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A COMPARATIVE STUDY OF VARIOUS DECALCIFICATION TECHNIQUES

Pathology

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ABSTRACT

Decalcification is a process of complete removal of calcium salt from mineralized tissues like bone and teeth and other calcified tissues. The physical hardness, which is, a unique characteristic of these tissues makes it necessary to "soften" them by removing the mineralized component. Human dentition and bone has been the subject of intense histological investigations for many years. Demineralization or decalcification of tissues is a routine process carried out in most laboratories by the use of various acids or chelating agents.In the manual method of decalcification, hard tissues are placed in a decalcifying agent at room temperature with changes of the solution at regular intervals till the end point is reached. Aim: The aim of the study is to evaluate commonly used demineralizing agents & to identify the best decalcifying agent.Materials and Method : The present study included decalcifying solutions: 10% formal nitric acid, 8% formal nitric acid, 10% nitric acid. Sixty (n=60) natural teeth (twenty in each group) will be decalcified in each of the decalcifying solutions and subjected to chemical end-point test. The decalcified teeth will be then routinely processed, sectioned, and stained with hematoxylin and eosin stains. Statistical Analysis: One way ANOVA will be used and compartive analysis by chi square test.Result: Considering preservation and staining of both hard and soft tissues, superior results have been obtained by 10% HNO3, followed by 8% Formal nitric acid and 10% formal nitric acid.

KEYWORDS

Decalcification,10% Formal nitric Acid, 8 % Formic acid, 10% HNO3,Teeth.

INTRODUCTION

Teeth are formed of both organic and inorganic components. The major part of the tooth is made up of dentin forming the main part of crown and central core within the dentin is formed by soft tissue component called pulp which is a specialized loose connective tissue containing fibers, cells, blood vessels, nerve terminations and ground substance .1

Organic component of teeth can be studied in ground sections, but decalcification is required to study the organic components. 2 Decalcification is routinely used process in most histopathological laboratories for the microscopic examination of calcified tissues . The need of decalcification is to remove calcium salts from mineralized tissue, resulting in preservation of organic components.3 Decalcification using chemical solutions like acids and chelating agents, while preserving the organic portions.4So, an ideal decalcifying agent should be fast, and good .

Henceforth, we present here the comparative evaluation of different decalcifying agents with respect , the effect of decalcifying agents on the dental tissue, and its influence on the staining characteristics.

MATERIALS AND METHODS

The study was conducted in the department of Oral & Maxillofacial Pathology at Bhojia dental college and hospital, Baddi, Solan, India. Extracted, non carious, non attrited, 60 human permanent teeth including, incisor, canine, premolar . The access opening was done for each tooth, using a high speed carbide bur with air rotor and apical 1/3rd of root was cut, for better penetration of fixative agent and decalcifying fluid. 10 % formalin was injected, without pressure, inside the root canal to fix the pulpal tissue. The teeth were fixed in the formalin for one day. After that, the specimens were exposed to different decalcifying solutions: 10% formal nitric acid, 8% formal nitric acid, 10% nitric acid.

The study was divided into three sets: set I, set II and set III containing each decalcification solution. Decalcification was carried out at room temperature by suspending the teeth in the container with the help of a thread in such a way that the teeth were completely immersed in about 100 ml of the solution. Time at the start of decalcification was noted. The solutions were subjected to repeated agitation and replaced by freshly prepared solutions every 24 h. The end point of decalcification was measured by chemical method. After confirming the decalcification by all the three methods, the teeth were removed from solutions and washed under running tap water for 24 h.The sections were cut, processed and stained with normal H& E. The stained sections were observed under light microscope by three independent observers and graded from 0-2 (0 - Total loss of tissue architecture, 1 – Partially preserved tissue architecture, 2 – Well preserved tissue architecture) for soft and hard tissue. (FIGURE 1)

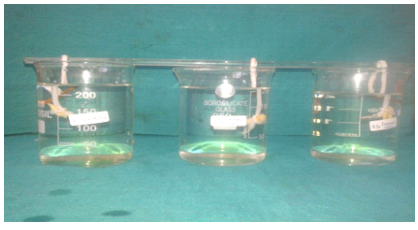

FIGURE 1: PLACEMENT OF TEETH IN DIFFERENT DECALCIFIED SOLUTIONS

The following criteria was considered

For hard tissue:

1 Preservation of dentinal structure
2 Clarity of dentinal tubules
3 Staining characteristics

For soft tissue:

1 Preservation of odontoblastic layer
2 Staining characteristics

RESULTS

Different decalcifying agents were evaluated and compared on the basis of selected parameters. Superior results were obtained with 10 % HNO3 > 8% FNA >10 % FNA (according to the respective mean values obtained by applying ANOVA test) . (Table 1: mean value comparison)

Table 1: mean value comparison

GROUPS	MEAN	SD	MIN	MAX	F VALUE	P VALUE
10% NITRIC ACID	94.04	15.54	64.00	120.00	6.76	0.0028*
10% FORMAL NITRIC ACID	77.88	17.17	48.00	100.00		
8% FORMAL NITRIC ACID	93.62	10.25	72.00	106.00		

Mean values of grading done by three observers for different decalcifying agents suggested that scoring for hard tissue preservation was better shown by 10 % HNO3 followed by , 8 % Formal nitric acid

and 10% Formal nitric acid. Similarly scoring for pulpal tissue was also in the same order. (Table 2 Dentin destruction, Table 3 Soft tissue preservation).

Table 2 Dentin destruction

DECAL. AGENT	PRESENT	ABSENT	χ2 VALUE	P
10% NITRIC ACID	4	16	0.53	.7672
8 %FORMAL NITRIC ACID	6	14		
10% FORMAL NITRIC ACID	5	15		

Table 3 Soft tissue preservation

DECAL. AGENT	PRESENT	ABSENT	χ2 VALUE	P
10% NITRIC ACID	17	3	3.24	.1979
8 %FORMAL NITRIC ACID	15	5		
10 %FORMAL NITRIC ACID	12	8		

Similarly, histopathological examination also revealed 10% NITRIC ACID staining better than 8 %FORMAL NITRIC ACID, 10 %FORMAL NITRIC ACID. Intercomparison has been shown in (FIGURE 2, FIGURE 3, FIGURE 4)

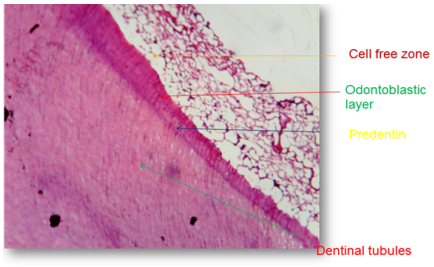

FIGURE 2: Histopathology of 10% NITRIC ACID

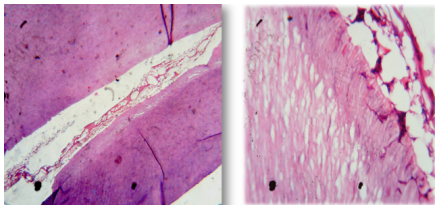

FIGURE 3 Histopathology of 8% Formal nitric acid

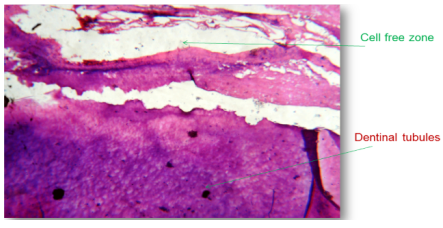

FIGURE 4: Histopathology of 10% Formal nitric aci

DISCUSSION

Decalcification is the commonly employed technique in most of the histopathology laboratories for the microscopic examination of calcified tissues including teeth and bones . In the present study, we have done comparison between three of decalcifying agents and found 10 % HNO3 better then 8% Formal nitric acidfollowed by10 % Formal nitric acid. The present study showed speed of decalcification was faster by 10% formal nitric acid hence causing more tissue destruction in comparison of 10% HNO3 which was compatible with the other studies by Geoffrey Brown and Bancroft which showed nitric acid (with formaldehyde fixative) to be the fastest decalcifying agent taking 2-3 days' time to decalcify Maurine William AB (1937) Reported that the time taken for decalcification by 5% HNO3 was 7-9 days for a single tooth , but in our study the time taken was less i.e. 3-5 days , this may be due to the increased concentration of HNO3 as the rate of decalcification also depends on the concentration used for the particular acid and varies accordingly.[5]

When efficacy was compared between 10% HNO3 and different strength of Formal nitric acid, no statistically significant difference was found but tissue integrity and staining was better observed with HNO3 ; the reason could be the rapid action of Formal nitric acid which can produce some alteration to the architecture of both hard and soft tissue components of tooth.3 Therefore, it is suggested that HNO3 should be preferred over Formal nitric acid .In contrast to our study, the study done by Zappa et al. HNO3 and FA were showing worst results after decalcification, for both hard and soft tissue components of tooth as compared to EDTA and other agents used in their study.6In contrast to our study where HNO3 was showing better tissue preservation and staining quality,the reason could be the rapid action of FNA which can produce some alteration to the architecture of both hard and soft tissue components of tooth.

CONCLUSION

Thus, for a decalcifying agent to be efficient it should preserve the tissue architecture with a reasonable speed of decalcification for the rapid diagnosis. So in our study HNO3 showed the most efficient result as it balances both tissue integrity and time factor making it clear that it can be used as a stable decalcifying agent for routine histopathological diagnosis.

However, further studies are required on a large sample size with consideration of individual factors to evaluate the effect of these agents on dental hard and soft tissues and to find a suitable decalcifying agent which provides reproducible results.

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