



Role of Aspiration Cytology in Diagnosis of Bone Tumors and Tumor Like Conditions

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ABSTRACT

Introduction: Diagnosis of bone tumor is based on clinical, radiological and laboratory investigations which includes histological examination. Open biopsy has its own drawbacks such as seeding of tumor cells in an avascular plane and making them resistant to curative radiotherapy and chemotherapy. Placing biopsy incision in area may interfere to final surgical planning, risk of anesthesia, necessity for hospitalization and time involved in preparing paraffin wax block & reporting, as well the cost involved. To avert above problems we banked on newer least invasive, low cost, quick reporting percutaneous fine needle aspiration cytology (FNAC). **Aim and Objective:** our aim was to standardize FNAC, find out its reliability and specificity in relation to basic pathological processes such as inflammatory, benign and malignant bone lesions. **Material and method:** Fine needle aspiration was performed as OPD procedure, under aseptic condition, without anesthesia by 10ml syringe with 20-22G needle. Aspirated material was spread over 2 to 8 slides. Slides were fixed in absolute Alcohol, then smear stained in H&E stain. The slides were examined under Microscope and Patients were given cytological report within next day. Diagnosis was based upon cellularity and findings were labeled as diagnosis of bone tumors as definite, indefinite, inflammatory and unsatisfactory smears. Repeat aspiration was done in indefinite and unsatisfactory smears. **Observation:** A total of 54 patients presenting as skeletal lesion were subjected FNAC. Based on cytological smear final diagnosis was Primary malignant tumor in 14 cases, osseous metastasis in 4, skeletal soft tissue malignancies 8, Primary benign bone tumors including Giant cell lesion 15 cases, primary benign non osseous skeletal tumors 1, inflammatory 6 and unsatisfactory 6 cases. More than 70% of primary malignant and benign bone lesions were less than 30 years age. Male to female ratio was 1.8:1, almost twice common in male. Among benign bone tumors, Giant cell lesions were more predominant. Lower extremity was commonest site for bone tumors. 73% long bone tumors were in Femur, Tibia, Fibula, while in flat bones Pelvis was commonest. No significant complication reported with FNAC. Repeat aspiration was done in 6 cases due to their discrepancy in clinical findings and cytological findings or unsatisfactory smears. **Discussion:** In our series of 54 patients having bony lesion were subjected FNAC. Cytological diagnosis confirmed 14(25.9%) as primary malignant bone tumors, 4(7.4%) metastatic bone tumors, 8(14.8%) soft tissue skeletal tumors, 15(27.8%) Primary benign bone tumors, 1(1.9%) primary non osseous tumors, 6(11.1%) inflammatory and remaining 6(11.1%) diagnosis could not be confirmed. Amongst primary malignant bone tumors, osteosarcoma and Ewing's tumor were common in first two decades and mostly long bones were involved. Giant cell tumors were predominant in benign lesions of bone and young adults. Overall accuracy of fine needle aspiration cytology was 88.9% as compared of needle biopsy which was 73% only. **Summary and conclusion:** Fine needle aspiration cytology is performed as OPD procedure with 20-22G needle, tissue immediately stained and reports are provided by the next day. With accuracy of 88.9%, FNAC can be better choice than open Biopsy. Even in doubtful cases or unconfirmed diagnosis it can be repeated without any major problems. This does not interfere with future surgical planning for tumor management. Thus we can conclude FNAC as simple, cost effective, safe, least time consuming procedure with better reliability in experts hand.

KEYWORDS : bone lesions, fine needle aspiration cytology(FNAC)

Introduction

Diagnosis of skeletal lesions is based upon clinical examination, roentgen graphic examination and laboratory investigations which includes pathological, bacteriological, chemical and histological tests. The clinically and radiologically doubtful lesions are subjected to Biopsy. The most protocols for the staging of primary malignant bone tumors require accurate histological diagnosis. In deep seated lesions, such as Bone, the open biopsy is almost a major procedure (Trott & Randall 1979; Young et al. 1981). The surgical exploration has its own drawbacks such as seeding of tumor cells in an avascular plane and making them resistant to curative radiotherapy and chemotherapy. Placing of biopsy incision in an area may interfere for subsequent surgical planning, risk of general or local anesthesia, the necessity for hospitalization, the time involved in preparing paraffin wax blocks, and of course cost involved in it. In spite of so many pitfalls, open biopsy is not 100 percent correct. Mankin and associates (1982) found 18.2% major diagnostic errors based on 239 cases done by 40 orthopedics surgeons who were members of musculoskeletal tumor society. To avert above problems we banked on newer procedures percutaneous needle biopsy and fine needle aspiration cytology (FNAC). Fine needle aspiration has been employed as a routine technique in diagnosis of soft tissue mass all over the world with high degree of reliability and acceptability. Its use in bone at the site of focal radiographic abnormality has been demonstrated in some earlier studies such as Coley (1934), Snyder & Coley (1945), Ottolenghi & Aires (1955). There has been a renewed interest in this procedure and a number of reports has been published from west such as Schazowicz & Derqui 1968; Hajdu & Melamed 1971; Stromby & Akerman 1973; Akerman & associates 1976; Thommesen & Frederiksen 1976; de Santos associ-

ates 1978; and El Khoury et al. 1983. However there is marked paucity of reports from India about its uses in skeletal lesions.

Aim and objectives:

1. To standardize the technique of FNAC.
2. To find out the reliability of aspiration cytology diagnosis in relation to basic pathological processes such as inflammatory, benign and malignant bone lesions.
3. To find out the specificity of the technique.
4. To find out the criteria for sub classification of giant cell lesions and osteosarcomas.
5. To find out the efficacy of aspiration cytology in grading of giant cell tumors.
6. To find out the draw backs and complications of fine needle aspiration.

Material and method

A total of 54 patients with suspected tumors and tumor like conditions of bone attending orthopedics departments of CIMS&H Lucknow, HIMS, Ataria, Sitapur Road and MIMS, Barabanki Road, Lucknow, between July 2012 To June 2015, were included in present study.

Inclusion Criteria:

Clinical and Radiological examination: the patients were examined in detail. The examination included the age, sex, location of tumor. Careful assessment of general condition of patient, local examination of swelling, and evidence of any concomitant lesion elsewhere, Radiograph of Local part and Chest X ray was taken. Routine Hematological and relevant Biochemical tests were carried out.

Procedure of Fine Needle Aspiration Cytology (FNAC):

It was done in all the 54 cases. Although several specially designed aspiration syringes with special attachment are available, for aspiration, but in this study an ordinary 10 ml syringe with 20 to 22 gauge has been used under all aseptic precautions without any anesthesia and mostly as OPD procedure. The site of aspiration was determined with the help of radiographs and the shortest path to the lesion was chosen. Due care was taken to avoid injury to Neurovascular structures. Periphery of lesion was usually selected in order to avoid the aspiration from central necrotic area. The best site for aspiration were those areas where there were break in continuity of cortex or where tumor as about to extend in soft tissues. Having cleaned skin with Savlon, Iodine solution and spirit the swelling was localized and held with left hand. The piston was completely pushed in the barrel of syringe and needle was hermetically sealed with syringe. The needle was then pushed inside the swelling keeping uniform firm pressure so as cortex pierced, and plunger was partially withdrawn creating negative pressure. Needle was kept inside cortex and with maintaining negative pressure it was moved in different directions. It was done so, to sample different areas of tumor and also to dislodge the hard tumor cells. Needle as given rotatory movement while maintaining the suction. vacuum then was released before withdrawal of needle to avoid sucking of other tissues in needle pathway. This ensures that only required tissue material is collected from lesion and aspiration is stopped once it is visible in hub. occasionally when aspiration found dry, then two or more areas were aspirated from different sites of lesion using separate needles. Needle was then detached from syringe and plunger was filled with air, then needle was reattached . gently plunger was pushed in and content of needle was blown over clean microscopic glass slide the content was gently spread by pushing other slide over it and pulling in opposite directions apart. Thus two slides were prepared from each aspiration. However those aspirations hen sample as blood mixed, six to eight slides were prepared. The wet slides were immediately put in 95% Alcohol for fixation as has been advocated by Kline and Neal[198]and Owen et al (1980)and then stained with Hematoxylin and Eosin stain. Having stained the smears by above method the slides were examined under microscope. All the cases the report of aspiration cytology was provided within 24 hrs. Hence, cytological diagnosis was unbiased with histological findings. The smears were assessed for both sensitivity and specificity. The aspiration smears has been studied in detail about their following cellular characterization:

1. **Cellularity:** this was assessed as scanty, moderate and marked. In mild only few small sized clumps and occasional isolated cells were seen under low power field. In marked cellularity , the smears were full of tumor cells, present in clumps, as well as isolated. The moderate has in between the two.
2. Presence or absence of osteoid/chondroid
3. Presence or absence of osteoblasts.
4. Presence or absence of Osteoclastic cells(giant cells)
5. Presence or absence of Pleomorphic cells and their counts
6. Presence or absence of spindle cells.
7. Tumor giant cells-multi or mono nucleated but bizarre pattern, nuclear overlapping present or absent.
8. Relation of Giant cells with stromal cells.
9. Presence or absence of inflammatory cells and types –Polymorph ,Lymphocytes, macrophage and Plasma cells.
10. Whether tumor cells were present in clusters and size of cell clusters.Cluster was small if 10 to 20 cells seen and it was large cluster if more than 20 cells were seen.
11. Presence or absence of debris and hemorrhage in background.

The above cytological characters have been noted in each case and the observation correlated with the histological findings. The aim was to find out whether one particular tumor present some specific cytologic features on which diagnosis could be based. Broadly the tumors were classified under following headings:

Definite Diagnosis of Malignancy—in malignancy cellular smears with large clumps, cells with larger than counterpart ,loosely packed, with cellular pleomorphism, and with osteonucleosis, prominence of nucleoli and nuclear membrane irregularity, has been regarded as malignant, the presence of debris being an indirect indicator of malignancy(Melcher et al.1981).Further exact characterization of tumor was also done wherever possible.

Indefinite diagnosis of malignancy: included two features of malignancy as mentioned above , but are not diagnostic of malignancy, as most of the time benign cells were seen to coexist and the suspicious ones were not in plenty (Webb 1970).

Benign Non-inflammatory smears; smears with no malignant cells and at the same time having sufficient number of cells with features of benign parent cells have been regarded as 'benign'. The features such as normal cell size with no pleomorphism, regular nuclear membrane and chromatin, absence of debris, have been the criteria for grading smears into this group (Kline 1981). In this also further characterization was done e.g if myxoid stroma with some binucleated cells were seen then tumor was diagnosed as Chondroma. Significant presence of multinucleated giant cells lead to the diagnosis of giant cell lesions.

Inflammatory smear: They are the smears which had either acute or chronic inflammatory exudates with or without any epithelial or mesenchymal cells. A smear has been labeled as acute or inflammatory (abscess), when it was full of innumerable polymorphs with occasional parent cell in the background. Chronic inflammatory smears showed polymorphous inflammatory cells with fair number of histiocytes. In specific chronic inflammatory cells , there were epithelioid cells and Langhans as giant cells as well.

Unsatisfactory smears: the term has been used for smears having an insufficient number or absence of cells.Some of these contained lot of blood and tissue debris (Duguid at al. 1979, Melcher et al. 1981).

Further the clinical significance of the cytological observation was evaluated according to Thommesen and Federiken(1976).the finding will be interpreted as:

- **Decisive**-which directly leads to further diagnostic and therapeutic procedures. It may be in contradiction with clinical diagnosis.
- **Supportive**-which supports but do not change the diagnosis.
- **Inconclusive** – No material in slide when there is strong clinical suspicion.
- **Misleading**- which are truly false positive or false negative.

Observation

A total of 54 patients presenting as skeletal lesion in the OPD and IPD in Department of Orthopedics were subjected to Fine Needle Aspiration Cytology(FNAC).Repeat aspiration was done in 6 cases. The verification of cytological diagnosis was done either by tissue diagnosis or clinical course of the disease including clinical and radiological findings.

Based on these assessment the final diagnosis was primary malignant tumors in 14,osseous metastasis in 4 ,skeletal soft tissue malignancies 8, primary benign bone tumors including giant cell lesions 15,primary benign skeletal (non osseous) tumors 1,inflammatory 6 and unsatisfactory in 6 cases. Age/sex ratio-more than 70% of primary malignant bone tumors and 50 % of non-osseous malignant skeletal tumors came within 30 yrs of age. Benign tumors were also more common in younger age groups. Almost 58% osteosarcoma and 82% Ewings' sarcoma presented in first two decades where multiple myeloma ,synovial sarcoma came later in life. The tumors were two to four times more common in males than females. In metastatic group all the patients were more than 40 years in age and male: female ratio was 4:3. In soft tissue sarcoma 68.4% were between 21-40 yrs of age and males were predominant 13:6 (M:F).

Primary benign tumors total 15cases,included 9 cases as giant Cell Tumors, 3 chondromas and one non-ossifying fibroma, one osteochondroma and one Unicameral Bone Cyst. About 80% Giant Cell Tumor peak incidence was 21-30 years age with overall prevalence in males(Table 4).The benign non- osseous tumors was neurofibroma age less than 30 years and male .

Location of tumor : lower extremity was commonest site for bone tumors. Ten (73%) of long bone tumors were in femur, tibia and fibula, in flat bones pelvis was commonest location. All the cases of osteosarcoma were in long bones 83% were around knee joint, in contrast 55% of Ewing's sarcoma and three cases of multiple myeloma were in

flat bones .Out of 7cases of metastasis 5 (71.42%) were in flat bones. 74% sarcomas were in long bones, 10% in short bones and 16% were in flat bones. Primary Benign bone Tumors 24(86%) were in long bones ,3(11%) in flat bone and short bones only 3% .In giant cell lesions the upper extremity was more involved than lower extremity. There were 2 cases with benign Nonosseous tumors ,whereas 6 cases were diagnosed as Inflammatory, 7 cases was reported as unsatisfactory.

Aspiration cytology finding in different groups: Primary Malignant bone tumors diagnosis was correlated with diagnostic cytology and X-ray findings, but confirmed by histology.

Complication of aspiration cytology: No significant complications except mild hemorrhage which was observed in three cases. This can be stopped after direct pressure bandage. One 60 year male fainted due to Transient Vasovagal stimulation or apprehension. And became normal after lying down in bed for 10 minutes.

Gross character of aspirate: Majority of aspirate was hemorrhagic except inflammatory lesions, unsatisfactory group and benign bone tumors. In unicameral bone cyst aspirate was watery and plentiful. Macroscopic examination gave some idea of cellularity. In 6 cases aspiration was repeated due to either discrepancy in cytological and clinical findings ,or very scanty malignant cells in aspirate, or unsatisfactory smears. It has been seen from the (table 1), that out of 6 repeat aspirations diagnosis changed. The smear having doubtful malignant lesions ,in first aspiration were confirmed by repeat aspiration in 5(83%)cases, whereas 1 case repeat aspiration was not helpful.

Cytological accuracy in different histological groups at a glance:

The correct and incorrect diagnosis in various groups is shown in (Table 2).Over all 43cases were diagnosed correctly, whereas 7 cases were unsatisfactory,2 false positive and 2 false negative. Among unsatisfactory 7 cases, 4 were found inconclusive on basis of clinico-radiological grounds, and one each were osteosarcoma, soft tissue sarcoma and GCT. The cytological accuracy ranged between 82.5% to 100%,whereas specificity ranged from75% to 100%.The benign non osseous and osseous lesion could not be specified except Chondromas. On comparing the cytological and needle biopsy findings with that of open biopsy it was seen that cytological biopsy was correct in 73.35% cases, wrong in 13.35% cases and unsatisfactory in 13.3%cases. In contrast needle biopsy gave correct diagnosis in 47% cases only. Out of 53% incorrect cases diagnosis was not correct probably due to insufficient tissue material.

Table 1: findings of first and repeat aspiration with comparison to Histological diagnosis:

S. No.	First aspiration	Repeat aspiration	Histological diagnosis
1	Nonspecific benign	No malignancy	Ossifying Fibroma
2	Unsatisfactory	GCT gr III	GCT gr 1
3	Blood clot	Aneurysmal bone cyst	Aneurysmal bone cyst
4	Unsatisfactory	Nonspecific smear	Chronic oesomyelitis
5	Unsatisfactory	Chondromas	Chondroma
6	? sarcomatus lesion	Osteosarcoma	Osteosarcoma

Table 2 : showing overall Cytological Accuracy in different groups.:

	Final Diagnosis	Correct Diagnosis	False negative	False positive	Unsatisfactory
1.	Primary malignant bone Tumor	%	%	%	%
	Osteosarcoma	82.5	8.75	-	8.75
	Ewing's sarcoma	100			
	Multiple myeloma	100			
	Synovial sarcoma	100			
2	Metastatic	85.7	14.3		

3	Soft tissue Sarcoma	95			5
4	Primary Benign bone tumors				
	Giant cell tumor	90		5	5
	Other lesions-ABC, etc	100			
5	Benign non osseous tumor	100			
6	Inflammatory	91.7		8.3	
7	Unsatisfactory	--			100

Table 3: showing incidence of primary malignant and benign bone tumors

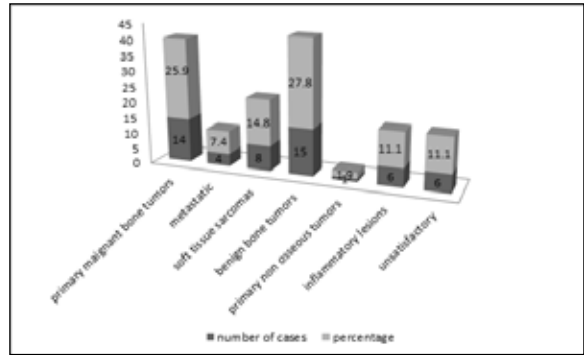
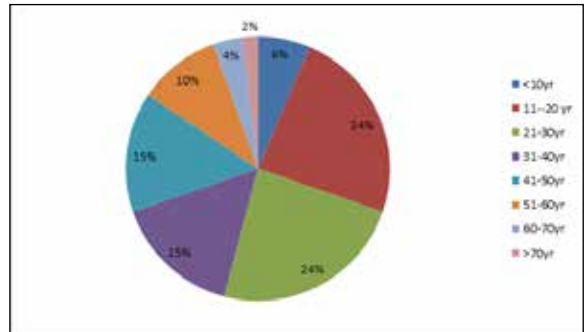


Table 4: Agewise distribution of cases



Discussion:

Amongst 54 patients having tumors and tumor like lesion of skeletal system 14(25.9%) were primary malignant bone tumors,4(7.4%) metastatic bone tumors,8(14.8%)soft tissue skeletal tumors,15(27.8%) primary benign bone tumors ,(1.9%) primary non osseous tumors,6(11.1%) inflammatory lesions and remaining 6 cases(11.1%) diagnosis could not be confirmed. Amongst primary malignant bone tumors osteosarcoma and Ewings' sarcoma were almost equally seen. However ,if we take into account of all specimen tumor lesions,12.6% were osteosarcoma,11.5% were Ewings'sarcoma,3.1% multiple Myeloma,1% synovial sarcoma and 7.3% were Metastatic tumors. Snyder and Coley (1945) reported on 474 bone tumors which comprised of 27.8% chondro &osteosarcomas,8.8% Ewings ' sarcoma,8.1% multiple myeloma,7.3% GCT,22.6% metastatic and 24.8% included other benign bone tumors and inflammatory lesions. Ottolenghi &Aires (1955) reported in 1061 bone lesions and of this 145 (13.67%)were primary malignant bone tumors. Out of this primary malignancies osteosarcoma were 28%,chondrosarcoma 10%,multiple myeloma 36%,Ewings 14%,reticulum cell sarcoma 12.3%.Thommesen & Fredriksen(1976) in his series of 85 cases found 12 primary malignant bone tumors,43 metastatic bone tumors,13 primary benign bone tumors and 17 miscellaneous lesions. Akerman et al (1976) reported on 150 bone lesions and of these 25.3% were primary malignant bone tumors,26% primary benign bone tumors and 48.7% metastatic tumors. Frible(1976) reported on 10 bone lesions ,of this 10% were primary malignant ,10% benign bone tumors and 80% metastatic tumors. De Santos et al.(1978) studied 91 bone lesions and of these 20% were primary malignant and benign bone lesions,74% were metastatic tumors and 6% were inflammatory lesions. El Khoury et al.(1983) found 13 primary and 57 metastatic bone lesions in 70 cases.

The extremely low incidence of metastatic bone tumors in our present study, as compared to others may be due to the reason that spinal lesions which are common site for metastasis were mostly not studied in this series. The other possibility may be that patients with advanced malignancy with bony metastasis usually has paralysis, pathological fractures limiting their transfer to the hospital.

In our series soft tissue sarcomas were 25.9% with known diagnosis. These patients presented as skeletal tumors with or without bone infiltration. Ottolenghi & Airds (1955) reported incidence fibro sarcoma in 11 cases out of 1061 bone lesion. Hadju & Melamed (1973) have reported on 190 bone and soft tissue tumors without any clarification. Hence the exact comparative incidence of soft tissue and bone tumors presenting as skeletal lesion can not be commented upon.

Age & sex ratio: maximum cases were between age group 11-30 years age. The primary bone tumors are known to occur in younger age group. Male were

almost twice more commonly affected as compared to females in present study. (1.8:1). The male predominance has been observed by all others also (Snyder & Coley 1945; Ottolenghi & Airds 1955; Moore et al: El Khoury et al. 1983).

Location of tumor: in present series 67.9% bony lesions were located in long bones, 22.9% in flat bones, 3.7% in short bones and 5.5% in joint. Snyder & Coley (1945) aspirated 567 sites in 474 patients and his series it was 49% long bones, 49% flat bones and 2% short bones. Thommeson & Fredriksen (1976) reported on 91 bone lesions and of these 40% were extremities and 60% were flat bones. In present study long bones were more commonly affected than flats in comparison to others. However lower extremity were more commonly affected than upper extremity in conformity to other reports also. Overall accuracy in present study was 82.7% by aspiration cytology as compared to needle biopsy it only was 73%. In other studies needle biopsy accuracy was in range of 66% to 97%.

SUMMARY AND CONCLUSION:-

A total of 54 patients both male and female, having skeletal lesions, and attending in and out patient department of orthopedics were performed fine needle aspiration biopsy followed by cytological examination (FNAC). 10ml syringe with needle size of 20-22 gauge used for aspiration under antiseptic precaution without any anesthesia. Prior X-ray was done to localize tumor lesion. All aspirated needle material was utilized for making slides. In general 2 to 8 slides were made in each patients. The slides were fixed in 95% ethyl alcohol for minimum of 15min to 24 hrs. The slides were stained by H&E stain in all cases. Reporting was done on aspiration slides on very next day. Final diagnosis in 54 skeletal lesions was 14 primary malignant bone tumors, 4 metastatic bone tumors, 9 soft tissue sarcomas, 14 benign bone tumors, 1 primary non osseous benign tumors, 6 inflammatory, 6 unsatisfactory in which the aspiration was found to be unsatisfactory. Repeat aspiration biopsy examination in 6 cases was done, in 5 cases diagnosis confirmed but no definite opinion could be made in one case.

Primary malignant bone tumors were more common in younger age group whereas metastatic bone tumors were frequent in middle or old age.

In primary malignant bone tumors 58% osteosarcoma and 82% Ewing's sarcoma were in first two decades, whereas multiple myeloma and synovial cell sarcoma were seen in later decades. soft tissue sarcoma were most common in 21th to 51st years age. Giant cell tumors presented most frequently 20 to 40 years. Male : female ratio showed male preponderance. 74% long bones in comparison to 25% flat bone with few short and joint tumors. In long bones Femur, tibia and Fibula accounted 50%.

Cytologically osteosarcoma was diagnosed irregular bizarre cells with osteoid, Ewings with high cellularity and scanty cytoplasm. Multiple myeloma were diagnosed by presence of typical or atypical Plasma cells, synovial cell sarcoma did not show biphasic pattern but mesenchymal malignant could be diagnosed. Soft tissue sarcoma were diagnosed on presence of malignant spindle shaped cells in clusters without presence of tumor giant cells or osteoid. The metastatic carcinoma

were diagnosed by presence of epithelial clumps having same features of primary tumors. large amount of eosinophilic cytoplasm was seen in squamous cell carcinoma. Amongst the benign lesions chondromas were diagnosed by presence of mono or binuclear cells against myxoid matrix. The Giant cell tumors were diagnosed due to presence of multinucleated Giant cells and stroma cells. The grading was correct in 75% GCT and incorrect in 25% cases. other benign tumors had nonspecific findings characterized by some osteoblasts, osteoclasts, histiocytes and small oval cells. Inflammatory lesions were diagnosed by presence of inflammatory cells with or without necrotic debris.

Diagnostic accuracy in aspiration cytology was 88.9% to 100%. Needle biopsy gave correct diagnosis in only 73% cases as compared to 80.7% of aspiration cytology. There were no significant complications in this study except minor pain, erythema local swelling in few, thus it can be concluded that fine needle aspiration cytology is a simple and reliable procedure in competent hands. It can be done as out-patient procedure thus saves hospital stay. The patient can be well prepared in advance and it helps in better planning of subsequent treatment modality. It is low cost and quicker time consuming procedure. In many cases it prevents the complicated surgical procedures which are often done only for establishing the diagnosis which is essential for definitive therapy.

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