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Evaluation of proliferative activity of gnathic low-grade centrosteosarcoma: A report of 10 cases and review of literature

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Abstract

Context: Low-grade central osteosarcoma (LCOS) is a clinically less aggressive form of osteosarcoma that may mimic benign fibrosseous lesions both clinically and histopathologically. The lesion also shows bland cytologic features, which makes it difficult to diagnose. Irrespective of its histologic features, it has the potential to metastasise, therefore posing a significant mortality risk to the patient.
Objective: The objective of the study was to highlight the proliferation index of low-grade central osteosarcoma using histochemical and immunohistochemical methods, using argyrophilic nucleolar organizer region (AgNOR) staining and MiB-1, respectively. In addition, the study aimed to assess the correlation of the histochemical and immunohistochemical methods.
Materials And Methods: The study was a cross-sectional study in which histologically diagnosed cases of low-grade central osteosarcoma seen over the last 30 years at the Lagos University Teaching Hospital were retrieved and analysed for their proliferative index using AgNOR staining and MiB-1 immunohistochemical staining.
Result: A total of 10 cases that met the selection criteria were retrieved. The mean PAgNOR of LCOS was 14% +/-4.97 while the PMiB-1 was 9.55% +/-4.49. Test of correlation between PAgNOR and PMiB-1 of LCOS showed a positive correlation (r=0.70).
Conclusion: The study showed that although histologically, LCOS may appear bland, they have significant levels of proliferative activity. The significant positive correlation of AgNOR staining and MiB-1 showed that when there are logistical challenges to the use of immunohistochemistry, special stains can be used to assess the proliferative activity of lesions, especially in resource-deprived settings.

Introduction

Low-grade central osteosarcoma (LCOS) is a rare variant of central osteosarcoma that was first described by Unni et al.[1] It is described as a central lesion in which the mesenchymal elements in addition to the production of malignant osteoid, appear bland and may display minimal cytologic atypia. This lesion poses diagnostic challenges because the neoplastic spindle-shaped, in addition to the minimal cytological atypia, mimics benign fibrosseous lesions like fibrous dysplasia, ossifying fibroma, and desmoplastic fibroma. Assessment of the proliferative activity of the neoplastic cells has been suggested as a reliable means to differentiate this lesion from its benign mimics like fibrous dysplasia and ossifying fibroma.
This study aims to report 10 cases seen at the Lagos University Teaching Hospital over a 30-year period from 1991 to 2020, as well as assess their proliferative activity using Argyrophilic nucleolar organizer region (AgNOR) and MiB-1. An additional aim of the study was to ascertain the correlation of histochemical and immunohistochemical methods of assessing the proliferative profile of low-grade osteosarcoma.

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Materials and methods

A review of the cases of osteosarcoma seen at the Oral

pathology diagnostic services of Lagos University Teaching Hospital over a period of 30 years from 1991 to 2020 was done. All the hematoxylin and eosin-stained slides of all the cases were retrieved from the archives. Two (2) trained oral pathologists reviewed all the slides independently to make a diagnosis of LCOS. In cases of disagreement, a third trained oral pathologist reviewed the slide before a consensus was reached.

The laboratory forms of the cases of LCOS were recovered from the Department of Oral and Maxillofacial Pathology/Biology. The collected data included the age, sex, and location of the lesion. The paraffin blocks of the selected cases were also retrieved, and sections were stained for AgNOR histochemical and MiB-1 immunohistochemical staining. AgNOR staining was done using the protocol described by Bukhari.[2]

The AgNOR staining protocol begins with preparing a working solution: one part of Solution A (500 mg gelatin in 25 ml deionised water at 37°C, plus 250 µl formic acid, shaken for 10 minutes until clear) mixed with two parts of Solution B (50% w/v silver nitrate in deionised water). Tissue sections (5 µm, paraffin-embedded) are deparaffinised in xylene, rehydrated through decreasing grades of alcohol, and rinsed in running water. Slides are incubated with the working solution for 38 minutes at 37°C in a dark humid chamber, toned in 10% sodium thiosulphate for 5 minutes, rinsed in deionised water, dehydrated in increasing grades of alcohol for 2 minutes, cleared in xylene, and mounted with Distyrene plasticizer xylene.

Immunohistochemical staining for MiB-1 was performed on 3 µm tissue sections. Sections were deparaffinised in xylene, rehydrated with decreasing alcohol grades, and endogenous peroxidase blocked with 3% hydrogen peroxide for 15 minutes. Antigen retrieval was done by placing the sections in a 10mM citrate buffer (pH 6.0) solution and microwave treatment at 92°C for 15 minutes, followed by cooling and PBS wash. After blocking with background stains by incubating in bovine serum albumin, the sections were incubated for 1 hour at room temperature with prediluted rabbit antihuman MIB-1 antibody (2 µg/ml). Detection involved BIOGENEX Super Enhancer reagent, PBS rinses, incubation with Polymer Horseradish Peroxidase, and visualization by 3-amino-9-ethyl carbazole for 15 minutes, then counterstained with haematoxylin. Negative controls omitted the primary antibody.

The AgNOR proliferative index (PAgNOR) measures the percentage of neoplastic cells with five or more AgNOR dots. Following Crocker et al.'s protocol, each slide was divided into four quadrants, with five points per quadrant identified under a 20X lens (north, south, east, west, centre). In each point, NOR staining was assessed in twenty consecutive cells at 100X magnification. Scores were calculated as NOR dots per twenty-five cells, then summed across quadrants for a total NORs score per one hundred cells per slide.

In addition, the MiB-1 Proliferative Index (PMiB-1); the percentage of stained neoplastic nuclei, was also evaluated. The Assessment protocol was adopted from the Ki-67 in Breast cancer workshop.[3] Five (5) random areas of the slides were selected. In cases where there is a hotspot, that hotspot was selected as part of the 5 random areas. The total number of neoplastic cells present in each spot was counted as well as the percentage of stained cells present.

Ethical approval was obtained from the Institutional Review Board of the Lagos University Teaching Hospital.

Result

A total of sixty-five cases of central osteosarcoma were reported over the thirty years. Of these cases, 10 were confirmed as LCOS. LCOS was more common in males (n=7) than in females (n=3) giving a male to female ratio: 2.3:1. It occurred more in the mandible(n=8) compared to the maxilla (n=2). The age range of the subjects was 21-54 yrs with a mean age of 33.2 +/- 10.0 years.

The mean PAgNOR of LCOS was 14% +/-4.97 while the PMiB-1 was 9.55% +/-4.49 Test of correlation

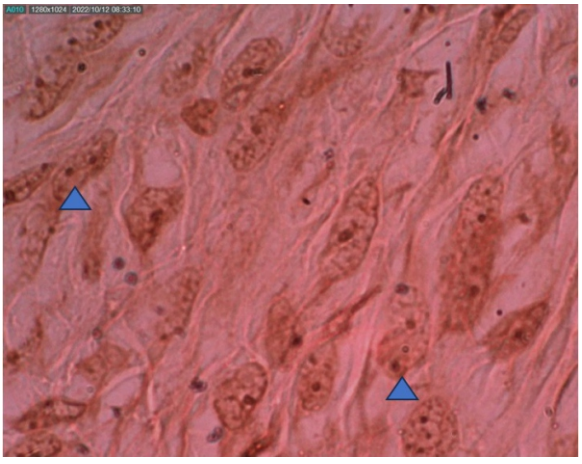

Figure 1: AgNOR staining show AgNOR dots (blue arrowhead) (x1000 magnification)

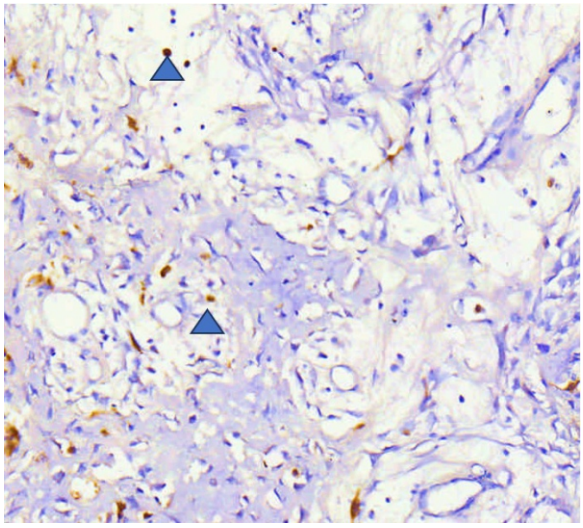

Figure 2: Photomicrograph showing proliferative index assessment for MIB-1 in LCOS.

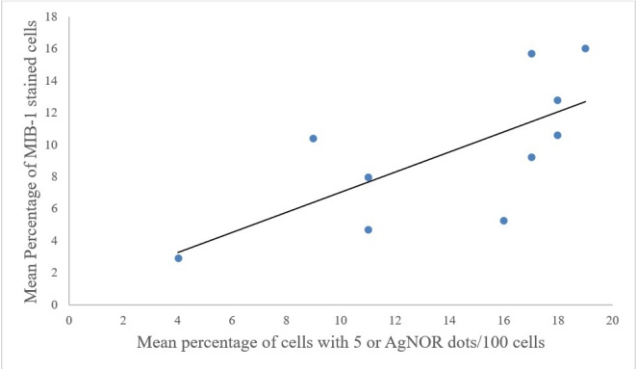

Figure 1: AgNOR staining show AgNOR dots (blue arrowhead) (x1000 magnification)

between PAgNOR and PMiB-1 of LCOS showed a positive correlation (r =0.70) (Fig. 1).

Discussion

Low-grade central osteosarcoma is a rare lesion. It is a less clinically aggressive variant of osteosarcoma. Histopathologically, this lesion lacks the bizarre cellular characteristics of conventional osteosarcoma. However, malignant osteoid is present like in conventional osteosarcoma, although the amount may vary from minute foci to heavy seams of osteoid.

It is difficult to ascertain the epidemiologic profile of LCOS as only a few cases have been reported. For instance, Ibikunle et al[4] reported 7 cases in Kano over 7 years, while Sasaki[5] reported 7 cases over 17 years in Japan. The mean age of presentation in this case was 33.2 years, which is similar to previous reports by Okada (33.8 years)[6] and Sasaki (36.2 years)[7] among a Japanese population, Bertoni (31.4 years)[8] among an Italian population, and Arslan (33.6 years.)[9] among a Turkish population.

The gender predilection appears to vary according to different authors. In this review, LCOS was common in males than in females (male-to-female ratio of 1.5:1), unlike Ibikunle et al, who reported an equal gender occurrence, while Sasaki reported a high female predilection (male-to-female ratio of 1:6).[4,7] This variance in the gender predilection is also seen in the site predilection. While a mandibular site preference was noted in this series, Sasaki reported a fairly equal site distribution.[7]

The diagnosis of low-grade central osteosarcoma involves the assessment of the margins for evidence of infiltrative growth pattern of the lesion, which is considered very crucial to distinguish it from benign mimics because of the low cytologic atypia seen in this lesion. The infiltrative growth pattern may, however, not be easily demonstrated, especially in small specimen sizes or incisional biopsies.[10]

The pMIB-1 score (9.55%±4.49) from this study is similar to previous assessments by Paul(9.9%)[11], Okada(10%)[12] and Diniz(7%)[13]. This score is also lower than the mean pMIB-1 score of 27% reported in conventional osteosarcoma by Jong et al. The authors did not find any previous report on the assessment of pAgNOR in LCOS.

Immunohistochemistry has some challenges that particularly affect laboratories in resource-poor areas. The antibodies used may require refrigeration, which requires electricity that may not be steadily available in rural communities of sub-Saharan Africa. In addition, sourcing of the antibodies may require extra financing that may also not be readily available. It therefore becomes important to have reliable alternatives for the assessment of the proliferative activity of malignancies. AgNOR staining is a reliable alternative, especially in rural environments. From the author's experience, the reagents do not require special storage conditions, as well as they are cheaper and more stable compared to immunohistochemical antibodies.

In this study, the significant positive correlation of PAgNOR of LCOS to PMiB-1 of LCOS suggests that PAgNOR can be used instead of Ki-67 immunohistochemical index when the need arises. This finding supports the finding of Mourad, who had stated PAgNOR is a viable tool for the assessment of proliferative index of tumours.[14]

Conclusion

This thirty-year review reports ten confirmed cases of low-grade central osteosarcoma (LCOS). LCOS is described as a rare, less aggressive variant, lacking overt cellular atypia but exhibiting malignant osteoid. It was found to be more frequent in males and in the mandible, with a mean age of 33.2 years.. The study demonstrates a strong association between AgNOR and MiB-1 indices, supporting the potential of AgNOR staining as a low-cost substitute for immunohistochemistry when evaluating tumour proliferation, particularly in low-resource environments.

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Author's contribution

Ernest E. Aforka contributed to conception, design, acquisition of data, analysis and interpretation of data; Ifeanyi C. Nwokike contributed to the conception, interpretation of data, revision of the article and Ifeoma W. Anusiobi contributed to revision and drafting of the article.

Conflict of interest: The authors declare no conflict of interest.

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