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ROLE OF FINE NEEDLE ASPIRATION CYTOLOGY AS AN INITIAL DIAGNOSTIC TOOL IN MUSCULOSKELETAL TUMOURS

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Keywords:

Fine Needle Aspiration, Histopathological Examination, Bone tumours, Light Microscopy, Tumour Cytology.

Abstract

Purpose: Inadequate sampling, equivocal cytological features and lack of confirmation with histopathological examination perpetuate long-standing controversy regarding the utility of FNAC in diagnosing skeletal neoplasms. In this study, we tried to conclusively determine its utility and limitations by analysing the diagnostic concordance between FNAC and open biopsy in each of 216 cases of bone tumours.

Materials and Methods: 216 consecutive cases of clinicoradiologically suspected bone tumours were evaluated by FNAC and followed up with histopathology of the excised tissue from suspected lesion. The cytological diagnoses were classified as conclusive, doubtful and inadequate and were compared with that of histopathological examination.

Results: 97.8% of diagnoses (n=178) in the conclusive group (n= 182) showed cyto-histological concordance. Sensitivity in diagnosing malignant bone tumours from conclusive smears was 98.78 while specificity was 88.89. Doubtful group (3.24%, n=7) mainly consisted of cartilaginous tumours. The inadequate group (12.5%, n=27) had either scanty cells (37.04%,n=10) or hemorrhagic smear (62.96%,n=17).

Conclusion: When the findings are conclusive FNAC provides accurate diagnosis in suspected bone tumours which can be safely relied upon for planning further management. However, further diagnostic procedures in the form of HPE or cytogenetic studies should be sought in cases of equivocal results or inadequate samping.

Introduction.

Fine needle aspiration cytology is a method in which cells and tissue fluid are extracted from a tumour or lesion using a syringe and fine needle. It is a simple, safe and quick procedure. Its role as a diagnostic modality has been well established in tumours in soft tissue organs like breast, thyroid and lymph nodes. In cases of bone tumours as well, it has gained wide acceptance. Martin and Ellis[1] first applied this technique to the diagnosis of bone lesions in 1930. Since then, several published series have yielded overall accuracy values ranging from 51% to 100%.[2–21] However it is yet to be established how accurately FNAC can give conclusive diagnosis of bone tumours. There is no clear consensus yet whether FNAC can be the final and definitive investigation to diagnose a skeletal neoplasm or deny its possibility. Several factors have led to this. Performance and interpretation of FNAC of bony lesions require considerable experience and training. Inadequate sampling and equivocal cytological interpretation have long been impeding its widespread usage. Above all, to establish strong evidence, comparison of result of each and every case with that of a gold standard procedure, namely histopathological study is required. We have carried out the study over a period of five years consisting of 216 cases of suspected bone tumour and correlated the findings of each case to its clinicoradiological and histopathological findings to understand the role of FNAC in its entirety.

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Materials and methods

A total of 216 consecutive patients with clinico-radiological features of bone tumours were selected for the study. Written informed consent was obtained from each of the patients. The radiological criteria were thinned out cortex of bone or/and cortical break with or without soft tissue involvement.

After explaining the procedure to the patient, the lesion was located by palpation. The site of needle placement was marked and its direction and trajectory was ascertained. The exact location and trajectory depended on a number of factors. It had to be ensured that the fine bore needle encountered least resistance while reaching the tumour site, by passing the needle through the area where the bone destruction was maximum. It had to be away from the plane of neurovascular structures. The puncture site was kept, as far as possible in the line of incision for future surgery so that the possible spillage of the tumour cells might be excised *in toto* during the surgery. Effort was always made to aim the needle at the edge of the lesion as the centre of the mass was likely to contain only the necrotic tissue.

Adult patients did not need any local anaesthesia or sedation, but for children sedation was required. Skin disinfection was carried out with 5% providone iodine and spirit. A fine needle with plunger was inserted with the bevelled end up. It was passed into the mass. A 20cc syringe was connected and suction was applied with small amplitude to and fro oscillations to scrape off small bits of tissue and push the same into the needle barrel. In case of suspected highly vascular lesions, the procedure was carried out rapidly to avoid damaging the blood vessels ending in useless bloody tap. The suction was immediately terminated if a drop of blood or fluid appeared on the translucent part of the needle. After releasing the suction, the needle was gradually withdrawn. The aspirated material was expelled to a minimum of two glass slides before it dried. For each aspirate, four smears were prepared. Of these, two were air dried and stained by May-Grunwald-Giemsa method. The other two were immediately fixed in ethanol and stained later by Papaniculau's method. In addition to the above mentioned routine stains; cytochemical stains like reticulin (Gomori's), alkaline phosphatase, Periodic acid Schiff (PAS) with without diastase and mucicarmine were employed to support the diagnosis wherever necessary.

Reporting: the reports of the pathological specimens were classified into following groups:

1. Conclusive: Cytological findings are clearly suggestive of a diagnosis.
2. Doubtful: The cytological findings are equivocal and fail to point to a diagnosis.
3. Inadequate smear: The amount or quality of smear obtained precludes proper cytological examination.

In each of the cases, biopsy was carried out. Some of them (n= 74) had to be performed before the definitive surgery. In others specimens were obtained from the excised or curetted tissues during the definitive surgery. For histopathological examination, tissues were embedded in paraffin blocks, sliced into 2-3 micron sections and stained with routine Hematoxylin and Eosin staining and examined under microscope. The histopathological examination was carried out in blinded manner so as to eliminate the possibility of a biased presumption based on the cytological findings. Finally, the three methods of diagnosis viz. Clinicoradiological , Histopathological and Cytological findings were compared.

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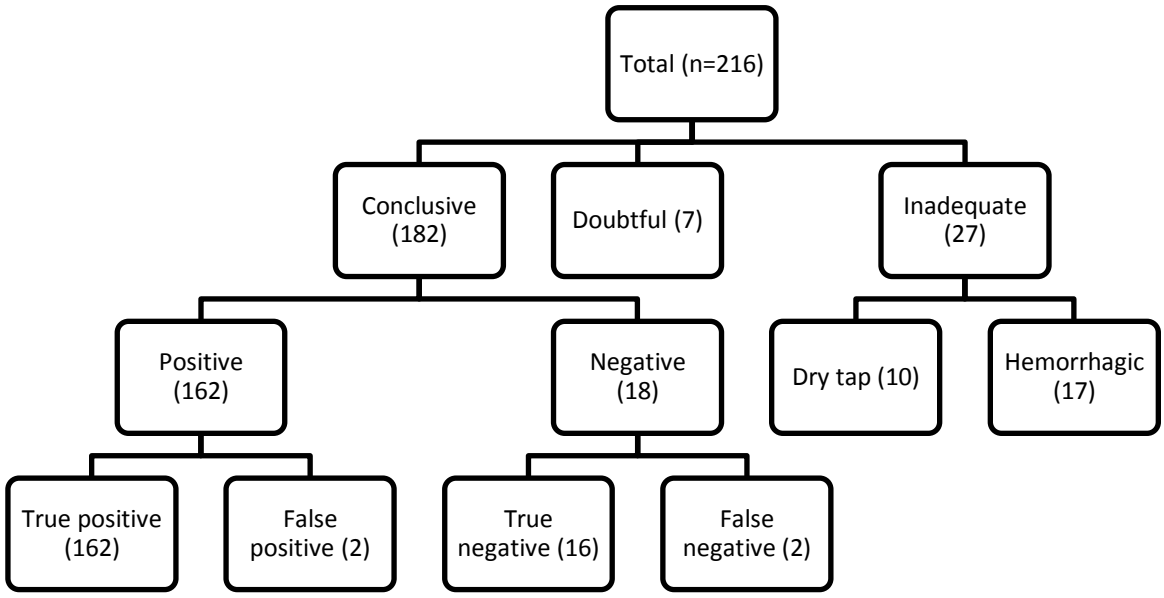

Fig:1.Flowchart Showing Study Design and Distribution of Results

Results and Discussion

The study population consisted of subjects from diverse age groups. The youngest subject was 4 years old and the oldest one was 76 years old (mean 31.4 years).It showed a slight male preponderance (57.41 % or 124 males v/s 42.59 % or 92 females. All the patients presented with swelling while some of them (96%) also had associated pain also. 6% of patients (n= 13) had pathological fracture. The mean duration of pain was 3 months (range: 14 days – 2 years). Figure 1 shows the distribution of the swelling and accordingly the site of aspiration

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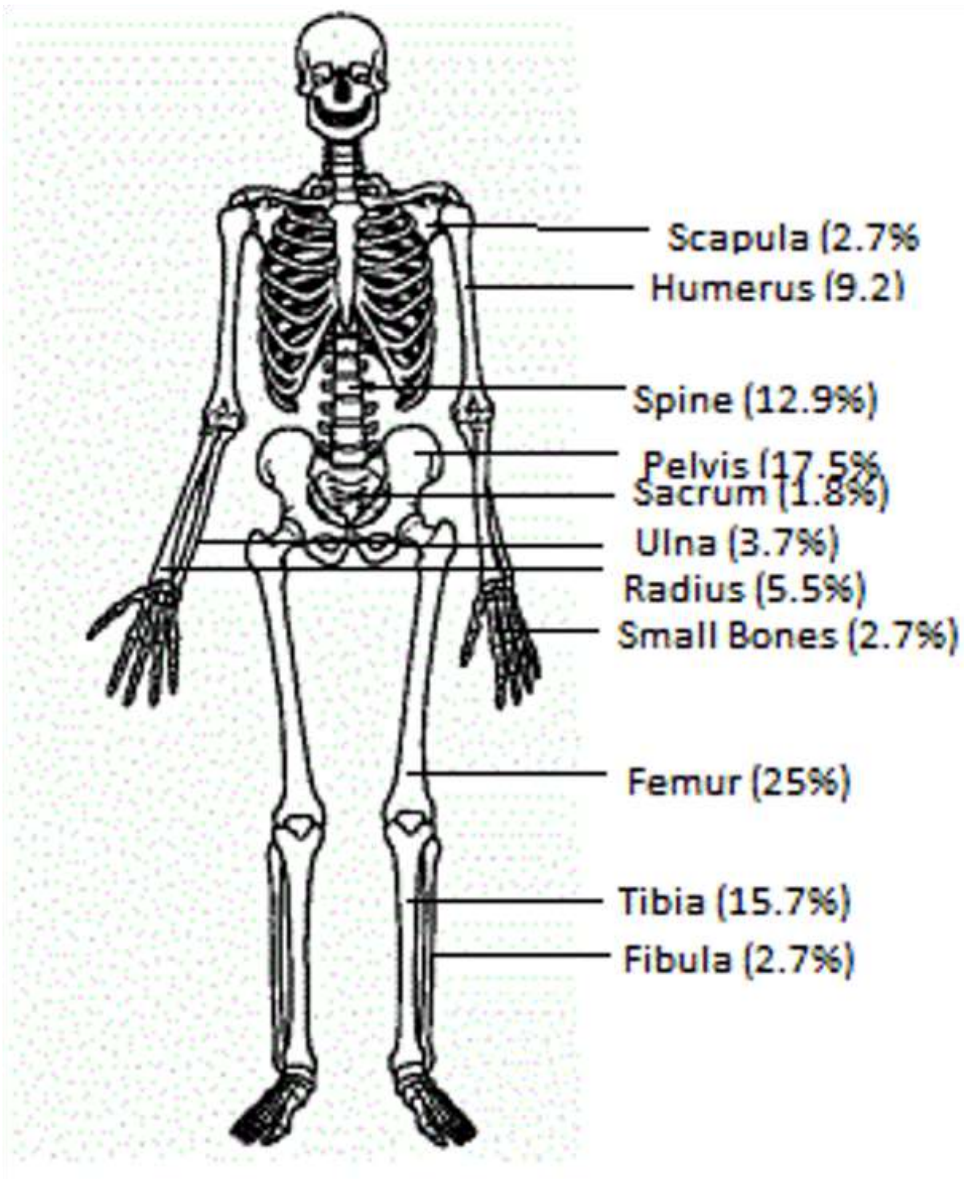

Fig2: Sites of Fine Needle Aspiration (with percentage of total)

Out of 216 aspirates, 189 (87.5%) yielded satisfactory smears. The rest 12.5% were unsatisfactory smears which consisted of blood in the smears (n=17) and scanty aspirated material (n=10).Out of the satisfactory smears the cytological examination conclusively diagnosed 182 cases (84.26% of total 216 cases), while in 7 cases the diagnosis was doubtful. On carrying out the histopathological examination, 178 cases out of 182 conclusive cases were verified to be correct with an accuracy of 97.8%. The cytological interpretation was 98.78% sensitive and 88.89% specific in diagnosing malignant bone tumours. The positive predictive value was 0.99 while the negative predictive value was 0.89. Table 1 shows the total final diagnosis of all the cases and those diagnosed by FNAC.

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Table 1 : The final diagnoses of all cases and correct FNAC diagnosis*		
Bone lesions as per Final Diagnosis (HPE)	**Final Diagnosis (n)**	**Diagnosed by FNAC**
Osteosarcoma	34	28
Metastatic Bone Tumour	39	37
Chondrosarcoma	28	19
Giant Cell Tumour	38	35
Ewing's sarcoma	17	14
Lymphoma of the Bone	13	12
Chondromyxoid fibroma	10	6
Synovial sarcoma	6	5
Plasmacytoma	6	4
Aneurysmal Bone Cyst	6	4
Fibrosarcoma	3	2
Osteomyelitis	9	7
Others	7	5
Total :	216	178
* All cases including conclusive, doubtful and inadequate smears.		

Osteosarcoma and giant cell tomours were the most common primary bone lesions. Figures 1-4 show their cytological and histological findings.

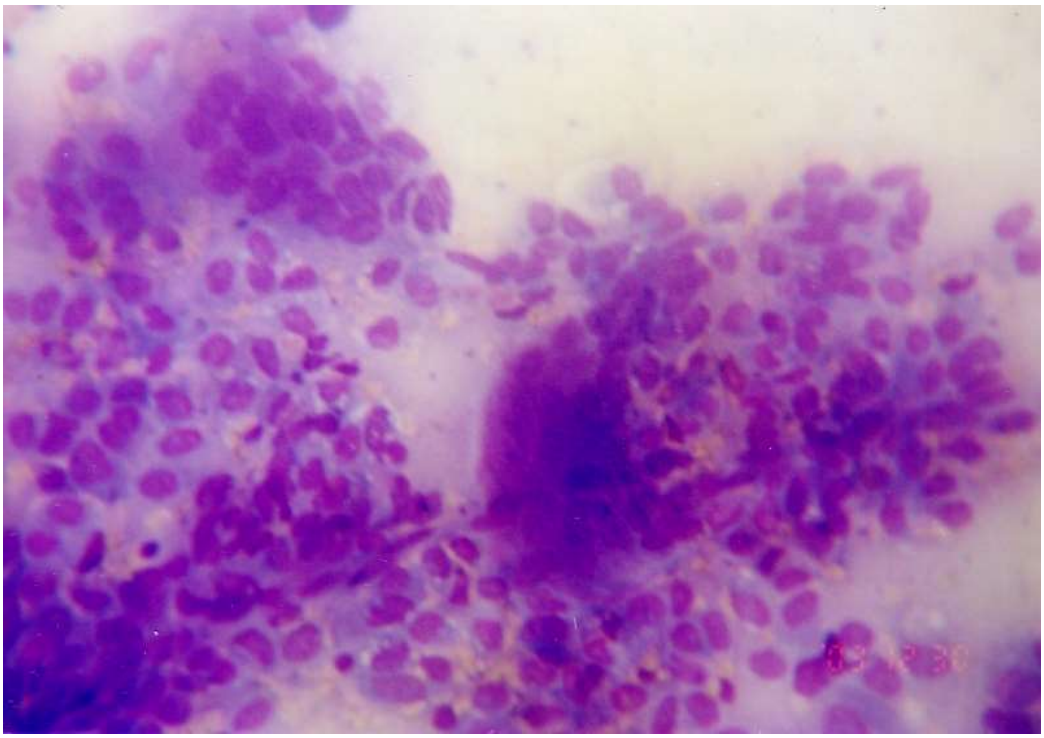

Fig:3 : Photomicrograph showing FNAC of Giant Cell Tumour MCG Stain (x200)

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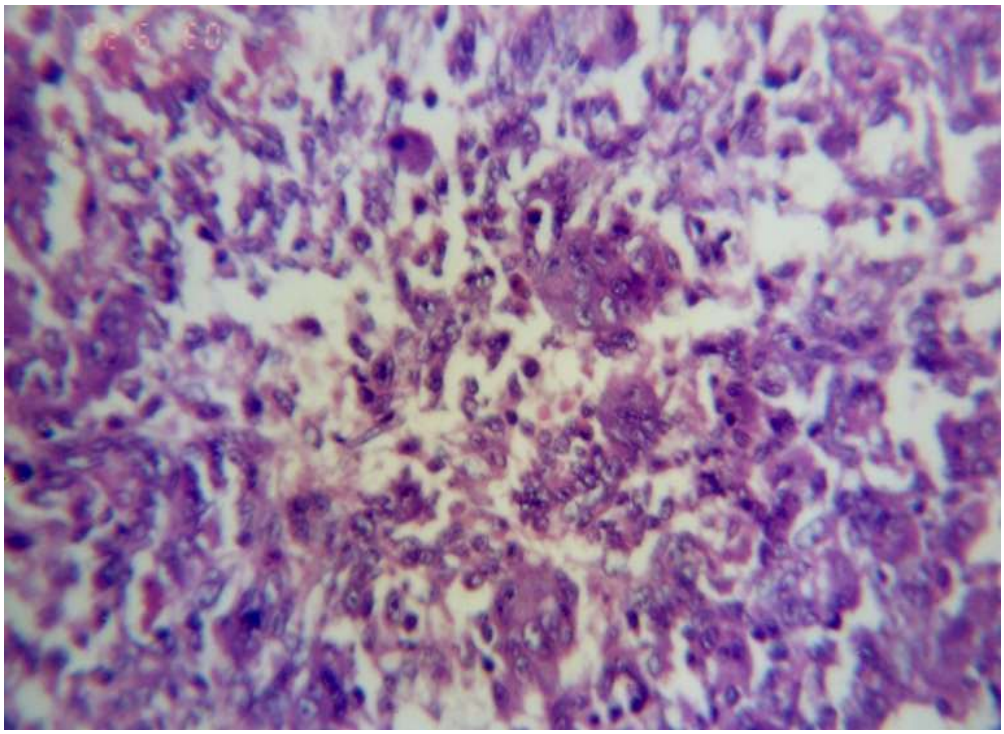

Figure 4: Photomicrograph showing histopathological section from the same lesion of Giant Cell Tumour, H&E (x200)

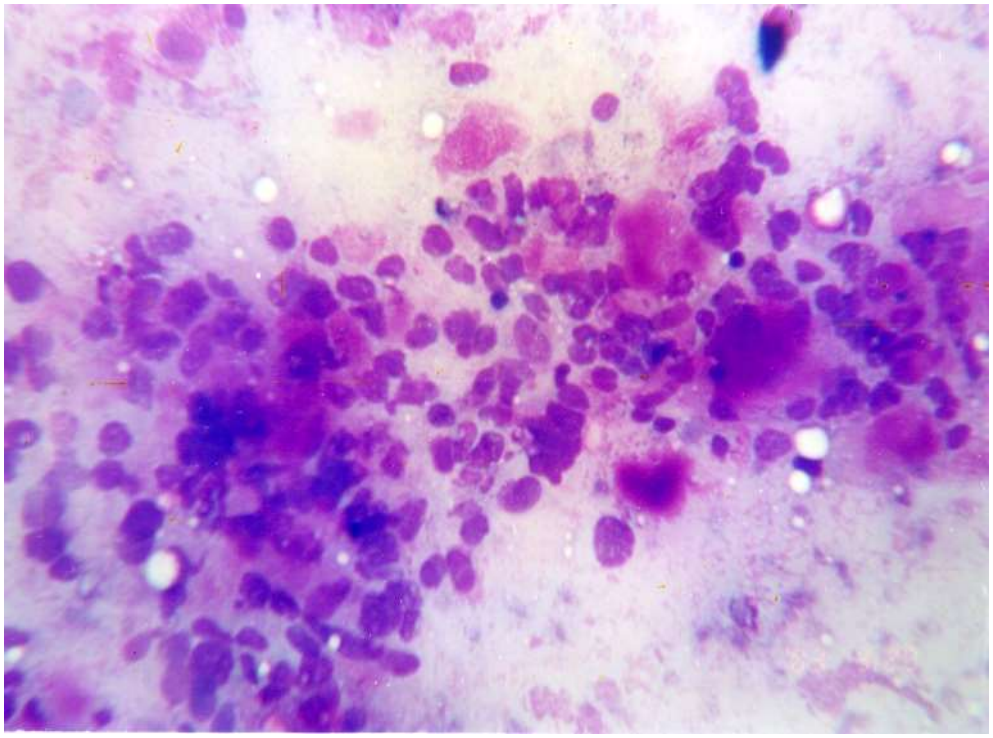

Figure 5 : Photomicrograph showing FNAC of osteocasrcoma MCG Stain (x200)

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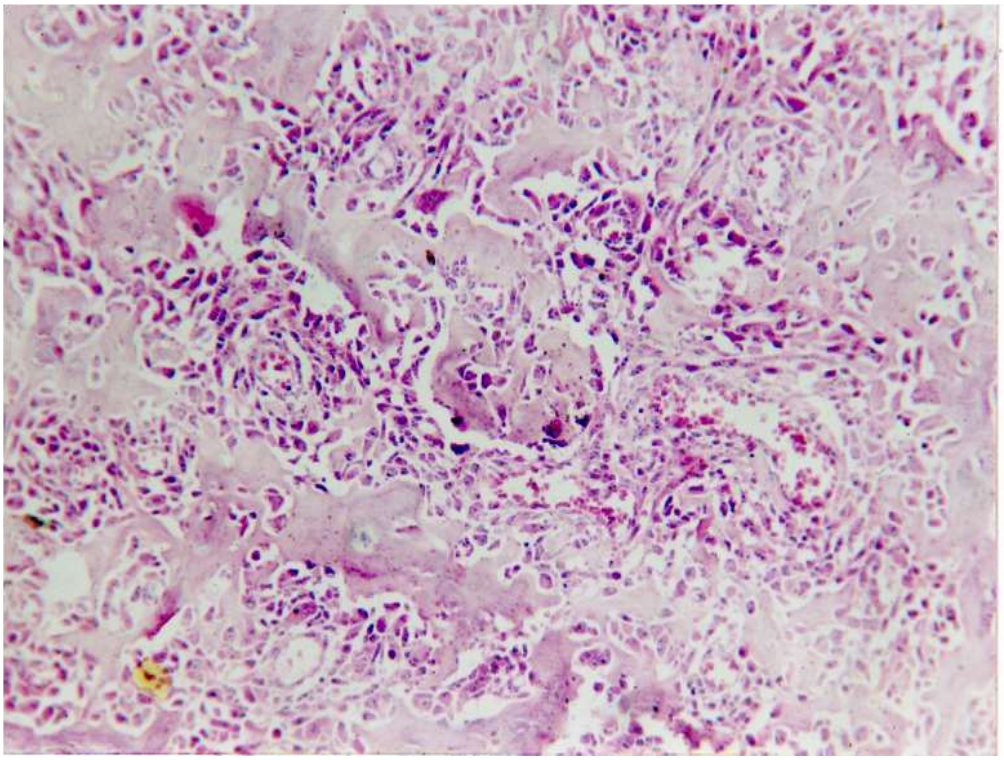

Figure 6: Photomicrograph showing histopathological section from the same lesion of osteosarcoma, H&E (x200)

There were two false positive cases. We investigated the discordant cases thoroughly to determine the contributory factors and limitation of the procedure. The first one was a case of osteolytic lesion in the D12 vertebral body which was suspected to be a metastatic lesion. Cytological features showed few mononuclear cells, some of which demonstrated moderate atypia and abundant necrosis, which should have been ideally classified as equivocal. Subsequent histopathological examination diagnosed it to be an inflammatory lesion. The second case was a suspected osteosarcoma in FNAC with immature woven bone at places. But in histopathological examination, it was diagnosed to be a heterotropic ossification.

There were two false negative cases in the study. The first case was a case of eosinophilic granuloma which was initially showed the charactarictics of inflammatory lesion in FNAC. The second case was diagnosed as a case of osteomyelitis in FNAC. But on histopathological examination it was diagnosed as Ewing's sarcoma.

All the metastatic tumours were diagnosed conclusively by FNAC.

The second group of the cytological interpretation consisted of those smears which revealed inconclusive findings. All those cases were diagnosed by the histopathological examination. Seven such cases were encountered. Table 2 shows the differential diagnoses in FNAC along with final diagnosis by subsequent biopsy.

Table 2 : Differential diagnoses in doubtful cases

SI No.	Differential Diagnoses	Final diagnosis (HPE)
1.	Giant cell tumour v/s Aneurysmal bone cyst	Giant cell tumour
2.	Giant cell tumour v/s Aneurysmal bone cyst	Aneurysmal bone cyst
3.	Giant cell tumour v/s Aneurysmal bone cyst	Giant cell tumour
4.	Chondroma v/s Chondrosarcoma	Chondrosarcoma
5.	Chondrosarcoma v/s Osteosarcoma	Osteosarcoma
6.	Chondroblastoma v/s Chondrosarcoma	Chondroblastoma
7.	Neuroblastoma of the bone v/s Ewing's sarcoma	Ewing's sarcoma

Many of the aspirations were exteremly paucicellular. Of these aspirations with scanty cells, the majority (78%) were oteosclerotic lesions. FNAC has a limited role in such cases unless supplemented by other techniques such as image guidance .The second category of the aspirations rendered inadequate smears were vascular lesions that led to a bloody tap, where blood obscured the whole tumour cytology. After clinicoradiological and histopatological correlation, such lesions were found to be simple bone cyst , aneurysmal bone cyst, telengiectatic osteosarcoma and giant cell tumour with large areas of heamorrage and necrosis.
Pain was the most common complaint after the procedure. 12% of cases (n=26) complained of severe pain while the rest experienced only mild to moderate pain. Hematomas appeared in 6% of cases (n= 13) all of which regressed spontaneously within ten days. The coagulation profiles of all patients were found to be normal. There was no incidence of nerve or vascular injury through the procedure.

Discussion

Tissue for diagnosis of bone lesions can be easily obtained by FNAC, core biopsy, or open biopsy as in lesions in other parts of the body. A closed biopsy is associated with a lower morbidity rate and is safer than open biopsy.[11] FNAC using a narrow bore needle is associated with a lower risk of tumour seeding in comparison to core[4, 6] or open biopsies.[11] Due to minimal disruption of the soft tissue barriers and spillage of the tumour cells, possibilities for the salvage of the affected limb improve compared to other more invasive procedures. As the aspiration wound is not endangered, treatment with radiation and/or chemotherapy can be initiated without any delay. It can drastically curtail the financial burden associated with bone tumours, as it can be performed as an outpatient procedure without the need of sophisticated equipments or anaesthesia. Complications are few and multiple specimens can be obtained without increased morbidity.[18] In addition, the adequacy of the specimens can be determined on spot by microscopic examination and repeat aspirations can be performed at the same sitting.
In worldwide literature review, it found that accuracy values for FNAC in diagnosing bone tumours have been from 51% to 100%. However in most of the studies, one to one histological confirmation was not performed, especially in those cases where cytological features gave a clear picture that corroborated with clinicoradiological suspicions. But that fails to provide a strong objective evidence of the accuracy of FNAC. This study differs from the previous ones as each of the cases included in the study was followed up with a histopathological examination regardless of the cytological findings or clinical and radiological charactaristics. Moreover the biopsy was performed in a blinded manner to preclude any bias. Even the smears which were found to be inadequate and those which gave false positive or false negative diagnoses were analysed so as to reach a conclusion as to understand the limitations of the procedure fully.
In a study by Agrawal et al involving 229 cases of FNAC, overall sensitivity and specificity were 86% and 94.7% respectively[23]. There was 1 false positive and 29 false negative report out of 159 specific morphologic diagnoses through fine needle aspiration.
In a study by Mehrotra et al involving 91 cases, authors found that sensitivity of FNAC was 93.3%, specificity 94.5%, positive predictive value 87.5% and negative predictive value 97.2% [24]. However only 65 cases out of total 91 cases underwent a histopathological examination.
Categorising cartilage lesions into benign and malignant groups posed particular difficulty in our study. Hypercellularity, plump nuclei, more than occasional binucleate cells, a permeative pattern, and entrapment of bony trabeculae aresome of the features that diagnose a malignant bone tumours cannot be conclusively observed in fine needle aspirations. Three of our seven cases in the doubtful group consisted of these lesions. Other lesions that can have confusing features in aspirated smears are those which reveal giant cells under the microscope. Whereas the presence of the cohesive mononuclear cells amidst giant cells suggests an osteoclastoma, the absence of same opens the possibility of several lesions eg aneurysmal bone cyst, brown tumour of hyperparathyroidism, chodroblastoma etc.. FNAC also failed to diagnose a case of expansile lytic lesion of the tibia of a five year old child whether it was Ewing's sarcoma or neuroblastoma of the bone.
Analysing the false negative cases, an inflammatory lesion as diagnosed by FNAC was later recognised as an eosinophilic granuloma in successive open biopsy. The second case was misdiagnosed as osteomyelitis of the femur as the cells were interpreted as inflammatory cells which on biopsy revealed characteristic cytology of small round cell. The first false positive case was a swelling over the proximal tibia, the aspirate of which revealed bits of osteoid matrix, interpreted as osteosarcoma. However biopsy revealed its true nature a heterotropic ossification with

abundant callus formation as a healing response to a pathological fracture at the site of a simple bone cyst. Some of these cases reflected the fact that the importance of clinical radiological findings cannot be overemphasised.
However, even core needle biopsy or open biopsy has considerable diagnostic limitations. The false positive and false negative cases in our study using FNAC correlate favourably when compared to those involving open or core needle biopsy.

Conclusion

Based on the finding of the study we propose the following recommendations :

1. FNAC should be performed as a first line diagnostic tool after clinico-radiological evaluation in cases of suspected bone tumours
2. If the smears are adequate and give definitive diagnosis, the morbidities associated with a separate preoperative histopatological examination can be safely avoided by reserving the latter for very exceptional cases.
3. Separate preoperative histopathological examinations are indicated in the following situations :
   a. When there is disparity between clinicoradiological and cytopathological diagnosis
   b. When no definitive cytopathological diagnoses can be reached upon
   c. When smears are inadequate and repeat aspirations are unlikely to improve the yield.

References

1. Martin HE, Ellis EB. Biopsy by needle puncture and aspiration. Ann Surg 1930; **92**: 169–81.
2. Coley BL, Sharp GS, Ellis EB. Diagnosis of bone tumors by aspiration. Am J Surg 1931; **13**: 213–24.
3. Snyder RE, Coley BL. Further studies on the diagnosis of bone tumors by aspiration biopsy. Surg Gynecol Obstet 1945; **80**:517–22.
4. Ottolenghi CE. Diagnosis of orthopaedic lesions by aspiration biopsy. J Bone Joint Surg Am 1955; **37**: 443– 64.
5. Schajowicz F, Derqui JC. Puncture biopsy in lesions of the locomotor system. Cancer 1968; **21**: 531–48.
6. Stormby N, Akerman M. Cytodiagnosis of bone lesions by means of fine-needle aspiration. Acta Cytol 1973; **17**: 166–72.
7. Schajowicz F, Hokama J. Aspiration (puncture or needle) biopsy in bone lesions. Recent Results Cancer Res 1976; **54**: 139–44.
8. Thommesen P, Frederiksen P. Fine needle aspiration biopsy of bone lesions: clinical value. Acta Orthop Scand 1976; **47**: 137–43.
9. Akerman M, Berg NO, Persson BM. Fine needle aspiration biopsy in the evaluation of tumor-like lesions of bone. Acta Orthop Scand 1976; **47**: 129–36.
10. deSantos LA, Murray JA, Ayala AG. The value of percutaneous needle biopsy in the management of primary bone tumors. Cancer1979; **43**: 735–44.
11. Murphy WA, Destouet JM, Gilula LA. Percutaneous skeletal biopsy 1981: a procedure for radiologists—results, review and recommendations. Radiology 1981; **139**: 545–9.
12. Agarwal PK, Wahal KM. Cytopathologic study of primary tumors of bone and joints. Acta Cytol 1983; **27**: 23–7.
13. El Khoury GY, Terepka RH, Mickelson MR, Rainville KL, Zaleski MS. Fine needle aspiration biopsy of bone. J Bone Joint Surg1983; **65**(A): 522–5.
14. Feldman PS, Covell JL. Cytodiagnosis of bone and soft tissue lesions by fine needle aspiration. Acta Cytol 1983; **27**: 558.
15. James LP, Frable WJ. Fine needle aspiration of bone lesions. Acta Cytol 1983: **27**: 559.
16. Xiaojing P, Xiangcheng Y. Cytodiagnosis of bone tumors by fine needle aspiration. Acta Cytol 1985; **29**: 570–5.
17. Layfield LJ, Glasgow BJ, Anders KH, Mirra JM. Fine needle aspiration cytology of primary bone lesions. Acta Cytol 1987; **31**:177–84.
18. Dollahite HA, Tatum L, Moinuddin SM, Carnesale PG. Aspiration biopsy of primary neoplasms of bone. J Bone Joint Surg 1989;**71**: 1166–9.
19. Layfield LJ, Armstrong D, Zaleski S, Eckardt J. Diagnostic accuracy and clinical utility of fine needle

aspiration cytology in the diagnosis of clinically primary bone tumors. Diagn Cytopathol 1993; **9**: 168–73.
20. Kumar RV, Rao CR, Hazarika D, Mukherjee G, Gowda BMG. Aspiration biopsy cytology of primary bone lesions. Acta Cytol 1993;**37**: 83–9.
21. Bommer KK, Ramzy I, Mody D. Fine needle aspiration biopsy in the diagnosis and management of bone lesions. Cancer (Cancer Cytopathol) 1997; **81**: 148–56.
22. Merce Jorda; Luis Rey; Andrew Hanly; Parvin Ganjei-Azar. Fine-needle aspiration cytology of bone: Accuracy and pitfalls of cytodiagnosis. Cancer. 2000;90(1):47-54
23. Agrawal PK, Goyal MM, Chandra T, Agrawal S: Predictive Value of FNAB of bone lesions. Acta Cytol 1997, 41:659-65
24. Mehrotra R, Singh M, Singh PA, Mannan R, Ojha VK, Singh P. Should fine needle aspiration biopsy be the first pathological investigation in the diagnosis of a bone lesion? An algorithmic approach with review of literature. CytoJournal 2007;4:9

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