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# Identification of mature and immature (osteoid production) in ossifying fibroma by Tetrachromic verdeluz orange G-acid fuchsin (VOF) stain versus hematoxylin and eosin stain

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**Abstract**

**Aim of Study**: Identification of mature and immature (osteoid production) in ossifying fibroma using histochemical study by hematoxylin and eosin (H&E) and tetrachromic verdeluz orange G-acid fuchsin (VOF) stains.

**Materials and Methods**: Forty five tissue sections divided into three groups made up the study's data. Twenty portions from the archives, which made up Group 1, exhibited ossifying fibroma, group 2 comprised fifteen psammatoid juvenile ossifying fibroma sections and group 3 comprised ten section of trabecular juvenile ossifying fibroma. Each block was divided into two 5 m sections, with one section stained with hematoxylin and eosin (H & E) and the other with the tetrachromic Verde Luz-orange G-acid fuchsin (VOF). Histomorphometric examination of tissues is based on quantitative measurements of microscopic osteoid production and has been used to provide information on tissue pathology in these lesions

**Results**: The results showed female predominance in OF cases comprising (70.0%).Whereas male predominance found in psammatoid and trabecular juvenile ossifying fibroma comprising (60.0%), (70.0%) respectively .Ossifying fibroma and juvenile ossifying fibroma were distributed between the maxilla and mandible in the following ways: (65.0%) of OF were in the mandible, (73.3%) of PsJOF were in the maxilla, and (60.0%) of TrJOF were in the mandible. By using ANOVA test however the result non-significant OF show high mean value OF osteoid followed by PsJOF then TrJOF in conventional H&E while in VOF stains PsJOF showed high mean value followed by OF and finally TrJOF. Using paired t-test H&E showed a high mean values of osteoid in OF, TrJOF and PsJOF, compared to VOF stain, however statistically non-significant compared mean value of osteoid between H&E stain and VOF in TrJOF there was a border line significant (0.055).

**Conclusion**: Our awareness of these lesions and our ability to distinguish and identify them is improved by a study of the histological characteristics of ossifying fibroma and its subtype. H&E staining necessitates numerous steps, whereas VOF only needs one step.

**Key word**: ossifying fibroma, osteoid, hematoxylin and eosin stain ,VOFstain.

## Introduction

A broad, fascinating, and difficult collection of disorders known as fibro-osseous lesions of the craniofacial complex provide challenges for both categorization and therapy[1]. The two types of ossifying fibroma are among the most perplexing benign fibro-osseous diseases both juvenile psammomatoid fibroma (JPOF) and juvenile trabecular fibroma (JTOF)[2]. When the typical clinical, radiologic, or pathologic characteristics of an ossifying fibroma are not present, overlapping characteristics amongst variants may be observed. The difficulty in diagnosing these lesions lies in both the fibrous stroma and the bone trabeculae.

For the diagnosis of such lesions, it is also essential to recognize all varieties of calcified formations in their early mineralization stages[3]. The precise histochemical nature of mineralized deposits must be determined since fibro osseous lesions have a high rate of recurrence and an aggressive potential; otherwise, "H and E" stain would only provide a diagnosis based on morphological characteristics, which may be deceptive[4,5].Histologically, fibrous connective cellular tissue and osteoblasts predominate over osteoclasts in ossifying fibromas, they are not enclosed, but the nearby bone effectively confines them. Myxomatous compositions are related to pseudocystic degeneration. Hemorrhagic regions and multinuclear large cells are frequently observed. Large, irregular osteocytes are housed in irregular agglomerates of highly cellular osteoid that are seen in trabecular ossifying fibromas. The psammomatoid form has spherical ossicles and concentric lamellas. These come in a variety of shapes and have basophilic centers with osteoid eosinophilic peripheral edges[6]. Osteoid is an organic tissue that has not yet undergone mineralization but eventually deposited as lamellae or layers in the bone matrix [7].

For decalcified bone sections, the common histological method of hematoxylin and eosin (H & E) does not clearly distinguish osteoid from calcified bone, lamellar bone and woven bone. Furthermore, pink coloration in both soft tissue and calcified tissue is possible. A potential method for distinguishing elements of both hard structures and soft tissues is the tetrachromic Verde Luz-orange G-acid fuchsin (VOF) stain[8].

## Materials And Methods

We received approval for our research from the institutional ethical review board. The Oral Pathology Laboratories/Oral Diagnosis Department at the College of Dentistry, University of Baghdad provided the samples for this retrospective analysis, and the Histopathological Laboratory in Al-Shaheed Ghazi hospital for specialized surgeries that were dated from the period of 2018 to 2020.

Forty five tissue sections divided into three groups made up the study's data. Twenty sections from the archives, which made up Group 1, exhibit ossifying fibroma, group 2 comprised fifteen psammatoid juvenile ossifying fibroma sections and group 3 comprised ten section of trabecular juvenile ossifying fibroma that were inspected for the presence of tumors. Each block was divided into two 5 µ pieces, one of which was stained with hematoxylin and eosin (H & E) and the other with the tetrachromic Verde Luz-orange G-acid fuchsin (VOF ).

### Preparing for VOF

To create the VOF stain, a 260 mg of light green, 140 mg of methyl blue, 500 mg of orange G , and 600 mg of acid fuchsin were dissolved in 100 ml of boiling distilled water. After cooling, 3 ml of glacial acetic acid and 1.5 g of phosphotungstic acid were added. 200 ml of Sigma pure ethanol was

then added. Prior to staining, solutions were made.

**Staining technique:**

All slides were deparaffinized, rehydrated for five minutes using a declining ethanol scale, and then washed for ten minutes in water. The sections were first stained with Harris' hematoxylin, then washed in water and dipped in acid alcohol, and then submerged in lithium carbonate for bluing then transferred to VOF stain about 4 minutes and 40 seconds then the slides were dehydrated in 70% and 90% ethanol, after that they washed with xylene, and mounted with DPX.

On the slides, hard tissue elements were present, and it was possible to see how they differ from the stroma. According to the histologic and morphologic traits of the mature and immature osteoid that were present, pathologists made observations and grouped the structures.

**Histomorphometric examination**

Histomorphometric examination of tissues is based on quantitative measurements of microscopic osteoid production and has been used to provide information on tissue pathology in these lesions. Image J is a scientific image analyzing program a health's national institutes and the laboratory for optical and computational instrumentation developed a Java-based image processing application. ImageJ was used as an image analysis library, and the ImageJ Download from internet link **ImageJ bundled with 64-bit Java 8** .(fig.1-2)

**Statistical Analysis**

The SPSS statistics software for Social Sciences was used to conduct the statistical analysis (version 20.0 for windows, SPSS, Chicago, IL, USA).

Quantitative data are represented as mean, SD, minimum and maximum.

To test differences between the disease groups, ANOVA test was used.

To test differences between stains Paired t-test was used.

P value of <0.05 was statistically significant.

**Results**

This study consisted 45 cases diagnosed histopathologically as ossifying fibroma(20) and psammatoid juvenile ossifying fibroma(15) trabecular juvenile ossifying fibroma(10). Female predominance was found in OF cases comprising 14 cases (70.0%) (Table I).Whereas male predominance found in psammatoid and trabecular juvenile ossifying comprising 9(60.0%),7(70.0%) respectively .

Ossifying fibroma and juvenile ossifying fibroma was distributed between maxillary and mandibular jaws as follows: thirteen cases (65.0%) of OF were in mandible, while for PsJOF eleven cases (73.3%)were in the maxilla and six cases 60.0% in mandible in TrJOF . The mean age of patients with OF, TrJOF and PsJOF was 27,20and 15 years respectively as demonstrated in (**table I)**.

After H & E staining, bone and osteoid in ossifying fibromas showed varying degrees of pink staining as well as soft tissue components including collagen. VOF stained collagen blue and osteoid and bone purple-red. **( Fig. 3).**

By using ANOVA test however the result non-significant OF show high mean value of osteoid followed by PsJOF then TrJOF in conventional H&E stains while in VOF stains PsJOF showed high mean value followed by OF and finally TrJOF. (**Table 2), ( Fig. 4).**
Using paired t-test H&E showed a high mean values of osteoid in OF, TrJOF and PsJOF, compared to VOFstain,however statistically non-significant compared mean value of osteoid between H&E stain and VOF in

TrJOF there was a border line significant (0.055)**(Table 2), ( Fig. 4).**

## Discussion

The craniofacial skeleton's ossifying fibromas (OF) are benign fibro-osseous lesions that replace healthy bone with a fibrous cellular stroma that comprises concentrated areas of mineralized bone trabeculae and osteoid that varies in volume and form [(9)]. Juvenile psammomatous ossifying fibroma (JPOF) and juvenile trabecular ossifying fibroma (JTOF) are two overlapping clinicopathological entities that have historically been identified based on their pattern of mineralization[(10)]. Ossifying fibromas are still difficult to diagnose due to their numerous histologic and radiographic characteristics, which are crucial for the diagnosis [(11)].

In this investigation, osteoid was identified and differentiated, which was necessary for precise histopathological diagnosis, using standard H & E and VOF stains. The limits of H & E staining have been overcome using a variety of stain combinations [(8)].

Tetrachromic VOF stain is a combination of dyes with varied molecular weights and sizes that is applied simultaneously to allow for the selective discrimination of different structures. While light green and acid fuchsin are amphoteric, orange G is acidic [(11)]. We compared H&E and VOF staining for both differentiation .

The mean value of H&E staining exhibited greater osteoid expression in all three lesions when compared with VOF stain but the difference it was non-significant and this result mean the use of this single simple step technique stain may be used as substitution to standard H&E stain when examine these lesions and this result come in accordance with Kunche et al., whose explained the higher contrast provided by VOF staining's blue, purple, and red staining, it was easier to identify the structures within peripheral and central ossifying fibromas [(12)].

Ossifying fibroma, as a benign fibro-osseous lesion of the jaw, is made up primarily of cellular fibroblastic tissue with various amounts of osteoid tissue, bone, cementum, and calcified tissue that resembles cementum. When clinical conditions are present, it appears that this lesion origin from the periodontal membrane, which contains pluripotent cells which under pathological condition capable of producing cement, bone, and fibrous tissue [(13)]. Mouselhya et al. offered additional justification for their claim that the stromal cells in OF are more immature [(14)]. According to our study this progenitor cell are more active and produce more osteoid in ossifying fibroma in compared with juvenile subtype.

## Conclusion

The assessment of histopathological features of ossifying fibroma and its subtypes increased our understanding of these lesions and helped us in differentiation and identification of these lesions. VOF is a single step technique, whereas H&E staining requires multiple steps .

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Table I. Clinical results of the investigated cases:

| | | Type | | | | | |
|---|---|---|---|---|---|---|---|
| | | O. F. P. (n=20) | | Psammatoid OF (n=15) | | Trabecular OF (n=10) | |
| | | Count | % | Count | % | Count | % |
| Gender | female | 14 | 70.0% | 6 | 40.0% | 3 | 30.0% |
| | Male | 6 | 30.0% | 9 | 60.0% | 7 | 70.0% |
| | Ratio | 2:1 | | 2:3 | | 1:2 | |
| Location | mandible | 13 | 65.0% | 4 | 26.7% | 6 | 60.0% |
| | maxilla | 7 | 35.0% | 11 | 73.3% | 4 | 40.0% |
| Age | | Mean (range) | SD | Mean (range) | SD | Mean (range) | SD |
| | | 27 (14-48) | 10 | 20 (8-50) | 11 | 15 (13-22) | 3 |

Table II: Comparison among ossifying fibroma, juvenile psammomatoid ossifying fibroma (JPOF) and juvenile trabecular ossifying fibroma by using ANOVA test and comparison between stains in each disease by using paired t-test.

| | | Disease | | | P value 1 |
|---|---|---|---|---|---|
| | | Oss. Fib. N=20 | Trabecular N=10 | Psmmatoid N=15 | |
| H&E | Mean | 0.033 | 0.026 | 0.032 | 0.550 |
| | SD | 0.014 | 0.011 | 0.020 | |
| | Minimum | 0.010 | 0.005 | 0.019 | |
| | Maximum | 0.062 | 0.045 | 0.090 | |
| VOF | Mean | 0.027 | 0.020 | 0.028 | 0.222 |
| | SD | 0.011 | 0.011 | 0.013 | |
| | Minimum | 0.013 | 0.005 | 0.010 | |
| | Maximum | 0.052 | 0.042 | 0.055 | |
| P value 2 | | 0.093 | 0.055 | 0.517 | |

P value 1: comparison between diseases using By using ANOVA test

P value 2: comparison between stains in each disease by using paired t-test

There was no significant difference in H&E or VOF comparison between diseases (P>0.05).

There was no significant difference between the stains in Oss. Fib. Or Trabecular or Psmmatoid diseases (P>0.05).

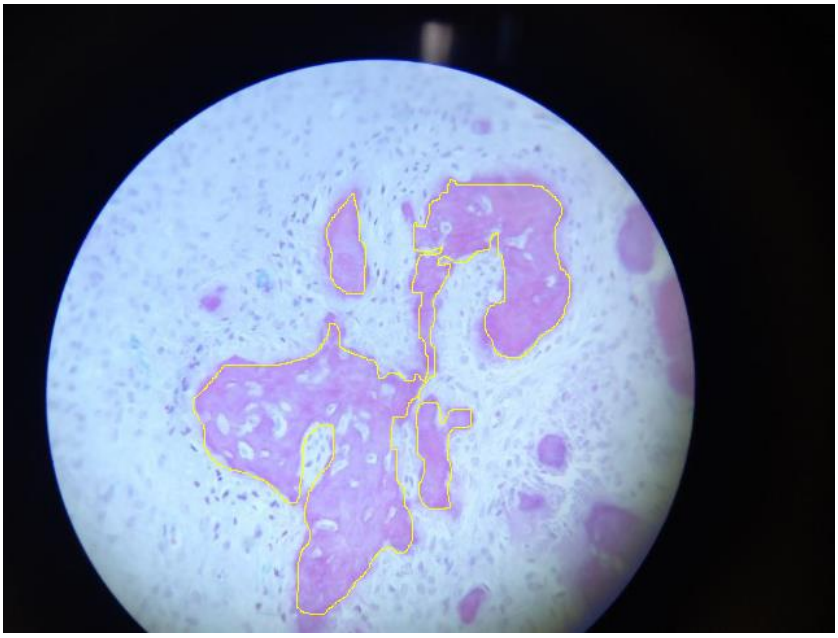

Fig1: Histomorphometric examination of tissues by using image j program

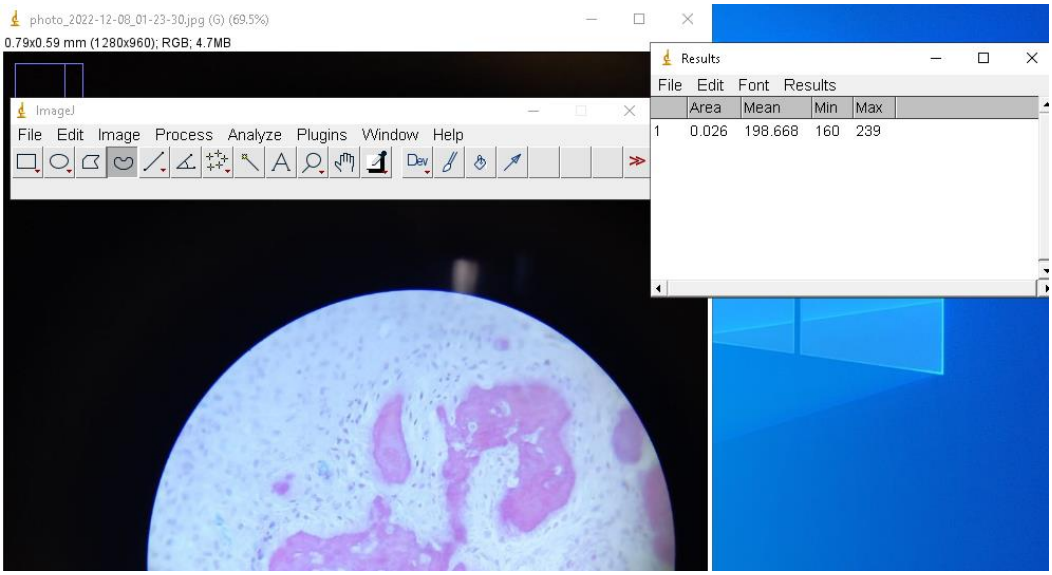


Fig 2:Set scale and measurement of area.

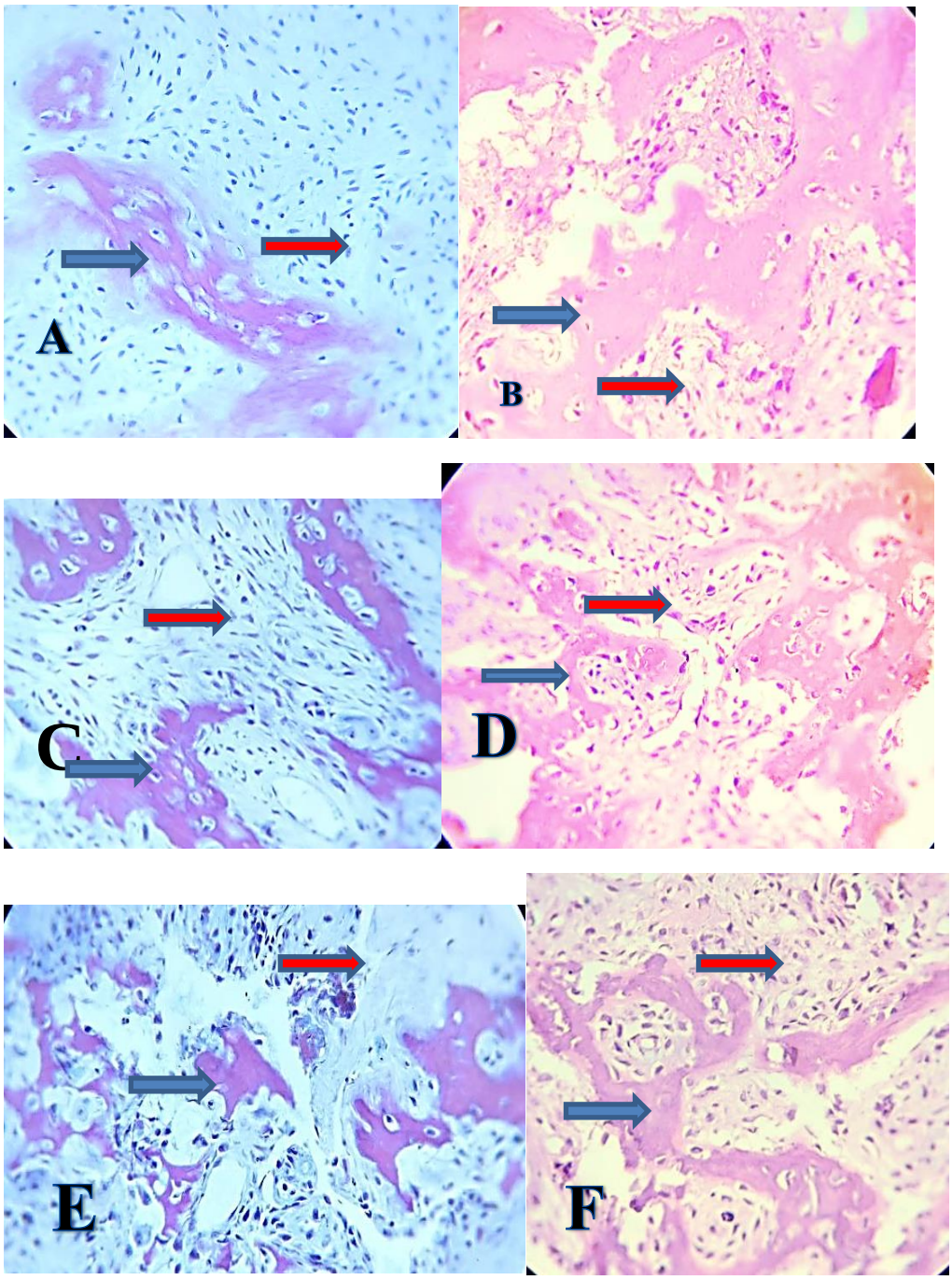


Fig. 3. (A,C,E) VOF purple-red staining of ossifying fibromas and PsJOF then TrJOF. Arrows indicate osteoid that stained reddish to purple with VOF stain. While (B,D,F) conventional H&E in ossifying fibromas, PsJOF and TrJOF respectively stained osteoid and collagen bundles varied degrees of pink. All pictures taken on Power × 40(Blue arrow: osteoid. Red arrow: stoma)

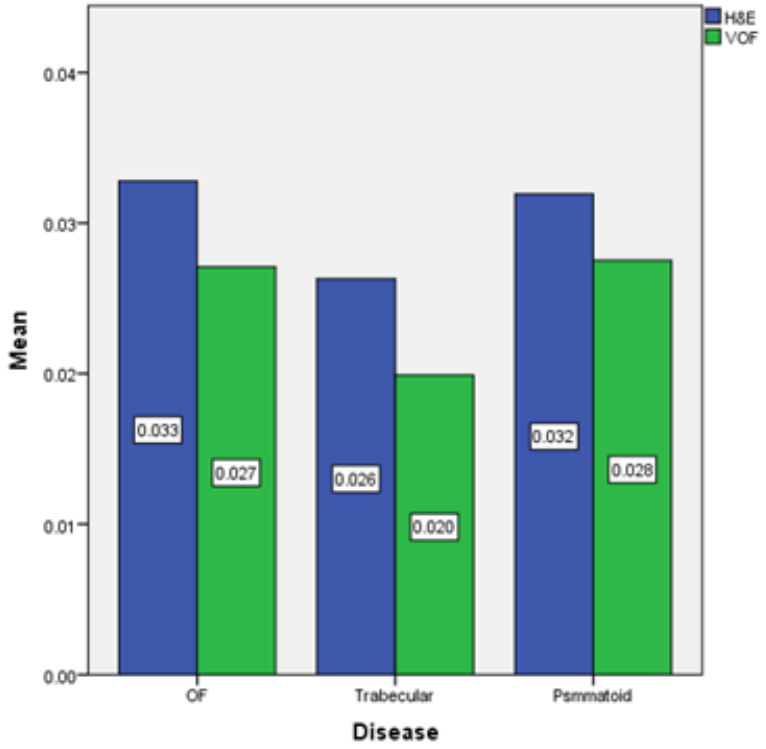


Fig 4. comparison between diseases by using hematoxylin and eosin (H & E) and the tetrachromic Verde Luz-orange G-acid fuchsin ( VOF ) and comparison between stains in each disease**.**