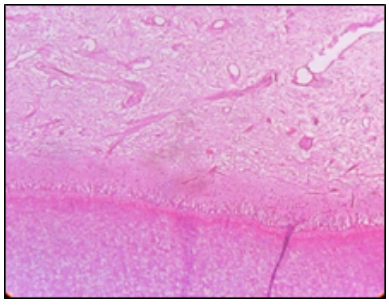


Figure 1: Tooth decalcified using neutral EDTA decalcifying solution under high power.

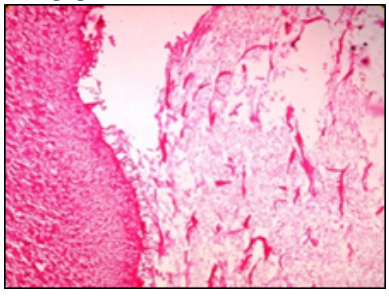


Figure 2: Tooth decalcified using 5% nitric acid under high power.

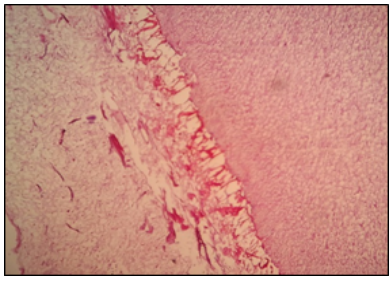


Figure 3: Tooth decalcified using 8% Hcl+8% Formic acid under high power

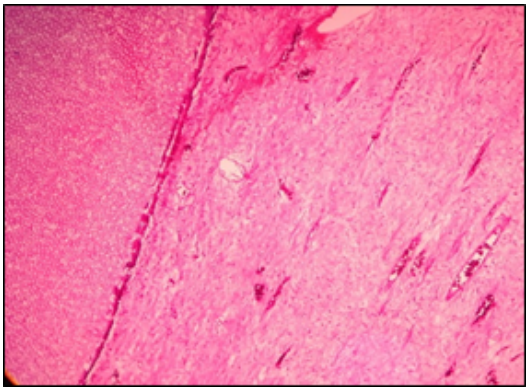


Figure 4: Tooth decalcified using 10% formic acid under high power

Pulp organization was found to be good in sections obtained by decalcification using both EDTA and formic acid than in nitric acid and Hcl+formic acid.

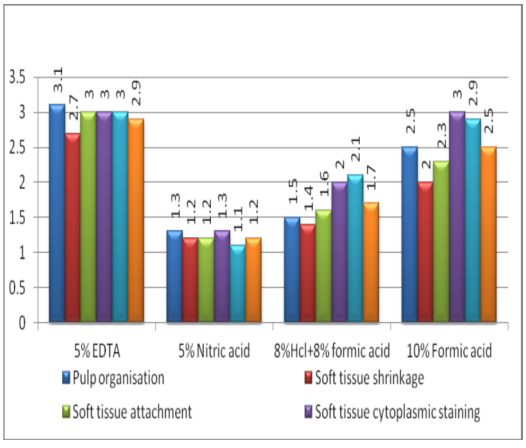
Soft tissue shrinkage soft tissue attachment, soft tissue cytoplasmic and nuclear staining were found to be better in slides obtained by decalcification using EDTA than in nitric acid and Hcl+formic acid. (Table 1)(Table 2)

Comparison between conventional and microwave assisted decalcification:

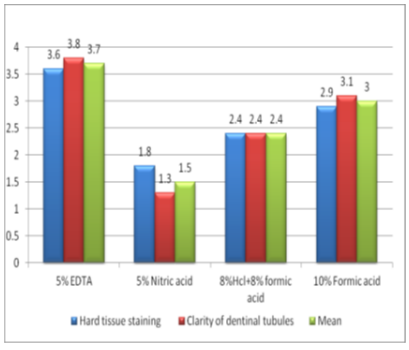
Number of days taken for decalcification, hard tissue staining, clarity of dentinal tubules, pulp organization, soft tissue shrinkage and attachment and soft tissue cytoplasmic and nuclear staining were found to be better in microwave method of decalcification using 5% nitric acid than in conventional method.(Table 3)

Table 1 & Bar Graph 1: Soft tissue evaluation of teeth decalcified by conventional method

Soft tissue evaluation	Decalcifying agent								p-value	Post-hoc test
	5% EDTA [A]		5% Nitric acid [B]		8%Hcl+8% Formic acid [C]		10% Formic acid [D]			
	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
Pulp organisation	3.1	.74	1.3	.48	1.5	.71	2.5	.85	<0.001 sig	A>B,C D>B,C
Soft tissue shrinkage	2.7	.67	1.2	.42	1.4	.70	2.0	.82	<0.001 sig	A>B,C
Soft tissue attachment	3.0	.95	1.2	.42	1.6	.70	2.3	.82	<0.001 sig	A>B,C D>B
Soft tissue cytoplasmic staining	3.8	.42	1.3	.48	2.0	.47	2.9	.32	<0.001 sig	A>B,C,D C>B D>B,C
Soft tissue nuclear staining	3.8	.42	1.1	.32	2.1	.57	2.9	.32	<0.001 sig	A>B,C,D C>B D>B,C
Total	16.40	3.03	6.1	1.73	8.6	2.67	12.6	2.72	<0.001sig	A>B,C,D D>B,C



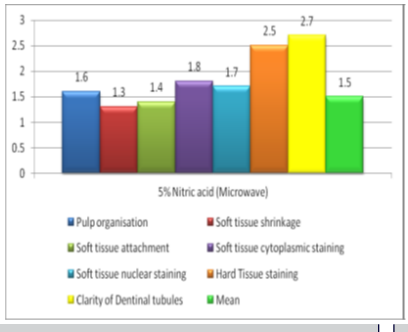
Hard tissue evaluation	Decalcifying agents								p-value	Post-hoc test
	5% EDTA [A]		5% Nitric acid [B]		8%Hcl+8 % Formic acid [C]		10% Formic acid [D]			
	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
Hard tissue staining	3.6	.52	1.8	.63	2.4	.52	2.9	.32	<0.001 sig	A>B,C,D D>B
Clarity of dentinal tubules	3.8	.42	1.3	.48	1.5	.53	2.5	.74	<0.001 sig	A>B,C,D C,D>B
Total	7.40	.84	3.10	.99	4.90	.99	6.00	.94	<0.001 sig	A>B,C,D C,D>B



ANOVA with post-hoc tukey's test; p value <0.001 Significant

Table 3 & Bar Graph 3: Comparison of soft and hard tissues of decalcified teeth between conventional and microwave assisted method

		Decalcifying agent				p-value
		5% Nitric acid (Conventional)		5% Nitric acid (Microwave)		
		Mean	SD	Mean	SD	
Days		11.30	2.21	9.50	1.43	0.045 sig
Hard tissue evaluation	Hard tissue staining	1.80	0.63	2.5	0.53	0.015 sig
	Clarity of dentinal tubules	1.30	0.48	2.7	0.48	<0.001 sig
Soft tissue evaluation	Pulp organisation	1.30	0.48	1.60	0.52	0.196
	Soft tissue shrinkage	1.20	0.42	1.30	0.48	0.628
	Soft tissue attachment	1.20	0.42	1.40	0.52	0.355
	Soft tissue cytoplasmic staining	1.30	0.48	1.80	0.63	0.062
	Soft tissue nuclear staining	1.10	0.32	1.70	0.67	0.025



Independent sample t test; p-value is borderline insignificant.

III. DISCUSSION

Decalcification is done to study the structure of tooth, pulp calcifications, evaluate the biological response of dental pulp to restorative materials.[4] Many surgical specimens contain calcified areas which need to be decalcified before processing and sectioning. Decalcification is commonly employed in most histopathology laboratories for the microscopic examination of teeth, bone and other calcified tissues.

Many authors have compared two to four decalcifying agents, sometimes varying the methods used and by employing a lot of permutations and combinations of the methods and agents used.

The choice of decalcifying agent used is largely dictated by the urgency of the procedure. Distortion and macerations are demonstrated by the use of chemicals. At a macroscopic level the tissue may appear unchanged, while microscopic evaluation reveals swelling, shrinkage, vacuolization, disruption, and fraying; not attributable to pathological conditions.

The present study was done to evaluate the effects of various decalcifying agents on human teeth in preserving the soft tissue morphology, to study the integrity of hard and soft tissues after decalcification and to compare the conventional and microwave assisted techniques of decalcification.

Decalcification achieved by using 5% nitric acid was the fastest where as EDTA took the longest time which was in accordance with the literature.

With respect to the integrity of hard and soft tissues and the staining qualities, results of our study revealed that pulp organisation was excellent in the teeth decalcified in neutral EDTA followed by formic acid, followed by Hcl +formic acid and then by nitric acid. Considering soft tissue shrinkage and soft tissue attachment the teeth decalcified using neutral EDTA exhibited best results than the ones decalcified in formic acid, Hcl+formic acid and nitric acid. In terms of soft tissue cytoplasmic and nuclear staining neutral EDTA and formic acid decalcified teeth stained the best followed by Hcl +formic acid and nitric acid.

Sanjai K et al (2012) in their study used 5% nitric acid, neutral EDTA, 10% formic acid and other acids. Their findings were in accordance with our study where in terms of efficacy of agents with respect to soft tissue integrity and hard and soft tissue staining excellent results were obtained with neutral EDTA. They also showed that 10% formic acid gave good results as it resulted in minimal soft tissue shrinkage and minimal loss of tissue, also in accordance with our study.[5] The overall superior results obtained by neutral EDTA may be attributed to the mechanism of capturing metallic ions like calcium which binds to the chelating agent.

Soft tissue attachment and soft tissue shrinkage as reported by **Zappa J et al (2005)** suggest that formic acid produced worst results in contrast to the results obtained from our study, wherein formic acid gave good results as it showed minimal soft tissue shrinkage after EDTA.[6] The better results of formic acid in our study can be attributed to the use of formic acid alone in 10% concentration whereas a combination of formic acid with 70% ethyle alcohol in 1:1 ratio was used in their study.

In a study done by **Singh S and Sicar K (2010)** on decalcification of teeth using 10% formal nitric acid, 10% formal formic acid and 5% formal EDTA, preservation of morphological characteristics was assessed on the basis of uniformity of staining and the clarity of dentinal tubules. EDTA was found to be significantly better than others in terms of clarity of dentinal tubules and uniformity of staining.[7] These findings coincided with our results too. The superior results obtained with EDTA can be attributed to the mechanism in which only the calcium ions from the external layer of apatite crystals are removed and are replaced by the ions from the deeper layer leading to the decrease in the crystal size gradually producing an excellent preservation of tissue components.

In our study using 5% nitric acid in microwave, the average time taken for decalcification was 9.5 days (mean) compared to conventional method which took 11.3 days (mean). In a similar study done by **Singh S and Sicar K (2010)** also stated that the decalcification time decreased considerably when microwave was used.[7] The reduction in time is attributed to the irradiation due to microwave, enabling better penetration and partly due to elevation of temperature resulting in better diffusion of decalcifying agent. Our findings are also in accordance with **Pitol DL et al (2007)** who concluded that the decalcification time was reduced with microwave, with preservation of tissue morphology and antigenicity.[8] Our findings also coincide with the study done by **Marr AL and Wong A (2009)** on optimal times for microwave decalcification of mouse and rat sternums and femurs and reported that decalcification of rat femurs with the combined use of microwave fixation and decalcification saved nearly 2 days of specimen turnaround times.[9]

In comparison to conventional method while using the microwave for decalcification, our study showed that clarity of dentinal tubules was best followed by hard tissue staining and soft tissue followed by cytoplasmic staining and nuclear staining. Even, pulp organisation, soft tissue shrinkage, soft tissue attachment, soft tissue cytoplasmic and nuclear staining were also better in microwave technique when compared to conventional method.

Our results are in accordance with the studies done by **Singh S and Sicar K (2007)** who used 5% nitric acid in microwave and conventional method and found that uniformity of staining and clarity of dentinal tubules was better in microwave than conventional technique. Our findings are also in accordance with the findings of **Maurine A.B.** who attributed the better staining and preservation of tissue structure in microwave assisted decalcification to irradiation of tissue, leading to better penetration of decalcifying fluid and also partly to elevation of temperature resulting in better diffusion of decalcifying agent.[7]

IV. CONCLUSION

To conclude the final impression led to the proposition that acids are better decalcifying agents when speed of decalcification is important. EDTA is indeed the best decalcifying agent currently available and should be advocated where time factor is relatively un-important. However, when the purpose of decalcification is the diagnosis and the surgeon is dependent on the pathologist for the biopsy report, a balance has to be struck.

It can also be concluded that the microwave assisted technique of decalcification was faster and also the uniformity of staining and preservation of soft tissue morphology were better in microwave assisted technique of decalcification when compared to conventional method of decalcification.

It can be comprehensively stated that the decalcification procedure depended largely on size of the specimen and the degree of mineralization. Thus it can be inferred that the choice of decalcifying agent and method would be largely dictated by the urgency of procedure, purpose of the study (either research or diagnostic), time and equipment available.

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