Original article

THE ROLE OF FINE NEEDLE ASPIRATION CYTOLOGY IN THE PRE-OPERATIVE ASSESSMENT OF AMELOBLASTOMA: A Preliminary Study

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ABSTRACT

OBJECTIVE: This study aims to determine the clinicopathologic features of ameloblastoma, and to determine the sensitivity, specificity and accuracy of fine needle aspiration cytology (FNAC) in pre-operative assessment of ameloblastoma.

METHODS: A prospective study of patients with jaw swellings over a 9-month period. FNAC procedure was performed using disposable 5ml plastic syringes and 21-gauge needles. Smears were fixed, stained and the cytopathological features and diagnoses documented. Surgical biopsy was performed afterwards and the histological sections reviewed. Patients with the histopathological diagnosis of ameloblastoma were selected for the study. Data on clinico-radiologic features, cytopathologic and histopathologic diagnoses were collected analyzed. The cytopathologic and histopathologic findings were compared. The sensitivity, specificity and accuracy of FNAC in the diagnosis of ameloblastoma were determined.

RESULTS: There were 6 males (66.7%) and 3 females (33.3%) with a male to female ratio of 2:1 and mean age of 36 $\pm$ 1.8 years. The most affected site was the mandible (n=6, 66.7%). Cytological smears of the ameloblastoma revealed mostly basaloid cells in clusters or discohesive patterns, occasionally associated with polyhedral or spindle cells. The most common cytological diagnosis was benign cystic tumour (n=6, 66.7%). Solid-multicystic ameloblastoma (n=6, 66.7%) was the most common histopathologic type. There was significant Pearson correlation of the cytological benignity and histopathological variants of ameloblastoma (p=0.013). The cytological benign nature of ameloblastoma showed sensitivity of 88.9%, specificity of 100% and accuracy of 88.9%.

CONCLUSION: There was significant association of the FNAC cytological diagnosis of benign lesion with the histopathologic variants of ameloblastoma, and a high sensitivity, specificity and accuracy for cytological diagnosis of the benign nature of the tumour.

KEY WORDS: Ameloblastoma, cytological diagnosis, histological diagnosis

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INTRODUCTION

Ameloblastoma is the most common epithelial odontogenic tumour of the jaws, comprising 1% of all jaw tumours and cysts.[1] Ameloblastoma arise from odontogenic epithelium without odontogenic ectomesenchyme.[2] It is a clinically persistent and aggressive lesion characterized by a benign histological appearance, but shows locally invasive behaviour, causing facial disfigurement, having a high risk of recurrence and potential for malignant transformation and metastases. Death due to ameloblastoma may occur from invasion of vital structures, superinfection, and distant metastases.[3,4] Etetafia et al (2014)[5] reported two cases of mortality associated with giant ameloblastoma due to delayed treatment as a result of ignorance, poverty and fear.

The incidence of ameloblastoma shows geographic variation and it is the most common odontogenic tumour in Africa[6-8] and Asia.[8,9] The diagnosis of ameloblastoma is often based on the clinico-radiological and histopathological features of this tumour. Some studies have attempted to evaluate the cytological diagnostic characteristics of ameloblastoma[1,10-13] and the malignant transformation that may occur in ameloblastoma.[14,15] However, in these studies, the confirmatory definitive diagnosis of ameloblastoma were based on histological examination.

The findings from fine needle aspiration cytology (FNAC) smears of ameloblastoma has been reported to consists of basaloid (ameloblast-like) cells arranged in clusters or palisading patterns, and squamous cells with spherical keratinized bodies in discohesive or whorl patterns, and stellate reticulum-like cells.[10,11] A report on FNAC in the diagnosis of granular cell ameloblastoma showed smears of granular cells along with spindle cells and basaloid cells.[12] Using imprint cytology in the diagnosis of ameloblastoma, Okada et al[13] reported three cell types in their smears: small polyhedral or spindle-shaped squamous epithelial cells with some projections thought to correspond to polyhedral or spindle cells histologically; naked cells with poorly defined cytoplasm in tubular and palisade patterns in some areas thought to correspond to cuboidal or columnar cells histologically; and large squamous epithelial cells with extensive wrinkled cytoplasm thought to be acanthomatous cells showing squamous metaplasia histologically.

Some other studies have reported findings in the cytology of malignant changes in ameloblastoma.[14,15] Matthew et al,[14] reported that smears of malignant ameloblastoma consists of two distinct cell population composed of small, hyperchromatic, basaloid-type cells and scattered larger cells with more open chromatin. The metastasizing lesions showed prominent pleomorphism, cellular crowding with molding and a high mitotic/karyorrhectic index. Furthermore, Parate et al[15] observed basaloid cells and squamous epithelial cells in a background of stellate and spindle cells in FNAC smear of malignant ameloblastoma. They recommended that FNAC be used as a useful diagnostic tool in the initial diagnosis and follow up of patients, when there is a primary ameloblastoma with possible metastasis to sites such as the lungs.

Although recent literature suggests a role of cytological examination in the diagnosis of ameloblastoma, the usefulness of cytological examination in the initial diagnosis of this tumour is yet to be ascertained in our environment where patients present mostly late in the course of their disease with large swellings. This study aims to determine the clinicopathologic features of ameloblastoma, and to determine the sensitivity, specificity and accuracy of FNAC in pre-operative assessment of ameloblastoma.

MATERIALS AND METHODS

This was a prospective study of patients who presented with jaw swellings at the Department of Oral Pathology and Medicine, University of Benin Teaching Hospital, Benin City, Nigeria, over a 9-month period. Approval was obtained from the Ethical Committee of the Hospital to perform fine needle aspiration cytology (FNAC) on consenting patients. The patients'

history was obtained and clinical examination was done. The FNAC procedure was performed using disposable 5ml plastic syringes and 21-gauge needles with an external diameter of 0.6 to 0.7mm. The swellings were aspirated and the aspirates were smeared on glass slides. Smears for Romanowsky Giemsa stain were air dried while smears for Papanicolaou (PAP) stain and hematoxylin and eosin (H and E) stain were immediately immersed in a coplin jar containing 95% ethyl alcohol. The slides were stained with hematoxylin and eosin (H and E), Giemsa and Papanicolaou and viewed under light microscope. The cytopathological features and diagnoses were documented.

Surgical open biopsy was performed afterwards under local anesthesia and specimens obtained were fixed with neutral buffered formalin in specimen bottles. The tissues were processed and stained with H and E, and the histological sections viewed under light microscope. The histopathologic features and diagnoses were documented. Patients with the histopathological diagnosis of ameloblastoma were selected for this study. The clinical and histologic classification of the ameloblastoma in this study was based on the 2005 histological Classification of odontogenic and allied Tumors by the World Health Organization. Ameloblastoma was classified into the following variants: solid/multicystic, extraosseous/peripheral, desmoplastic, and unicystic.[2,16]

Data on patients' age, gender, site, size of lesion, duration of lesion on presentation, the clinico-radiological features, cytopathological nature and diagnosis, and the histological diagnosis were analyzed. The cytopathological findings and the histological diagnosis were compared. Pearson's correlation significance was set at p value of <0.05 (2 tailed).

The sensitivity, specificity and accuracy of FNAC were determined using the formulae below adopted from Abdul G.A, 2008[17] and Omitola et al., 2010[18] to determine the benign nature of the ameloblastoma:

$$\text{Sensitivity} = \frac{\text{True-Positive (TP)}}{\text{True positive (TP) + False Negative (FN)}}$$

$$\text{Specificity} = \frac{\text{True Negative (TN)}}{\text{False positive (FP) +True Negative (TN)}}$$

$$\text{Accuracy} = \frac{\text{True Positive + True Negative}}{\text{Total}}$$

TP: Benign cytologic diagnosis consistent with the histological diagnosis.

FP: Benign cytology but malignant or inflammatory in histology.

TN: No benign either cytologically or histologically (malignant or inflammatory).

FN: Malignant or inflammatory cytology but benign histology

Sensitivity: Is the ability of a test to detect who has the disease being tested for.

Specificity: Is the ability of a test to detect those who do not have the disease being tested for.

RESULTS

Forty-nine patients had FNAC performed within the study period among which 9 (18.4%) cases were histopathologically diagnosed as ameloblastoma. Of these ameloblastoma, there were 6 males (66.7%) and 3 females (33.3%) with a male to female ratio of 2:1. The ages of the patients range between 5 to 57 years with a mean age of 36 $\pm$ 1.8 years and the peak age group was the third decade of life (n=4, 44.4%). The age to gender relationship was not significant (p=0.345) [Table 1].

Three patients (33.3%) presented with painful swellings. The most common site was the mandible (n=6, 66.7%), while 2 (22.2%) lesions were found in maxilla and one (11.1%) in the maxillary gingiva. The mean duration of the tumours on presentation was 4 years. The mean diameter of the tumour mass was 6.6cm $\pm$ 1.8 (SD).

Table 1: Age and gender distribution of the patients diagnosed with ameloblastoma

Age	Gender		Total (%)
	M	F	
5	1	-	1
21	1	-	1
28	1	1	2
30	1	-	1
44	1	-	1
56	-	2	2
57	1	-	1
Total	**6**	**3**	**9 (100)**

P = 0.345

The jaw lesions were mostly radiolucent masses (n=9, 100%), among which were 6 (66.7%) multilocular radiolucent lesions and 2 (22.2%) unilocular radiolucent lesions. There was a gingival lesion (11.1%) without obvious bone involvement. The aspirates were adequate for cytological evaluation and were mostly straw coloured (n=5, 55.6%) [Table 2]. The most common clinical impression was ameloblastoma (n=4, 44.4 %).

Cytological smears of the patients revealed mostly basaloid cells arranged in clusters or dyscohesive patterns, occasionally associated with polyhedral cells or spindle cells. There was a case with a large (glandular-like) cell and hyperchromatic basaloid cells. The background of the smears was loose with occasional infiltrates of acute and chronic inflammatory cells (Figure 1 and 2). The most common cytological diagnosis was that of a benign cystic tumour (n=7, 77.8%) [with copious cystic aspirate]. Only 2 (22.2%) patients had specific cytological diagnosis as cystic ameloblastoma (Table 2).

The histological types of ameloblastoma in this study were: unicystic ameloblastoma (n=2, 22.2%) [Fig 3]; solid/multicystic ameloblastoma (n=6, 66.7%) [Fig 4] consisting of plexiform variant (n=3, 33.3%) and follicular variant (n=3, 33.3%); and 1 (11.1%) peripheral ameloblastoma (follicular variant) [Table 3].

There was significant Pearson correlation of cytopathological diagnosis of benign lesion (benignity) with the histopathologic variants of ameloblastoma (p=0.013). The FNAC diagnosis of the benign nature of ameloblastoma in this study showed a sensitivity of 88.9%, specificity of 100% and accuracy of 88.9%.

Table 2: Relationship between the nature of aspirate, the radiological features and the cytopathological diagnosis

Number of cases	Nature of Aspirate	Radiologic features	Cytological diagnosis
1	Straw coloured	Multilocular radiolucency	Cystic ameloblastoma
2	Straw coloured	Multilocular radiolucency	Cystic ameloblastoma
3	Straw coloured	Multilocular radiolucency	Benign cystic tumour
4	Straw coloured	Multilocular radiolucency	Benign cystic tumour
5	Straw coloured	Multilocular radiolucency	Benign cystic tumour
6	Dirty brown	Multilocular radiolucency	Benign cystic tumour
7	Grey coloured	Unilocular radiolucency	Benign Cystic tumour (infected)
8	Grey coloured	Unilocular radiolucency	Benign tumour (inflamed)
9	Bloody	No demonstrable finding	Adenocarcinoma

Table 3: Correlation of the cytopathological diagnosis of benignity and histopathological variants of ameloblastoma

Cytopathological diagnosis	Histopathologic variants of ameloblastoma, n (%)				p=0.013
	Follicular ameloblastoma	Plexiform ameloblastoma	Unicystic ameloblastoma	Peripheral ameloblastoma	Total %
benign lesion	3(33.3)	3(33.3)	1(11.1)	0	7 (77.8)
inflammatory lesion	0	0	1(11.1)	0	1 (11.1)
malignant lesion	0	0	0	1(11.1)	1(11.1)
Total n (%)	3 (33.3)	3 (33.3)	2 (22.3)	1 (11.1)	9 (100)

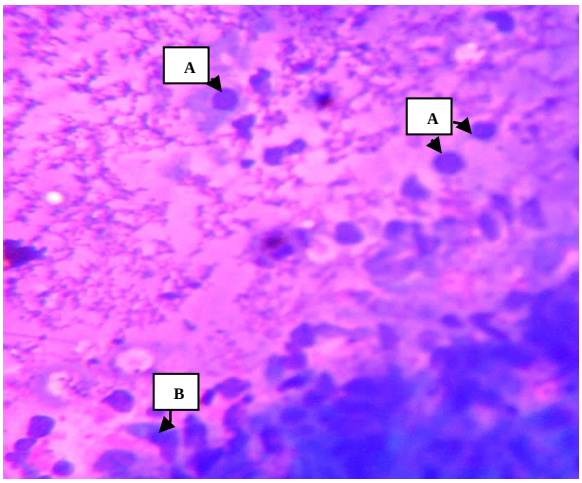

Figure 1: Smear showing cluster and discohesive basaloid (A) and spindle cells (B) in a loose fibrillary background with red cell extravasations [Giemsa x400]

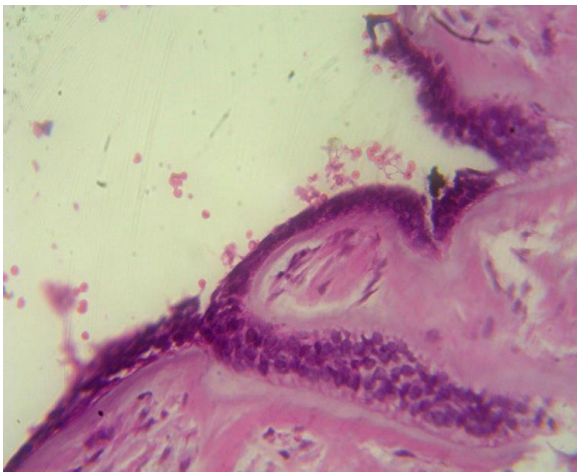

Fig.3. Photomicrograph of unicystic ameloblastoma lined by odontogenic epithelium and outer fibrous connective tissue capsule (H&E x400).

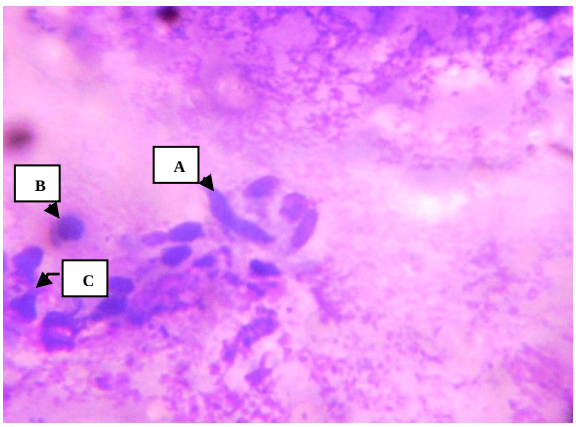

Figure 2: Smear showing discohesive basaloid cells (A), spindle cells (B), polyhedral cells (C) in a loose background with red cell extravasations [Giemsa x400]

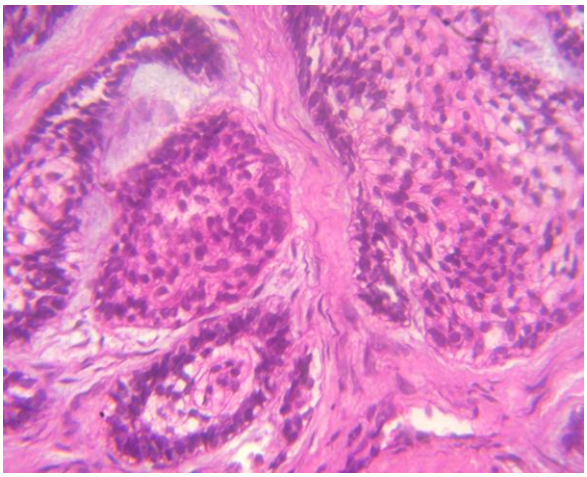

Fig 4. Photomicrograph of solid ameloblastoma showing odontogenic epithelial cells arranged as

islands palisaded basal ameloblast-like cells and central stellate reticulum-like cells in a fibrous connective tissue stroma (H&E x400).

DISCUSSION

Ameloblastoma is a locally infiltrating and aggressive epithelial odontogenic jaw tumour known to be common in the African sub-continent as shown by previous studies that reported high incidence of ameloblastoma among Nigerian population. This prevalence underscores the need for early diagnosis and treatment to avert facial disfigurement, high recurrence rate and the likelihood of mortality associated with ameloblastoma in this population.[5] It has been reported that FNAC of this tumour may help to avoid hasty or unnecessary surgical biopsy, provide helpful information about the lesion, and ensure adequate period to prepare the patient for treatment.[20]

Most patients in this study presented late for treatment with long standing swellings. The tumours at presentation were large, mostly intrabony and occurred most commonly in the mandible. Only one peripheral tumour was found. There was predilection of the tumour for young adult males and the predominant imaging feature was multilocular radiolucency. These findings are comparable to previous reports on the clinical and radiological features of ameloblastoma.[6-8,21,24] Studies have recommended that clinical and radiological findings must be considered, to rule out entities with similar cytological findings, before a diagnosis can be made by FNAC. [22,23]

In this study, aspirates were mostly straw-coloured fluid. There were 2 (22.2%) cases of infected cystic ameloblastoma with grey coloured aspirates which on cytology consist of numerous inflammatory cells and necrotic debris. Dense inflammatory cell infiltrates and debris can obscure tumour cells in smears which may make FNAC challenging to use for cytological diagnosis of some lesions.[21] This was encountered in the cytological assessment of the 2 cases (22.2%) in this study which were however diagnosed cytologically as infected benign cystic tumour and inflamed benign tumour.

The cytological cellular patterns found in this study were similar to those previously reported in the cytological smears of ameloblastoma.[11-13] There was however a case that was cytologically misdiagnosed as a malignant salivary gland tumour (salivary gland adenocarcinoma) due to the presence of a few large cells among the hyperchromatic basaloid cells, which were thought to be glandular cells, derived from salivary gland parenchymal cells. It has been reported that large cells are occasional cell types found along with hyperchromatic basaloid cells in malignant ameloblastoma.[14] The patient was diagnosed histologically as ameloblastoma. However, a long-term follow-up was planned for the patient to detect any recurrence, malignant transformation or metastasis often associated with malignant ameloblastoma.[14,15]

In this study, the ameloblastoma was mostly cytologically diagnosed as benign cystic tumour following FNAC due to the slow growing nature, cystic/locular radiolucencies on imaging, aspiration of mostly straw-coloured fluid and the benign cytologic appearance of the basaloid cells. Only two (22.2%) cases had specific cytological diagnosis of cystic ameloblastoma consisting predominantly of basaloid and spindle cells. This is consistent with previously reported cytologic features of ameloblastoma.[12] Cytopathological diagnosis of ameloblastoma as a benign lesion was significantly associated with histopathological variants of ameloblastoma ($p=0.013$). A high sensitivity (88.9%), specificity (100%) and accuracy (88.9%) of FNAC for diagnosing ameloblastoma as a benign tumour were observed in this study. The sensitivity of 88.9% and specificity of 100% are comparable to the sensitivity of 86.6% and specificity of 100% reported by Kaliamoorthy (2013).[25] In contrast, the sensitivity in this study was lower than the 93.5% reported by Uçok et al, (2005)[26] in their diagnosis of ameloblastoma.

This preliminary study is limited because of the small sample size; however, this study recommends FNAC as an early preoperative diagnostic tool to assess the benign nature of tumours suspected to be ameloblastoma based

on the clinico-radiological features observed in the patients. The cytologic findings should be combined with the clinic-radiological features of tumours to preoperatively predict the histopathological diagnosis of ameloblastoma and for early preparation of the patients for treatment.

However, using FNAC for early cytological diagnosis of intrabony ameloblastoma at the initial stages of the tumour may be difficult to perform. Until the tumour causes thinning or destruction of the cortical plate of the jaw bone to allow for needle penetration.[27] This may be a draw back for FNAC diagnosis in early intrabony hard jaw tumours. Due to late presentation of most of the patients in this study, the tumours have destroyed and thinned-out bone at the tumour sites, from which aspirates were easily obtained.

In conclusion, the comparison of the findings of FNAC with the histopathological diagnosis of ameloblastoma showed significant association and a high accuracy for cytologically assessing and diagnosing the benign nature of ameloblastoma. Clinico-radiologic appearance should be considered in making the FNAC diagnosis. FNAC is useful as an initial early diagnostic tool for evaluating ameloblastoma or for excluding it as a locally aggressive benign lesion from other aggressive lesions suspected clinically as malignant lesions. Further studies with larger number of cohorts is suggested to further highlight the cytologic features of ameloblastoma and the accuracy of FNAC in the diagnosis of ameloblastoma.

Conflict of interest: None declared

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