INTERNATIONAL JOURNAL OF MULTIDISCIPLINARY RESEARCH AND ANALYSIS

ISSN(print): 2643-9840, ISSN(online): 2643-9875

Volume 08 Issue 01 January 2025

DOI: 10.47191/ijmra/v8-i01-15, Impact Factor: 8.22

Page No. 117-122

Von Ebner's Fluid for Decalcification of Osteoma: Case Report

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ABSTRACT: Osteomas are benign osteogenic tumors composed of mature bone tissue, often asymptomatic and diagnosed incidentally during imaging for unrelated conditions. Histological evaluation is essential for accurate diagnosis, with decalcification being a critical step in processing calcified bone tissue for microscopic analysis. This case report presents the challenges encountered in decalcifying a mandibular osteoma from a 21-year-old female patient. The patient exhibited localized pain and swelling, and imaging revealed a well-defined exophytic bony lesion on the left mandible. Initial decalcification attempts using formic acid, hydrochloric acid (HCl), and trichloroacetic acid were ineffective in adequately softening the specimen. The decalcification process was subsequently optimized using Von Ebner's fluid, which successfully softened the osteoma and preserved its histological integrity. Microscopic examination revealed trabeculae of mature lamellar bone surrounded by a vascular fibrous stroma, confirming a diagnosis of cancellous osteoma. This case represents the first documented application of Von Ebner's fluid for decalcifying human mandibular osteoma, demonstrating its superior efficacy in preserving tissue structure while facilitating sectioning. These findings highlight Von Ebner's fluid as a potentially valuable decalcifying agent for challenging bone specimens, with implications for improved histopathological analysis in osteoma diagnosis.

KEYWORDS: Osteoma, mandibular osteoma, decalcification, Von Ebner's fluid, histopathology, case report, cancellous osteoma, bone tumor.

INTRODUCTION

Osteoma are non-malignant tumors formed from mature bone cells, They are commonly asymptomatic and discovered by chance during scans for unrelated conditions. Osteomas can develop in various locations within the skull, including the jawbone and paranasal sinuses [3,2]. Typically there are three main types of osteoma; compact osteoma , spongy(Cancellous) osteoma osteoma and combined osteoma. Compact osteoma is composed of very dense bone material, while Spongy osteoma, resembles normal bone and often contain bone marrow. Combined osteoma, also known as, mixed osteoma, which have characteristics of both compact and spongy bone [1].

Histological techniques are crucial diagnosing osteoma. The Histological techniques applied for diagnosing osteoma involves careful tissue collection, fixation, decalcification (if necessary), processing, sectioning, and staining to ensure accurate identification of the tumor's structure. Histological evaluation typically reveals well-organized bone tissue, confirming the benign nature of the osteoma[5]. It should be highlighted, that . Tissue decalcification is considered a vital step in preparing bone tissue for histological examination, particularly when dealing with mineralized tissues like osteoma. The decalcification process includes removing calcium salts from bone to make it soft enough for sectioning without altering the tissue structure. There are various methods for decalcification, each with its advantages and disadvantages.

There are several types of tissue decalcification which are acid decalcification, chelating agent decalcification, electrolytic decalcification and combination methods. In acid decalcification strong acids, such as hydrochloric acid (HCl) or formic acid (CH_2O_2), are used to dissolve calcium salts from the bone. The acids work by breaking down the calcifacted calcium phosphate

($Ca_3(PO_4)_2$) in the bone, making the calcifacted $Ca_3(PO_4)_2$ pliable enough for sectioning. Acid decalcification is a quick method and can decalcify tissues in hours to a day, depending on the size and thickness of the specimen. However, acid decalcification might lead to over-decalcification that can cause tissue damage, and the acid may interfere with subsequent staining, particularly with hematoxylin and eosin (H&E) stains [6].

Chelating agent decalcification requires Chelating agents, for example, ethylenediaminetetraacetic acid (EDTA) to bind to calcium ions in the bone and removing them through a process of ion exchange. Although, this method is slower than acid decalcification as it can take few days, chelating agent decalcification causes less tissue damage and maintains better structural integrity of the specimen compared to acid decalcification. Thus, Chelating agent decalcification in general, EDTA decalcification in particular is often used when immunohistochemistry or other delicate staining techniques are required. The main drawback of Chelating agent decalcification is the limited effect on large and thick specimens [7].

Electrolytic decalcification is applied to prepare large bone specimens as it uses electric current to accelerate the removal of calcium salts from the bone. The specimen is placed in a solution containing a decalcifying agent, the electric current is passed through it to increase the rate of decalcification. The main disadvantage of Electrolytic decalcification is the complexity of the technique and the risk of tissue damage which limits the usage [8].

Combination Method combines both acidic and chelating decalcification methods are to optimize the decalcification process. This may involve initial treatment with acid, followed by chelation with EDTA to ensure thorough decalcification without damaging tissue integrity. Combining both methods can provide the speed of acid decalcification with the gentleness of chelation, offering an optimal balance for certain specimens. Combination Method Requires careful monitoring to avoid over-decalcification or incomplete decalcification.

Moreover, gentle decalcification can be achieved via Von Ebner's fluid, typically composed of HCl and sodium chloride (NaCl), which is advantageous when preserving delicate tissue structures in bone specimens. It is widely used in histological labs where the preservation of tissue morphology is crucial, as it minimizes tissue distortion during the decalcification process. However, the time required for decalcification with Von Ebner's fluid can vary depending on the size of the specimen and the concentration of the reagents [9].

CASE REPORT

Patient information and medical history.

A 21-year old female was referred for evaluation after been diagnosed. The patient was suffering from localized pain and swelling. The patient had no significant past medical history, and there was no known genetic medical history. The clinical examination showed a left maxillary sinus mucosal thickening, the rest of sinuses & both OMC were unremarkable. Also, the epiglottis and epiglottic folds were normal and the vascular and osseous structures in the neck were intact. A dense sclerotic bony lesion seen at the left half of the clivus was noted, alongside homogenous enhancement of submandibular and parotid glands **(Figure 1).**

Furthermore, the medial impression demonstrated a left mandibular exophytic bony lesion with subtle periosteal reaction, no soft tissue component or local invasion, associated with multiple bilateral deep cervical lymph nodes, features could represent (Osteochondroma/non-ossifying fibroma, however, osteosarcoma could not be ruled out owing to the subtle periosteal reaction), for histopathological correlation.

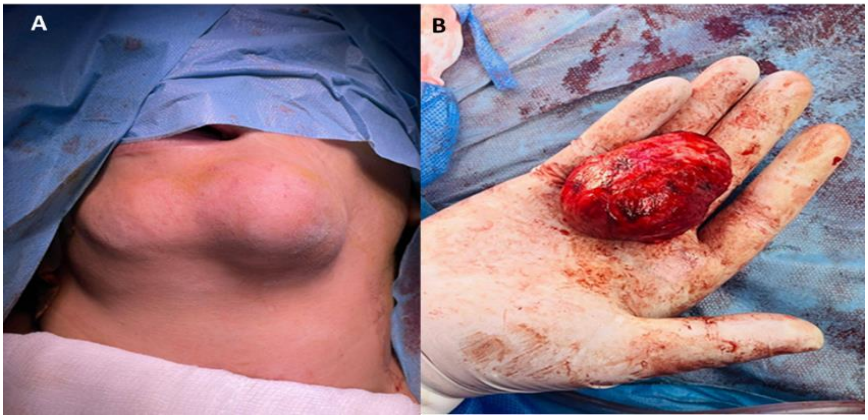

Figure 1: Images of left mandibular exophytic bony lesion with subtle periosteal reaction (A) before extraction and (B) after extraction.

CT-Scan:

A well-defined exophytic bony lesion, seen arising from the lower aspect of the outer alveolar surface of the left mandible with extension from the left premolar to the ipsilateral wisdom tooth & compressing the ipsilateral buccal space. the lesion appeared incompletely covered with cortex having a subtle periosteal reaction however the medulla is seen continuous with the medulla of the mandible, and no evidence of extra-osseous soft tissue components or local invasion was observed.The lesion measures approximately (5.8x4x4.8cm) (APxWxCC).

bilateral deep cervical lymph nodes, involving almost all levels (la, lb, lla, lib, III, bucco-facial& occipital), averaging in long axis about (1-2cm). ely enlarged complex right thyroid lobe with cystic component, measures 6x5cm) (APxWxCC), causing mass effect with partial tracheal narrowing & alateral shifting, as well as subtle retro-sternal extensions.

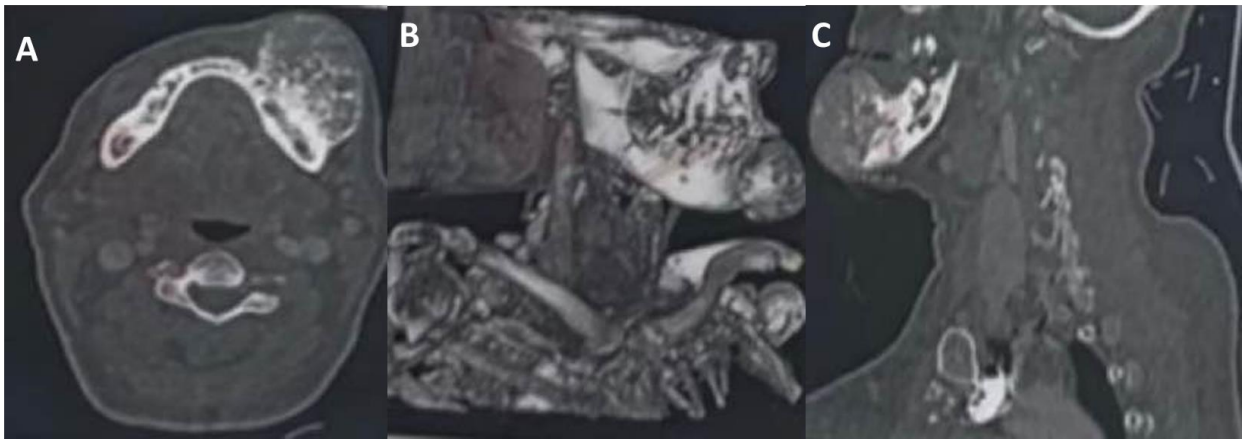

Figure 2: Images from (A-C) illustrates CT-Scan images of the presented of left mandibular exophytic bony lesion with subtle periosteal

HISTOPATHOLOGY

Formic acid was applied for 7 days with daily changes but it was insufficient compared to the hardness of the sample. After that, 10% HCl was applied and it was ineffective. Trichloroacetic acid was also applied for 3 days and was not effective. When von Ebner's fluid was applied, it became soft and ready for gross and cutting.

Specimen was obtained left mandible, bony mass Excisional Biopsy. The microscopic examination shows trabeculae of mature lamellar bone surrounded by vascular fibrous stroma.

Figure 3: Image (A) shows the lesion prior to adding von Ebner's fluid. Image (B) shows the lesion post adding von Ebner's fluid.

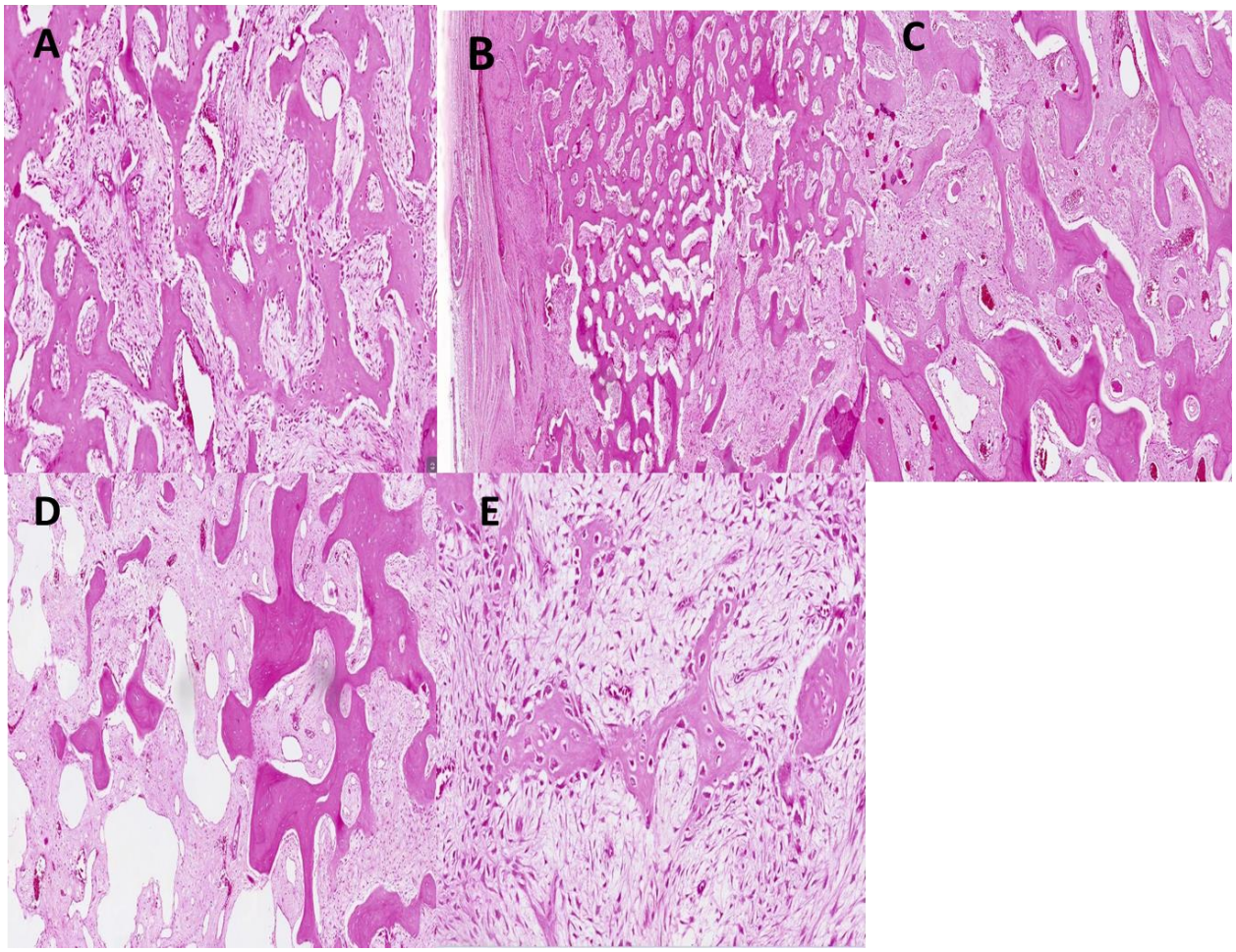

Figure 4: The histopathology analysiss indicates that the patients had Cancellous Osteoma. Images from (A-E) illustrates trabecular of mature lamellar bone surrounded by vascular fibrous stroma.

Ethical Approval and Patient Consent Statement:

This study was approved by the Ethics Committee at Al-Jalla Hospital, Benghazi, Libya.

The patient was fully informed about the purpose of the research and the potential publication of their clinical case in a medical journals. Written informed consent was obtained, ensuring the patient understanding of the use of their clinical data and images for academic and research purposes. All efforts were made to maintain confidentiality and anonymity throughout the process.

DISCUSSION

The main challenge in histological analysis of boney lesion is preparing the sample in short time as decalcification of tooth and boney lesion is a time-consuming procedur that might take days of not weeks [10]. Persevering the histological structure and morphology of tooth and boney tissues is another obstacle to tackle. In this case three different acid decalcification techniques were used on Osteomas specimen via adding Formic acid, HCl and When von Ebner's fluid.

Even though, Formic acid was proven to be functional decalcification for tooth as demonstrated by Khangura et al [11]. which indicated that tooth specimen exposed to Formic acid were easy to section. However, the time taken for decalcification tooth specimen was approximately 22 days. Which is in agreement with this report as treating Osteomas specimen with Formic acid for 7 days was not effective in this case to soften the calcified specimen. In contrast to the current observation Savi et al. [12] animal study illustrated 10% formic acid can decalcify Rat Mandibles almost 5 days post exposure. This contradiction might due to the fact that human mandibular Osteomas is significantly calcified and harder than Rat Mandibles 10-20 mm and 1.2 mm subsequently [13,14].

Hydrochloric acid is thought to be a strong inorganic solvent and rapid decalcification agent particular for bone tissue as illustrated by Cornelison et al [15]. as HCl provided the fastest decalcification time of cranial fracture sample, almost 3.57 days, compared to nitric acid and EDTA, 10.35 days and 78.97 days respectively. These results are in agreement with Salih. 2020[16]. In vivo study as

both HCl had a rapid decalcification rate for of the bone tissue affected with mycetoma infection. These experimental findings are countered in this case report where HCl produced no significant impact on mandibular osteoma specimen. This observation could be aligned with Sabolová et al [17] trial demonstrated that the HCl decalcification reaction varies among anatomical regions.
The best decalcification observation in current case was obtained after treating the sample with Von Ebner's solution. The hard and large osteoma converted into soft and cuttable lesion when exposed to Von Ebner's. The decalcification effect of Von Ebner's solution is evidenced by several studies were Von Ebner's solution was applied on different anatomical regions. [18] It should be highlighted that to the current state of knowledge this case report is the first to demonstrate the functional effect of Von Ebner's solution on preparing mandibular osteoma specimen for histological analysis. Thus, further studies are required investigate the effectiveness of Von Ebner's solution on varies osteoma samples and on optimizing the protocol for Von Ebner's solution to balance decalcification efficiency with tissue preservation and explore its comparative effectiveness with other decalcification agents.

CONCLUSION

This case is the first to compare the effectiveness of Formic acid, HCl and When von Ebner's fluid on decalcification human mandibular Osteomas. Von Ebner's solution outperformed both Formic acid, HCl as Von Ebner's solution successfully softened the specimen, preserving tissue structure and enabling sectioning.

ACKNOWLEDGMENTS

The authors extend their sincere gratitude to Dr. Hoda Mohammed and Dr. Malek Idris Al-Mahdawi at Al-jalla Hospital for their invaluable clinical support and insightful contributions to this study. We also wish acknowledge the Department of Histopathology at the Medical Center of the Libyan International Medical University for their meticulous histopathology analysis, which were crucial in establishing the diagnosis.

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