INTRODUCTION

Ossifying fibroma (OF) is a benign, locally aggressive fibro-osseous neoplasm of the jaw in humans and animals [1] that most frequently affects the mandible, causing distortion of the normal contour, displacement and loss of teeth and difficulty in mastication.

OF is the most common benign fibroosseous lesions (FOLs) neoplasm of the oral and maxillofacial region. It was described by Menzel in 1872 but was reported by Montgomery in 1927 [2]. The determination of the precise global frequency of OF still poses a challenge due to the dearth of reported epidemiological studies and varying terminologies that have been used to describe the lesion [3,4].

Observation from the scientific literature shows that classification of FOLs remains a challenging task both for the clinician and the pathologist and have therefore been constantly subjected to frequent renaming and reclassification [5]. However, in an attempt to unify various classifications, the World Health Organization (WHO), came up with an objective histological definitions of tumours which will facilitate the wide adoption of uniform terminology. The most recent WHO classification of odontogenic tumours and allied bone lesions, classifies OF into ossifying fibroma, cementoossifying fibroma and cementifying fibroma [6].
addition, another recently recognized variant of OF is the juvenile active OF (JAOF) which consists of the two subvariants namely; psammomatoid and trabecular JAOF [7].

The aim of this study, therefore, is to review the clinico-pathologic and radiological pattern of maxillofacial OF and to further perform special histochemical analysis using Masson’s trichrome (MT) stain and AgNOR stains at the Lagos University Teaching Hospital, Iyi-Araba, Lagos, Nigeria.

It has been established from the literature that Masson’s trichrome stain has specific affinity for collagen tissues such as bone, muscle and cartilage. Since collagen is a major constituent of bone and that the arrangement of collagen differs between matured (lamellar) and immature (woven) bone, the stain will impart different colour on the collagen present [8].

AgNOR staining is an accurate and easily performed technique. Studies using this technique have suggested that quantitative analysis of AgNORs can be useful in estimating proliferative activity of neoplasms in surgical pathology. Specifically, it has been utilised as a diagnostic tool in comparing the proliferating activities of fibrous dysplasia and ossifying fibroma [9].

MATERIALS AND METHODS

This is a retrospective study of cases of histologically diagnosed maxillofacial OF in the department of Oral Biology and Oral Pathology, Lagos University Teaching Hospital, Iyi-araba, Lagos, which is one of the foremost tertiary health care institution in Nigeria from 1970 to 2009. Lagos state is cosmopolitan with an estimated population of 9.5 million (NPCN 2006). It consists of people from diverse ethnic groups that are involved in inter-ethnic and inter-religious marriages. Record of oral biopsies of OF cases diagnosed in the oral and maxillofacial bone region were retrieved for analysis.

Approval for this study was sought and obtained from the ethical committee of Lagos University Teaching Hospital for use of the past medical/dental records of material of subjects used for the study.

Inclusion Criteria

All cases of OF in the maxilla or mandible with complete clinical and radiological data, complete record of histological materials and data, centrally located in the jaws, and OF that have been re-confirmed histologically after review.

Exclusion Criteria

Cases not re-confirmed histologically as OF after review and cases for which histological materials such as paraffin blocks, could not be retrieved.

Data Collection Tools and Techniques

Bio-data and clinical parameters such as information on age, sex and site of each lesion, as well as radiographic information, which consisted, of lesional border and bone density, were retrieved from the past record of patients, using the oral biopsy request forms, case notes and X-ray films (where available).

Haematoxylin and Eosin (H and E) histological glass slides and all available paraffin sections of all cases that satisfied the inclusion criteria were retrieved and re-evaluated to confirm diagnosis of FD. Cases that did not satisfy the criteria were excluded from the study.

HISTOCHEMICAL STUDY

Materials for Masson’s Trichrome Stain

1. Bouin’s solution: Picric acid (saturated) 75 ml, Formaldehyde (37-40%) 25 ml, Glacial acetic acid 5 ml. This solution improves the quality of Masson Trichrome stains.
2. Weigert’s Iron Haematoxylin Solution: Mix equal parts of Solution A and B.
3. Stock solution A: Haematoxylin (1 g), 9% Alcohol 100 ml.
4. Stock Solution B: 30% Ferric chloride in water - 4 ml, Distilled water - 95 ml and Hydrochloric acid (concentrated) 1 ml.
5. Acidified Ponceau-Fuchsin Solution: Ponceau (2R) 1% aqueous - 90 ml, Acid fuchsin, 1% aqueous -10 ml, Acetic acid, glacial - 1 ml.
6. 1% Phosphomolybdic acid in distilled water.
7. Aniline Blue Solution: Aniline blue - 2.5% ( water soluble), Acetic acid, glacial - 2.0%, Distilled water - 100 ml.
8. 1% Acetic Acid Solution: Acetic acid, glacial - 1 ml, Distilled water - 99 ml.

Materials and for AgNOR Stain

AgNOR Reagents, Silver Nitrate Solution-50%, Gelatin-2%, Formic Acid-1%, Deionised Water.
Assessment of Each Stain

**Haematoxylin and eosin (H and E) stain**

H and E was routinely assessed for the lesion initially diagnosed. It served as the histological standard for the masson’s trichrome (MT) and AgNOR stained sections.

**Masson’s trichrome (MT) stain**

MT stain was utilized in the characterization of hard tissue component of the lesions into woven bone or lamellar bone [8,10]. Lamellar bone trabeculae stained red with excellent display of lamellate structure and parallel arrangement of collagen (concentric ring) while the woven bone trabeculae stained bluish-red with bluish areas of osteoblasts, with bone trabeculae edges merging imperceptively with the surrounding stroma tissue. Woven and lamellar bone (mixed) stained reddish in area and bluish in another.

**AgNOR stain**

Cells that stained positive for AgNOR showed black intranuclear dots which were best demonstrated at X 1000 magnification under oil immersion objective lens.

Data was analyzed using IBM SPSS Statistics for windows Version 20 (Armonk, NY: IBM Corp) and results were presented as simple frequencies and descriptive statistics.

**RESULTS**

One hundred and fifty seven cases of OF, representing 52.0% of FOL were seen during the study period. OF was most commonly found in the age group of 20-29 years, where 45 cases (28.7%) were observed with a mean age of 30.14 ± 13.72 years (Table 1). OF occur more frequently in females, 100 cases (63.69%) than males 57 cases (36.31%) at a female to male ratio of 1.75:1 (Figure 1). OF occur more commonly in the mandible, 117 (74.52%) cases than maxilla 40 (25.48%) cases. Expansion of bone was noticed in 40 (25.48%) cases. In general well defined radiographic border occurred in 70% of OF. H and E stain showed osteoblastic rimming of lamella bone (Figure 2) and well demarcated and clear border of the OF (Figure 3). High proportion of lamellar bone (31% and above) as demonstrated by Masson’s Trichrome Stain was more commonly observed in OF, 21 cases (52.5%) (Figure 4). There is low proportion of mixed type of bone trabeculae in OF, 20 cases (50%). The mean AgNOR score of 130.4069 ± 32.3392/100 cells (1.304 ± 0.3234/cell) observed for OF with numerous intranuclear AgNOR dots in the fibroblasts (Figure 5).

**Table 1.** Ossifying fibroma.

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Ossifying Fibroma (No)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-9</td>
<td>2</td>
<td>1.3</td>
</tr>
<tr>
<td>10-19</td>
<td>38</td>
<td>24.2</td>
</tr>
<tr>
<td>20-29</td>
<td>45</td>
<td>28.7</td>
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<tr>
<td>30-39</td>
<td>36</td>
<td>22.9</td>
</tr>
<tr>
<td>40-49</td>
<td>19</td>
<td>12.1</td>
</tr>
<tr>
<td>50-59</td>
<td>11</td>
<td>7</td>
</tr>
<tr>
<td>60-69</td>
<td>4</td>
<td>2.5</td>
</tr>
<tr>
<td>TOTAL</td>
<td>157</td>
<td>100</td>
</tr>
</tbody>
</table>

**Figure 1.** Graph showing proportion of male to female.
DISCUSSION

Ossifying fibroma is well thought-out by most to characterize a benign neoplasm arising from undifferentiated cells of the periodontal ligament tissues \cite{11}. However, there is controversy over such an origin, since tumours of similar histology have been reported in bone lacking periodontal ligament and not located in the tooth bearing region, such as ethmoid bone, frontal bone or even long bones of the body (cementiform fibrous dysplasia) \cite{12-16}.

Due to its origin from multipotent mesenchymal cells, it can produce bone, cementum, spheroidal calcifications and fibrous tissues.

In this study, there is female preponderance of OF with a female: male ratio of 1.75:1. This agrees with reports of similar studies in the scientific literature, although higher female to male ratio have been reported. Williams et al. \cite{17} reported 2:1 for OF,
Abdulai et al. [18] reported 2:1 for OF, while Worawongvasu and Songkampol, [19] reported 6.8:1 for OF. Some Nigerian studies have also documented female to male ratio prevalence of OF, that are similar to findings in this series. Odeku et al. [20] reported 2.1:1 for OF, while Ajagbe and Daramola, [21] reported 2:1 for OF. Literature search revealed that there is generally female preponderance. It could be argued that females are more conscious of their appearance than males and therefore tend to present themselves early for treatment than the males would do. On the contrary, MacDonasld-Jankowski, [4] have reported no gender bias as it was found to affect males and females equally in his series.

Observation from present study revealed well defined radiographic border in 70% of OF at the mixed radiolucent/radiopaque radiographic pattern. This observation agrees with reports in the scientific literature where border definitions were best recognized at this stage of radiographic presentation [5,19,22]. It is important to note that no association was established between border definition and type of lesion, with respect to radiolucent as well as radiopaque radiographic patterns. This suggests that mixed radiolucent/radiopaque radiographic pattern is the most important radiographic pattern in considering border definition in OF.

Surgical exploration usually reveals a well-demarcated mass that often is easily shelled out from the surrounding bone, either intact or in several large pieces. A significant feature of OF is its proclivity to completely shell out during surgery [23]. This is as a result of clear well demarcated histological border observed from the H and E stain (Figure 3).

From the Masson’s trichrome (MT) stain in the present study, it was discovered that presence of lamellar bone was relatively higher in OF. This could be an important criterion in identifying OF histologically and distinguishing it from fibrous dysplasia (FD). The bone in OF has been documented to consist of lamellar bone and woven bone in varying proportions [24-26]. However, the present study have emphasised presence of lamellar bone as significant in identifying OF.

Findings from the AgNOR stain showed higher AgNOR score for OF (AgNOR score of 130.4069 ± 32.3392 per 100 cells (1.304/cell)). This higher score have been documented in Japanese [26] as well as Iranian populations [9]. This high score indicates higher proliferative fibroblastic activity in this lesion compared to similar activity in FD. Furthermore, the finding supports the neoplastic nature of OF. Studies in the scientific literature which reported application of AgNOR histochemical staining technique as a parameter to differentiate OF of the jaws from FD of the jaws are still rare [9,26]. Only one African study has applied the AgNOR stain technique as a parameter to differentiate FD of the jaws from OF of the jaws [27]. Previous studies from Japan and Iran have documented mean AgNOR score to range between 1.304/cell and 1.75/cell for OF. [5,26] It can therefore be postulated that OF may be identified by mean AgNOR score/cell that ranges between 1.304/cell and 1.75/cell.

CONCLUSION

This present study showed a female predilection and that OF was most commonly observed in the 3rd decade of life. It also highlights the greater proportion of OF present in the mandible. In addition, it showed presence of lamella bone as predictor of OF using Masson’s trichrome (MT) stain.

REFERENCES