

## Comparison of Conventional and Microwave Bone Decalcification Methods by using 10% Nitric Acid: A Research Protocol.

### Abstract:

**Background:** In bony specimens, elimination of calcium is obtained by a method known as “Decalcification” . It is completed through the means of chemical agents such as acids, chelators etc that combine with ions of calcium. Decalcifying agent is used in regular conventional method where the hard tissue is placed at a room temperature (20- 250C) with modifications of the solution at orderly intervals until the final cutoff point is obtained. Usage of microwave oven for the process of decalcification is a new and fast method in contrast to the routine conventional method of decalcification. In this study, an attempt has been made to regulate and compare the conventional procedure of decalcification with decalcification done by microwave oven of hard tissue specimens by using nitric acid of 10% concentration with regards to decalcification speed, conservation of tissue architecture as well as productiveness of staining.

**Methodology:** This prospective analytical study, will include decalcification of 30 hard tissue specimens by microwave method and conventional method. The results will be compared in terms of decalcification speed, conservation of tissue architecture and staining productiveness. Necessary tests will be applied to analyze the data.

**Expected Results:** Significant advantages of microwave method are expected over conventional method of decalcification.

**Conclusion-** The conclusion will be drawn based on careful analysis of the results.

**Keywords:** Microwave, Bone, Decalcification, Collagen, Paraffin, Architecture, Staining.

### Introduction:

Preservation of bony tissue near to viable state is truly crucial for the comprehension of tissue information and functions. Sections which are cut by regular procedures to obtain fine sections is not possible in instances of specimens which include bones, odontomas, teeth and lesions which have undergone calcification. In these kind of bony specimens, elimination of calcium is obtained by a method known as “Decalcification” which construct the tissue smooth with the use of a instrument named microtome<sup>[1]</sup>

Decalcification of bony specimens is a maximum touchy procedure within side of the histopathology lab.<sup>[2]</sup> This procedure is usually accomplished by chemical retailers, such as acids which are inorganic to form salts of calcium which are soluble and chelator’s that combine with calcium ions<sup>[3]</sup>

The average duration taken for routine procedure of processing of tissue by Conventional Processing(CP) and routine staining is roughly 6-9 hrs and time duration taken for Conventional method is 24-41 hrs (based on the measurements of the specimen). In malignancy

cases, as the issuing of report has a time limit, the necessity of a faster process of tissue is required.<sup>[4]</sup>

For faster and accurate diagnosis several methods have been proposed with the usage of automated tissue processor. The standard of final specimen is improved, but the extent of time span stays constant i.e; 6-9 hours<sup>[4]</sup>. Temperature acceleration decreases the thickness of the fluid which is used for processing, permitting high speed entrance into the tissue. With the application of conventional heat to the processing fluid, it will result in irregular tissue penetration leading to in dappled staining<sup>[3]</sup>.

Some studies have suggested that the use of microwave oven cause a temperature elevation which results in enhancement of decalcification by the process of diffusion of the solution which is used for decalcification . Also few studies have suggested that the use of microwave oven do not expand the process of diffusion of the decalcifying solution but rather aid a greater deposition of calcium attributable to the already established magnetic field.<sup>[5]</sup>

Diffusion process inside the system will be increased by rise in the temperature. But, a highly elevated temperature(55-60<sup>0</sup>C) is harmful to tissue morphologically. If the calcium deprivation in the tissue happens too quick, it results in hydrolysis and swelling of calcified matrix. A great rise of temperature generated in microwave can be rectified by ice bath while fixing the samples.<sup>[5]</sup>

Decalcifying agent is used in regular conventional method where the hard tissue is placed at a room temperature(20- 25<sup>0</sup>C) with modifications of the solution at orderly intervals until the final cutoff point is obtained. Usage of microwave oven for the process of decalcification is a new and fast method in contrast to the routine conventional method of decalcification. In microwave method, bony tissues are placed in a microwave oven in the decalcifying agent for irregular periods with orderly modifications of the solution till the final cutoff point is arrived. Irradiation of the bony specimens in a microwave has appeared to fasten up the process of decalcification from few days to hrs<sup>[6]</sup>

In 1970 Mayers, proposed usage of microwave in laboratory. He promoted usage of microwave to speed up the hour span of tissue fixation.<sup>[4]</sup> Aside from reducing the time of diagnosis of tissue specimen, Microwave Decalcification (MD) has also eliminated the disclosure to possible toxins. Several workups have revealed that processing in the microwave results in minor amount of alteration in the nucleic acid.<sup>[7,8]</sup>

Microwave oven produces steady heat and increases the charge of tissue piercing maintaining the good calibre of the bony tissue specimen.<sup>[4,7,8,9]</sup> Altering electromagnetic fields are produced by non-ionizing radiation of microwaves which leads to the revolving of molecules which are dipolar like proteins as well as water. This produces molecular dynamics which leads to the causation of energy flux that will be continued till radiation come to an end.<sup>[8]</sup> Analysis showed that at ambient temperature, decalcification of bone by microwave technique is hastened approximately for 10 times in contrast with conventional method of decalcification.<sup>[10]</sup>

Various decalcifying agents can be used for decalcification process. The prime agent to be used will be based upon the emergency of technique. When various chemicals are use they may result in deformation and maceration of tissues. the tissue appears unchanged grossly, but under

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microscopy it shows shrinkage, disruption, swelling and vacuolization which are not related to pathological conditions.<sup>[11]</sup>

The decalcification agents which can be use are formal nitric acid of 10%, Ethylenediaminetetraacetic acid, formal nitric acid of 8 %, perenyi's fluid , etc. Ethylenediaminetetraacetic acid is the slowest agent but the sections can be taken easily and are also easy to handle and not fragile .Formal nitric acids of 8 and 10 percentages are usually the fastest decalcifying agents, easy to handle and dissections can be taken easily. 8% formic acid may result in friability of the tissue. the specimen decalcified with perenyi's fluid may result in over staining and under staining of sections, difficulty in sectioning and the effect of chromic acid and nitric acid may result in tissue friability <sup>[3]</sup>.

A perfect decalcifying agent must 'be quick', 'be superior' and should 'be satisfactory'. An ideal agent must make certain of total detachment of calcium, and should result in mild harm to tissue, and should not lead to any staining impairment and should decalcify the tissue at reasonable speed.<sup>[6]</sup>Some decalcifying agents may take out the ions of calcium completely but they can damage these tissue components and also affect the staining characteristics.The rate of decalcification and the quality of sections which are decalcified relies upon various factors such as temperature, fixation concentration of the agent used for decalcification, pressure blending microwave radiation, electric current, tissue suspension, size of tissue and type of tissue.<sup>[3,12,13]</sup>

The cutoff point for the process of decalcification can be assessed through various methods as follows:

- 1) Radiographically where if the opacity suggests incomplete decalcification .
- 2) Physical method of probing the bony specimen with a needle .
- 3) Chemical methods by using the strong liquor ammonia.<sup>[3,14]</sup>

In this study, an attempt has been made to regulate and compare the conventional procedure of decalcification with decalcification done by microwave oven of hard tissue specimens by using nitric acid of 10% concentration with regards to decalcification speed, conservation of tissue architecture as well as productiveness of staining.

### **Aim & Objectives**

The aim and objective of present study is to regulate and compare the conventional procedure of decalcification with decalcification done by microwave oven of hard tissue specimens by using nitric acid of 10% concentration with regards to decalcification speed, conservation of tissue architecture as well as productiveness of staining.

**Comment [DOS5]:** Repetition of previous information.

### **Materials and Methods**

Place of study - Division of Histopathology, Department of Pathology, Jawaharlal Nehru Medical College, Sawangi, Wardha.

Study duration - 2 years.

Study design- It is a prospective analytical study.

Sample Type-Hard tissue specimens received in Division of Histopathology.

### **Techniques.**

1.Steps for conventional method of decalcification are as follows-

- i. Hard tissue specimens are to be collected.
- ii. The specimens will be labeled properly.
- iii. These bony specimens will be immersed in 10% nitric acid for decalcification process.
- iv. The solution will be changed periodically till the bony tissue gets softened.
- v. The duration of the entire process will be recorded.
- vi. These specimens will be exposed to routine tissue processing as well as respective staining.

2.Steps for microwave method of decalcification are as follows-

- i. Hard tissue specimens will be washed with water for 10 minutes.
- ii. These specimens are then put in a container consisting of 10% Nitric acid.
- iii. This container is kept in domestic microwave oven and set for one-minute cycle at 700 W.
- iv. After 1 minute of cycle, the bony specimen will be taken off outside and settled to cool off for 45 minutes.
- v. This entire process is repeated for several times (average- 7 cycles/day).
- vi. The nitric acid solution is changed every 3 hrs. till decalcification is achieved.

### **Inclusion Criteria**

All hard tissue specimens received in Division of Surgical Pathology.

### **Exclusion Criteria**

All soft tissue specimens received in Division of Surgical Pathology.

### **Expected Outcome/ Results**

In this study, as we are comparing and contrasting two different decalcification methods with respect to speed of the procedure for completion of decalcification process, the ability to preserve the tissue architecture, the staining productiveness and good morphology under microscope, and also as microwave oven is a novel method and also has all advanced characteristics for decalcification process, we are expecting a better outcome with use of microwave oven when compared to conventional method. This will be drawn after the proper assessment of study conducted after the clearance from the institutional ethics committee.

### **Discussion**

Decalcification process is a time taking process. Many specimens contain numerous calcified areas which needs to be decalcified before the tissue processing and sectioning. Usually it takes several weeks to decalcify a hard tissue specimen. This process is based on the quality and rate of demineralization. **the application** of microwave energy in the form of nonionizing radiation results in rotation of water and proteins (polar side chains). This molecular kinetics result in production of energy variability<sup>[15]</sup>.

R Sangeetha et al (2013) did a study and have concluded that decalcification done in microwave is quicker than conventional method. And also the tissue conservation and staining productiveness was better in microwave procedure of decalcification compared to conventional method of decalcification method.<sup>[15]</sup>

A novel method using a domestic microwave oven has seen to speed up the decalcification method. The microwave have been proved to be functional in decreasing the time which is necessary for decalcification. The energy which is produced by microwave combines with molecules which are dipolar by transmitting kinetic energy as well as alteration of electric field<sup>[5]</sup>.

A Thirumal Raj et al (2016) in a study comprising of 240 hard tissue specimens have concluded that a combination of both microwave and conventional decalcification is affective to speed up the time spent in laboratory with minute settlement over quality of the tissues.<sup>[16]</sup>

Archana Srivasyaiah et al (2016) in a study comprising 72 premolars and the decalcifying agent used were trichloroacetic acid, nitric acid, and formic acid with concentrations of 5% and 7%. They have concluded nitric acid of 5% concentration used in microwave method demonstrated as the perfect agent due to its speed. This also resulted in fine histological details and characteristics if various stains used.<sup>[17]</sup>

Ahmad Danish Rehan et al (2017) did a study comprising 30 hard tissue specimens and the decalcifying solutions used were diluting 5% of formic acid, 5% of nitric acid, 14% of EDTA. The study concluded that usage of nitric acid of concentration 5% was the quick agent when used in microwave oven for the decalcification process. The results also demonstrated that the structural features and better staining productiveness were good when 5% nitric acid is used for decalcification as when compared to other solutions.<sup>[18]</sup>

Sanjay k et al (2012) conducted a study using neutral EDTA, 10% of formic acid, 5% of nitric acid, and other acids. They have concluded that neutral EDTA showed better soft tissue coherence and good staining of hard bony tissues as well as soft tissue. They also concluded that minimal shrinkage of soft tissue and minute tissue loss is shown by 10% formic acid.<sup>[19]</sup>

Singh S and Sicar K (2010) conducted a study and they have demonstrated that preservation of morphological characteristics is based on staining uniformity. The various decalcifying agents used in this study were as follows-10% of formal formic acid, 10% of formal nitric acid, and 5% of formal EDTA. They have concluded that EDTA is found to be much better when compared to others with respect to uniformity of staining. They also stated that

the decalcification time is reduced when microwave was used.<sup>[20]</sup> Few of the related articles were reported<sup>[21-37]</sup>.

In the present study, for decalcification of hard tissue specimens, microwave method, and conventional method will be done. The results obtained by these methods will be compared in regards to decalcification speed, conservation of tissue architecture as well as productiveness of staining.

### **Limitations**

Few limitations which will be there in the study are improper biopsy technique, inadequate biopsy material and improper tissue processing and staining.

### **Conclusion**

It will be discussed and conclusion will be drawn in correlation with observation and results.

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