Evaluation of Different Decalcifying Agents on Oral Hard Tissues: A Comparative Study

Introduction Decalcification of bone and teeth is often an essential and important step during tissue processing. The rate of decalcification and the effect of various decalcifying agents on the tissue and their staining characteristics are two important parameters influencing the selection of decalcifying solutions. Some decalcifying agents, although they completely and rapidly remove the calcium ions also adversely affect the staining characteristics and may cause damage to the organic components. This study aimed to evaluate the efficacy of the commonly used demineralizing agents to identify the best decalcifying agent.

Materials and Methods Three decalcifying agents namely, 10% nitric acid, 10% hydrochloric acid and 10% formic acid were used to decalcify 30 natural teeth. The endpoint of decalcification was evaluated by physical and chemical methods. The decalcified teeth were subjected to routine processing and staining with hematoxylin and eosin stains.

Result Formic acid of 10% was the most considerate to the hard tissues and 10% hydrochloric acid was the least considerate to the tooth structure.

Conclusion Formic acid of 10% though being the slowest decalcifying agent, gave excellent results for soft-tissue integrity and staining characteristics.

KEYWORDS 10% nitric acid, 10% hydrochloric acid, 10% formic acid, decalcifying agents

INTRODUCTION

Decalcification is a process of complete removal of calcium salts from mineralised tissues like bone and teeth and other calcified tissues. It is carried out by chemical agents like acids to form soluble calcium salts or with chelating agents that bind to calcium ions.

The pulpal soft tissue can only be assessed in decalcified sections which otherwise in ground section is not possible as it is lost. Decalcification of hard tissues is one of the most important technique sensitive procedures in the histopathology laboratory.

Here we present the comparative evaluation of different decalcifying agents with respect to the rate of decalcification, effect of decalcifying agents on the dental tissue and its influence on staining characteristics.

AIMS AND OBJECTIVES

The aim of the study was (1) to evaluate the fastest decalcifying agent amongst the three, (2) to evaluate the staining characteristics of the teeth decalcified in the different decalcifying agents and (3) to compare and contrast the different decalcifying agents.

MATERIALS AND METHODS

Freshly extracted, non-carious, non-attrited, 30 natural teeth were obtained from patients. The teeth were fixed in 10% formalin and premolars and molars were used to analyse the three different decalcifying agents namely 10% nitric acid, 10% hydrochloric acid and 10% formic acid. Each decalcifying agent was used to decalcify these teeth separately.
Decalcifying agents were subjected to repeated agitation and replaced by freshly prepared agents daily. End point of decalcification of solutions was assessed using physical method of probing the teeth. The speed of decalcification was graded from 1–4 (1 - slowest and 4 - fastest).

All the teeth were washed under running tap water and continued with routine processing and staining with hematoxylin and eosin stain. The stained sections were then graded from 1–4 (1 - poor and 4 - excellent) based on the staining quality – both cytoplasmic and nuclear staining.

RESULTS

Data was entered into SPSS version (21.0) (IBM, Chicago). Descriptive statistics were applied to calculate mean and standard deviation for the continuous data and frequencies. For categorical data, intergroup comparison was done using one-way Anova and Krukal-Wallis test. P value ≥0.05 was considered statistically significant at 95% confidence interval.

Parameter 1
Speed of decalcification: 10% nitric acid decalcified teeth the fastest and 10% formic acid was the slowest (Table 1).

Parameter 2
Staining quality: 10% formic acid decalcified teeth stained the best and the teeth decalcified by 10% hydrochloric acid did not retain good staining characteristics (Table 2).

Overall, 10% formic acid scored over the other agents and 10% hydrochloric acid scored the least.

DISCUSSION

Decalcification is commonly employed in most histopathology laboratories for the microscopical examination of bone and other calcified tissues.

The criteria for a good decalcifying agents are:
1. Complete removal of calcium
2. Absence of damage to tissue or fibres
3. Non-impairment of subsequent staining techniques
4. Reasonable speed of decalcification

In the present study, we have compared the efficacy of three decalcifying agents, their rate of decalcification, effect on dental tissues and their staining characteristics.

The speed factor amongst the decalcifying agents was the highest with 10% nitric acid and lowest by 10% formic acid decalcifying solution (Table 1). In terms of effect on dental tissues and staining characteristics excellent results were actually obtained with the slowest decalcifying agent i.e. 10% formic acid (Fig. 1) and unsatisfactory results by 10% HCL (Fig. 2). Nitric acid of 10% also showed good staining characteristics (Fig. 3).

The quality of decalcified sections and its rate of decalcification depend on factors like concentration of

**Table 1** Number of days required for decalcification.

<table>
<thead>
<tr>
<th>Decalcifying agents</th>
<th>N</th>
<th>Mean</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>10% Nitric acid</td>
<td>10</td>
<td>4</td>
<td>0.667</td>
</tr>
<tr>
<td>10% HCL</td>
<td>10</td>
<td>11.9</td>
<td>0.737</td>
</tr>
<tr>
<td>10% Formic acid</td>
<td>10</td>
<td>21.5</td>
<td>0.971</td>
</tr>
</tbody>
</table>

*P value less than ≥ 0.05, considered to be significant.

**Table 2** Comparison of staining characteristics of different decalcifying agents.

<table>
<thead>
<tr>
<th>Decalcifying agents</th>
<th>Grades</th>
<th>Frequency</th>
<th>Per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitric acid</td>
<td>Fair</td>
<td>5</td>
<td>16.7</td>
</tr>
<tr>
<td></td>
<td>Good</td>
<td>5</td>
<td>16.7</td>
</tr>
<tr>
<td>HCL</td>
<td>Fair</td>
<td>2</td>
<td>6.7</td>
</tr>
<tr>
<td></td>
<td>Poor</td>
<td>8</td>
<td>26.7</td>
</tr>
<tr>
<td>Formic acid</td>
<td>Good</td>
<td>3</td>
<td>10.00</td>
</tr>
<tr>
<td></td>
<td>Excellent</td>
<td>7</td>
<td>23.3</td>
</tr>
</tbody>
</table>

*P value less than ≥ 0.05, considered to be significant.

Fig. 1 Tooth decalcified with 10% formic acid.

Fig. 2 Tooth decalcified with 10% hydrochloric acid.
Various methods have been published for determination of end point of decalcification which are (1) mechanically by running a sharp needle into the specimen, (2) weight (Lillie et al. 1951)\(^6\), (3) chemical method (Morse)\(^9\), (4) radiological method (Miles, 1949; Molenaar, 1957)\(^10,11\).

Verdenius and Alma in their investigation of decalcifying methods used weight loss as an indicator of the rate at which calcium salts are removed. Control of decalcification using weighing method has not been widely used\(^12\).

In a study done by Waerhaug, bone and teeth were decalcified rapidly under vacuum. By this method the time taken for decalcification was reduced to one-tenth\(^13\). By creating a vacuum the process of decalcification is characterized as:

\[
\text{Insoluble calcium salts + acids } \rightarrow \text{ soluble calcium salts + CO}_2
\]

The carbon dioxide disturbs the chemical equilibrium resulting in acceleration of the reaction.

Microwave-aided decalcification proved to be more effective than the traditional methods in aspects such as reduced time for decalcification, good preservation of tissue, staining efficacy and an increase of calcium release\(^4\). Pitol et al. (2007) showed that there was a 30-fold increase in decalcification speed compared to the traditional method when the material was irradiated in a microwave oven\(^14\).

In our study, 10% formic acid showed the most efficient results suggesting that it can be used as a stable decalcifying agent for routine histopathological diagnosis.

**CONCLUSION**

Formic acid though being the slowest decalcifying agent among the three agents used in the study, gave excellent results in both soft-tissue and hard-tissue staining. Hence it can be regarded as the best decalcifying agent where time factor is relatively insignificant.

**REFERENCES**