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THYROID FNAC: EXPERIENCE WITH THYROID BETHESDA SYSTEM IN PRACTISE

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ABSTRACT

The Bethesda system of classification of thyroid lesions as proposed by National Cancer Institute (NCI) [1] is excellent for reporting thyroid fine needle aspiration and demonstrates that the additional category follicular lesion of undetermined significance (FLUS) on the diagnosis of follicular lesions helps in better classification of thyroid fine needle aspiration cytology (FNAC). This prospective study was conducted on a series of 151 cases over a period of 2 years from 2007 to 2009 in the department of Pathology, Dr. B.R. Ambedkar Medical College, Bangalore to define the accuracy of FNAC based on Bethesda system. The distribution of the lesions was as follows: unsatisfactory 8.6%, benign 76.8%, FLUS 3.9%, follicular neoplasm 2.6%, suspicious for malignancy 0.6% and malignant 7.2%. There was an excellent association between the categories in predicting benign and malignant lesions. However, one case of follicular carcinoma and papillary carcinoma each was reported negative for malignancy on FNAC, hence two false negative cases were found. There were no false positive cases reported on cytology in our series. Given only 39% of patients underwent surgery, the sensitivity of thyroid FNAC for diagnosing the malignant thyroid nodule was 100%. But the sensitivity of thyroid FNAC as a screening test for all neoplasms was 91.0%. The specificity for diagnosing a malignant thyroid nodule and the specificity of thyroid FNAC as a screening test for all neoplasm is 100%. This positive predictive value and negative predictive value in a neoplastic process was 100% and 94.8% respectively, while in malignancy showed 100% each. The diagnostic accuracy of FNAC on Bethesda system was concluded as 96.6%.

Key Words: Bethesda System, Follicular lesion of undetermined significance (FLUS), Thyroid FNAC

INTRODUCTION

Fine needle Aspiration cytology (FNAC) has aroused interest since 1949 [2] and is the principal method of preoperative diagnosis in both children and adults. Depending upon the nature of the thyroid nodule, FNA biopsy cytology can function as a diagnostic tool or a triage tool. [3] As a diagnostic tool, FNA biopsy cytology can be used to diagnose carcinoma, metastatic malignancy, thyroiditis, benign nodular goiters and cysts. As a triage tool, FNA biopsy cytology can be used to distinguish thyroid nodules that might have a higher risk of malignancy and would thus require surgical excision. [4]

Diagnosis of follicular lesions has long been considered a diagnostic grey area in thyroid cytology which often leads to false negative and false positive cases. Until recently various diagnostic terminologies, including 'atypical', 'indeterminate' were used to describe diagnostic challenge cases. [5,6] Further until recently there has been no uniform criteria established for various diagnostic categories and sample adequacy. [7] In an attempt to establish various standard diagnostic categories/classification system and morphological criteria for reporting thyroid FNAC, the National Cancer Centre (NCI) sponsored the NCI thyroid FNAC state of science conference, an interdisplinery programme, which convened a group of experts in Thyroid FNAC at Bethesda MD, in October 2007. [8,9]

We adopted this new classification in studying thyroid FNAC and hence we report our experience employing this system for thyroid FNAC in a 24 month period, including the incidence and histological outcome of each diagnostic category.

MATERIALS AND METHODS

An institutional review was carried out on 151 patients

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from June 2007 to June 2009 on patients visiting outpatient department with the swelling of the thyroid. FNAC was performed by Pathologists with 22 to 23 Gauze needle using non aspiration technique, also called Cytopuncture. Three or more different sites were preferred in any case to avoid false negative cases and to increase the yield of cellularity for the correct diagnosis and interpretation. Direct smears were prepared from each pass and were either air dried and stained with Giemsa or fixed in 95% alcohol and stained with Papanicolaou stain.

Cytologic diagnostic Terminology and interpretation [7,8]

At our institution, a six tier classification was developed according to the recommendations by 2007 NCI Thyroid FNAC state of science conference with minor modifications. The lesions were classified into 6 diagnostic categories, Inadequate, Benign, Follicular lesion of undetermined significance (FLUS), Follicular neoplasm, Suspicious, Malignant.

Unsatisfactory: Smears demonstrated inadequate cellularity, poor fixation and preservation, obscured by blood or a combination of these factors. Inadequate cellularity was defined as less than 6 groups of well preserved follicular cells on each of at least 2 slides. Aspirates from nodules that demonstrated abundant colloid and or macrophages with little or no follicular cells were not considered unsatisfactory and these findings were correlated with the clinical and radiologic impression as colloid nodule or cystic lesion.

Benign: where not showing any criteria for malignancy and are categorized as nodular goitre, nodular goitre with hyperplastic nodules, colloid nodules, cyst contents with or without benign follicular cells, macrofollicular adenoma and lymphocytic thyroiditis. The adenomatous goitre shows high cellularity (30%), honeycomb pattern, few microfollicular pattern (5-10%) with colloid (10-20%) and degenerative changes (30%). Risk of malignancy in these cases is 1-3%, and the patients are kept for follow up.

Indeterminate/follicular cells of undetermined significance: This overlapping cytological feature between hyperplastic nodule and follicular neoplasm forms the grey zone or the indeterminate category which makes the diagnosis difficult. The indeterminate category accounts for about 5-42% of cases of FNAC.

The cellular lesion is defined by high cellularity, scant colloid, admixture of flat sheets and syncytial / microfollicles (50%) fragments, with minimum nuclear overlapping. Risk of malignancy in these cases is 5-15%, and the patients are followed up for repeat FNAC.

Follicular neoplasm: Constitute about 29% of all cytological lesions. The differential diagnosis of follicular lesions includes hyperplastic/adenomatoid nodules, follicular adenomas and follicular carcinomas and follicular variant of papillary carcinomas. The general principle was followed that abundant colloid, it is more likely benign; the more cellularity, more likely the lesion is neoplastic. The follicular neoplasm is defined by the presence of high cellularity, scant colloid, prominent microfollicles (>75%) and syncytial fragments (>50-75% of cells) and nuclear overlapping, crowding and nuclear enlargement (2 x size of RBCs) and atypia, coarse chromatin and prominent nucleoli on high power. The microfollicles were also defined as <15 cells arranged in a circle that is at least two third complete. Risk of malignancy in these cases is 20-30%, and the patients are treated by lobectomy.

Suspicious for malignancy: This category applied to the specimens that demonstrated features of a malignant neoplasm but were quantitatively or qualitatively insufficient to a definitive diagnosis of malignancy. These features included, but were not limited to, an occasional intranuclear inclusion, nuclear grooves, or psammoma calcifications (calcospherites). Risk of malignancy in these cases is 60-75%, and the patients are treated by lobectomy or thyroidectomy.

Positive for malignancy: This category applied to cellular specimens with unequivocal cytologic evidence of a malignant neoplasm. Risk of malignancy in these cases is 100%, and the patients are treated by thyroidectomy.

Follow up: Histological diagnosis of patients who had undergone surgery was used as a gold standard for co-orelation with the cytological interpretations. The slides with discrepant cytology and where biopsy was done for histological diagnosis to determine the plausible

explanations of these discrepencies.

Statistical analysis: Descriptive statistical analysis has been carried out in the present study. Results on continuous measurements are presented n mean +/- SD (Min-Max) and results on categorical measurements are presented in number (%). Diagnostic statistics viz. Sensitivity, Specificity, PPV, NPV and Accuracy were computed. [10]

RESULTS

A total of 151 thyroid FNACs were evaluated at our institution during the 24 months study period. Table 1 summarizes the distribution of cytological diagnosis according to the patients. Overall 59 cases (39.0%) underwent total or partial thyroidectomy. Table 2 summarizes the cyto-histo co-orelation.

Table 1 : Classification of thyroid lesions according to Bethesda system

Cytological Diagnosis	Patients	Percentage
Unsatisfactory	13	8.6%
Benign	116	76.8%
Indeterminate/follicular cells of undetermined significance	6	3.9%
Follicular neoplasm	4	2.6%
Suspicious for malignancy	1	0.6%
Positive for malignancy	11	7.2%

Table 2: Cytological and histological co-orelation

	Histological diagnosis						
Cytological Category	MNG /HT	FA	PC	FC	Others	Total operated cases	% of operated cases out of 151
Unsatisfactory	1		1			2	1.2 %
Benign	34	02		01		37	24.5 %
Indeterminate/follicular cells of undetermined significance		03		01		04	2.6 %
Follicular neoplasm		03		01		04	2.6 %
Suspicious for malignancy			01			01	0.6 %
Positive for malignancy			05		06	11	7.2 %
Total	35	08	07	03	06	59	39.0 %

Fig 1: Follicular cells in clusters and acini in benign category

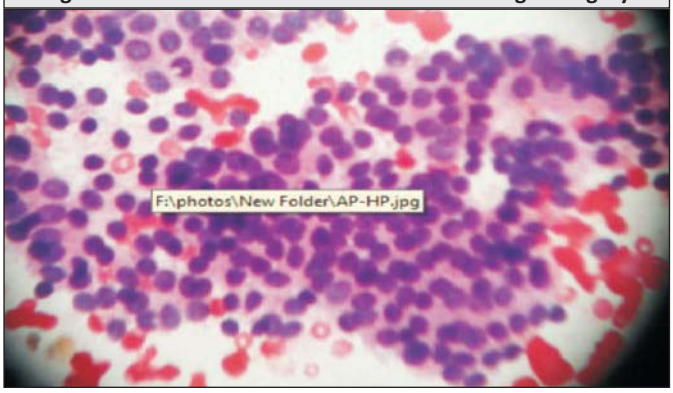


Fig 2: Both monolayered sheets and microfollicular pattern in FLUS (Follicular lesion of undetermined significance)

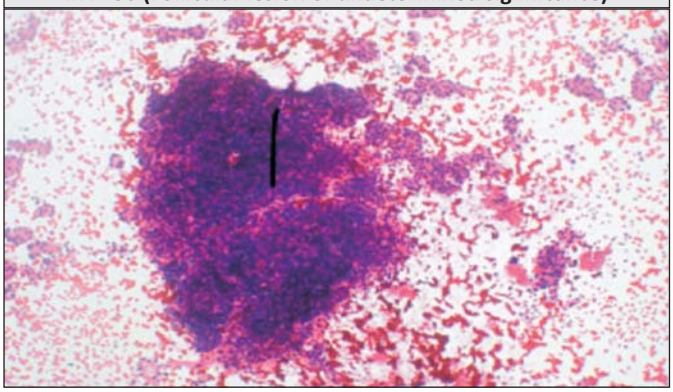


Fig 3: Follicular neoplasm: Highly cellular and microfollicular pattern

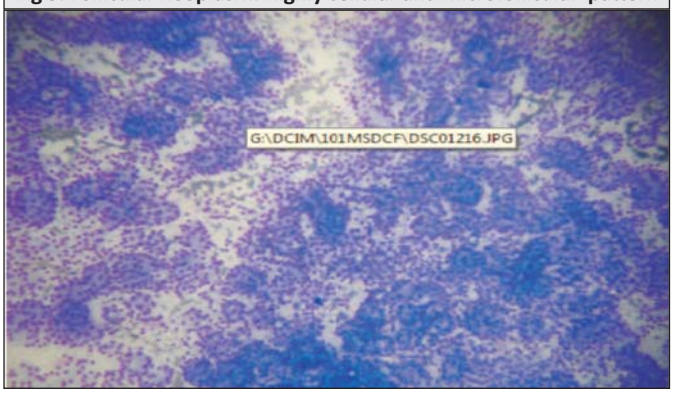
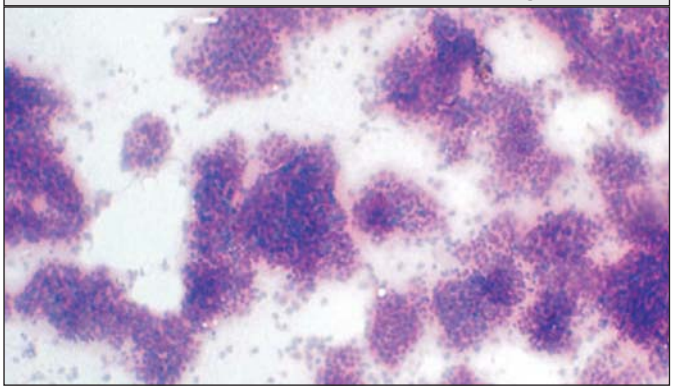


Fig 4: Suspicious for aspirate: papillary fragments with occasional intranuclear Inclusion and nuclear grooves



MNG: Multinodular goiter; HT: Hashimoto's thyroiditis; FA: follicular adenoma; PC: Papillary carcinoma; FC: follicular carcinoma/hurthle cell carcinoma; Others indicate medullary carcinoma, anaplastic carcinoma or metastatic carcinoma

Of the 13 patients with an unsatisfactory diagnosis, 05 patients had material on repeat FNAC, out of which 04 were negative for malignancy, and 1 positive for papillary carcinoma. The surgery was performed on only two patients and they were showing co-rrelation on histopathology. In all, 116 of the 151 (76.8%) thyroid nodules were interpreted cytologically as negative for malignancy; the majority of them were nodular goiters, followed by lymphocytic thyroiditis and colloid nodules. One case of papillary carcinoma was associated with Hashimoto's thyroiditis.

Six out of 151 patients who underwent thyroid FNAC were interpretated as indeterminate; of these five patients had abundant cellularity with both macrofollicular and microfollicular pattern while the sixth one had low cellularity with predominately microfollicular pattern with no significant atypia. 4 patients underwent partial thyroidectomy due to pressure symptoms or large size of thyroid. Interestingly, all the first three cases were reported histologically as follicular adenomas. The last case was follicular carcinoma with capsular invasion. The cases with size of more than > 4cms, were considered predictors of malignancy.

Four of 151 (2.6%) patients were diagnosed as follicular neoplasm; 3 were subclassified as with low cellularity, predominately microfollicular pattern and absence of colloid and the demonstrated nuclear features not characteristic of benign lesions. All patients underwent lobectomy, 1 was found to have follicular carcinoma and other three was reported as follicular adenoma. Therefore follicular neoplasm at our institution carried 25% risk of being malignant.

Only 1 patient was diagnosed with suspicious for papillary carcinomas and was reported as the same on histopathologic examination. 11 patients of 151 patients were classified as positive for malignancy: 4 patients were diagnosed cytologically as papillary carcinoma, 1 medullary carcinoma, 1 anaplastic carcinoma, 1 diffuse large B cell large cell lymphoma, 1 hurthle cell carcinoma and 1 metastatic squamous cell carcinoma. No false positive were identified in positive for malignancy diagnostic category.

Overall, there has been excellent co-rrelation between the six diagnostic categories and the histological outcomes in predicting non-neoplastic and neoplastic thyroid nodules. (Table 2) Based on cyto-histo co-orrelation of 59 cases, there have been no false positive cases. We calculate the specificity of a thyroid FNA as a diagnostic process, that is, in predicting a malignant process is 100% and the specificity of thyroid FNAC as a screening test, in predicting a neoplastic process is 100%. Only 39% of 151 patients underwent surgery, sensitivity of thyroid FNA as a screening test was 91.0% and in malignant process is 100%. Given the above, the positive predictive value of a malignant cytological diagnosis is 100%, of neoplastic process as a screening test is 100%. The negative predictive value of cytological diagnosis of the neoplastic lesion is 94.8%, while it was 100% in case of malignancy. (Table 4) Diagnostic accuracy of FNAC in our study was calculated as 96.6%. A larger study need to be done to get better statistical co-orelation.

Table 3: Risk of malignancy per Bethesda category in our study

Thyroid FNA cytological diagnosis (% of patients that underwent surgery in each category)	Percentage malignant on Histology [NCI recommended rate][a]
Benign / negative for malignancy (37/116 or 31.9%)	2.7% [2-3%]
Indeterminate cells of undetermined significance (4/6 or 66.7%)	25% (1/5)[b] [10-15%]
FN (4/4 or 100%)	25% (1/2) [20-30%]
Suspicious for malignancy (1/1 or 100%)	100% (1/1) [75%]
Positive for malignancy (11/11 or 100%)	100% (1/1) [100%]

a: NCI (National Cancer Institute) guidelines on thyroid FNAC include recommendations as to an expected range of malignancy risk per diagnostic category.

b. These values may in fact be lower given that that a subset of patients with the diagnosis that underwent surgery demonstrated increased risk of malignancy.

Table 4: Operating characterstics of thyroid fine needle aspiration

	As a screening test for neoplasm	As a diagnostic test for malignancy
Sensitivity	91.0 %	100%
Specificity	100 %	100%
Positive predictive value	100 %	100%
Negative predictive value	94.8 %	100%

Table 5: Comparison of the present study with other studies in each Bethesda category

Category	Yang J [9] (2007)	Constantine GA [16] (2009)	Current study (2011)
Unsatisfactory	10.4%	1.1%	8.6%
Benign	64.6%	73.8%	76.8%
FLUS	3.2%	3.0%	3.9%
Follicular Neoplasm	11.6%	5.5%	2.6%
Suspicious for Malignancy	2.6%	1.3%	0.6%
Malignant	7.6%	5.2%	7.2%

DISCUSSION

The current study attempted to evaluate the efficacy of the newly proposed six-tier diagnostic classification system in reporting thyroid FNAC results. Based on a study of 151 thyroid FNACs, 8.6% of the thyroid nodules were classified as unsatisfactory, 76.8% benign, 3.9% follicular cells of undetermined significance, 2.6% follicular neoplasm, 0.6% suspicious for malignancy, and 7.28% positive for malignancy. In a recent study that also utilized the six tier classification system, the authors reported 10.4% as unsatisfactory, 64.6% classified as benign, 3.2% were classified as atypical cells, 11.6% classified as follicular neoplasm, 2.6% as suspicious, and 7.6% classified as malignant. [11] Although we observed lower number of cases observed as follicular neoplasm, suspicious and positive for malignancy, there was no statistically significant difference among the diagnostic categories between the two studies. One of the major limitations of thyroid FNAC is the relatively high rate of unsatisfactory/non diagnostic specimens. The majority of the reported unsatisfactory categories is between 08-20%. [12,13] The rate of non diagnostic specimens may be influenced by the nature of the thyroid nodules, experience of the aspirators, whether on site adequacy assessment is performed, and the criteria to define the specimen adequacy. On site cytological adequacy of the smear is assessed directly under microscope or after staining with toluidine blue which have shown to significantly decrease unsatisfactory rates. [14,15]

The aspirates classified as benign/negative for malignancy accounted for the majority of thyroid FNACs forming 76.8% of cases, which is comparable to the study done by Constantine GA. [16] This category consisted of heterogeneous group of lesions such as nodular goitre, hashimoto's thyroiditis and colloid nodules. Most of the patients were followed up clinically. However, a small number of 37 of 116 patients with benign cytological interpretation underwent surgery because of the size of nodules, presence of family history of thyroid cancer or history of irradiation of the head and neck area. These patients have a higher risk of thyroid malignancy than the general population. There were two false negative cases in our current series. Typically the reported cases of false negative cytology diagnosis reported in the literature are rather low. [12,17,18]

The diagnostic category indeterminate includes cases that does not fit into either benign or follicular neoplasm and is equivalent to follicular lesion of indeterminate category according to NCI guidelines. We have approached the follicular lesions based on three different parameters. The first parameter based on the assessment of colloid and cells ie more the colloid, more likely the lesion is benign, and conversely more the cellularity and less colloid more likely the lesion is

malignant. The second parameter is assessment of architecture and background ie. range of follicle size either predominantly honeycomb sheets or microfollicles. The lesions with mixed architecture with features of both macro-and microfollicular pattern (50% of each), were placed in follicular lesion of undetermined significance. While the combination of high cellularity and microfollicular pattern (more than 75%) were placed in follicular neoplasm. The third parameter is based on the assessment of cytological features ie. extremely crowded, 3 dimensional groups of disorganized follicular cells and irregular follicles, large pleomorphic nuclei (3-4 x normal), abnormal chromatin with prominent nucleoli. Cytologically, it is not possible to distinguish between follicular adenoma and hyperplastic adenomatous nodule. We do not consider these cases to be discrepancies because there is only minimal risk to the patients.

In our study, FLUS constitutes the second lowest (only 3.9%) of all thyroid FNACs. 4 out of 6 in this category underwent surgery, 3 cases were reported as follicular adenoma, 1 case was reported as follicular carcinoma. Based on this histological follow up, 25% cases turned out to be malignant. Currently we believe that nodules with indeterminate category, FNAC diagnosis should be re-biopsied and not surgically resected. We also consider a targeted, well sampled repeat FNAC in borderline cellularity cases.

The diagnostic category follicular neoplasm was 2.6% of the total FNAC cases. Yang J [9] and Constantine [16] reported 11.6% and 5.6% of the cases respectively, which was slightly higher than my study. This may be because of the less number of cases, perhaps a bigger data can give a clear picture. Because of the risk of malignancy all four cases underwent lobectomy. About three cases were reported as adenomas and other as follicular carcinoma. The risk of malignancy was 25% and was considered slightly higher due to less number of patients categorized in this category. Because of the inability of the FNAC to differentiate between follicular or hurthle adenomas from their malignant counterparts, the role of FNAC shifts from diagnostic to a more screening test.

0.6 % and 7.2% cases underwent cytological diagnosis for suspicious of malignancy and positive for malignancy respectively. Yang J [9] also reported 7.6% of the total cases as malignant, which was similar to mine study. The case was specific for papillary carcinoma in suspicious for malignancy category, which was reported as positive for papillary carcinoma. All the cases in the category definite for malignancy underwent thyroidectomy. Histological follow up did not reveal any false positive for cases that were reported definite for malignancy cytologically, that is, there were no false positive in the positive for malignancy category. The risk of malignancy for a diagnosis definite for malignancy was 100%, that is, consistent with the data published in the literature. [9]

The only limitation in our study is limited number of cases, risk of malignancy in FLUS and follicular neoplasm cannot be estimated accurately. This may be because of the heterogeneity in its use and the associated variable risk of malignancy. [19,20] Many patients were lost in benign category, so the risk of carcinoma reported may be slightly lesser than true value, 2.7% in our series, though Bethesda also reported 2-3%. Some studies and including ours, have demonstrated that the risk of malignancy associated with FLUS is similar to that for the cases diagnosed as FN, thus calling into question its role in thyroid nodule management; however, the meta-analyses of the published literature have indicated that the risk is different. [21]

CONCLUSION

Our study shows that the six tier diagnostic approach of reporting FNAC proposed by NCI. This helped in reducing the number of false negative patients and because there were no false positive cases, Bethesda System allowed good co-orelation between cytological and histopathological diagnosis and prevented the unnecessary surgeries performed on the patients. It also allows the cytopathologists to express their level of concern of the possibility of underlying malignancy to guide subsequent management to the surgeons.

REFERENCES

1. Guidelines of the Papanicolaou society of Cytopathology for the examination of fine needle aspiration specimens from thyroid nodules. The Papanicolaou society of Cytopathology Task force on Standards of Practice. Med Pathol 1996; 9(6): 710-5.
2. Soderstrom NJ, Berman LH, Grant JW. US guided core needle biopsy of the thyroid gland. Radiology 2003; 226: 827-32.
3. Gharib H. Fine needle aspiration biopsy of the thyroid nodules; Advantages, Limitations and Effect. Mayo Clin Proc 1994; 69: 44-49.
4. Kendall CH. Fine needle aspiration of the thyroid nodules. Three year experience J Clin pathol 1989; 45: 23-27.
5. Baloch ZW, LiVolsi VA. Fine needle aspiration of thyroid nodules: past, present and future. Endocr Pract 2004; 10: 234-241.
6. Eedes CR, Wang HH. Cost effectiveness of immediate specimen adequacy assessment of fine needle aspirations. Am J Clin Pathol 2004; 121: 64-69.
7. Abati A. The National Cancer Institute Thyroid FNA state of science conference: “wrapped up”. Diagn Cyto Pathol 2008; 36: 388-389.
8. Baloch ZW et al. The National Cancer Institute thyroid fine needle aspiration state of science conference: a summation. Cytojournal 2008; 5: 1-17.
9. Yang J, Schnadig V, Logrono R, Wasserman PG. Fine needle aspiration of thyroid nodules, a study of 4072 patients with histological and clinical co-orelations. Cancer 2007; 111: 306-315.
10. Altman DG, Bland JM (1994). "Diagnostic tests. 1: Sensitivity and specificity". *BMJ* 308 (6943): 1552. PMC 2540489. PMID 8019315.
11. Yader BJ, Redman R, Massoll NA. Validation of a 5 tier cytodiagnostic system for thyroid fine needle aspiration biopsies using cyto-histological co-orelation. Thyroid 2006; 16: 781-786.
12. Nasuti JF, Gupta PK, Baluch ZW. Diagnostic value and cost effectiveness of on site evaluation of fine needle aspiration specimen: a review of 5688 cases. Diagn Cytopathol 2002; 27: 1-4.
13. Zhu W, Micheal CW. How important is on site adequacy assessment for thyroid FNA: An evaluaton of 883 cases. Diagn Cytopathol 2003; 35: 183-186.
14. Amikachi M, Ramzy I, Rubenfeld S, Wheeler TM. Accuracy of fine needle aspiration of thyroid. Arch Pathol Lab Med 2001; 125: 484-488.
15. Gharib H, Goellner JR, Johnson DA. Fine needle aspiration cytology of thyroid. A 12 year experience with 11,000 biopsis. Clin Lab Med 1993; 13: 699-709.
16. Constantine G.A.Theoharis, Kevin M. Schofield, Lynwood Hammers, Robert Uhelsman and David C.Chhieng. The Bethesda Thyroid Fine Needle Aspiration Classification System: Year 1 at an Academic Institution. Thyroid 2009; 19: 1215– 1223.
17. Sclabas GM, Staerkel GA, Spario SE et al. Fine needle aspiration cytology of the thyroid and its correlation with histopathology in a contemprory series of 240 patients. Am J Surgery 2003; 186: 702-709.
18. Baloch ZW, Sack MJ, Yu GH et al. Fine needle aspiration of thyroid: an institutional experience. Thyroid 1998; 8: 565-869.
19. Theoharis CG, Schofield KM, Hammers L, Udelsman R, Chhieng DC. The Bethesda thyroid fine-needle aspiration classification system: year 1 at an academic institution. Thyroid. 2009; 19: 1215-1223.
20. Walts AE, Bose S, Fan X, et al. A simplified Bethesda System for Reporting Thyroid Cytopathology using only 4 categories improves intra- and inter-observer diagnostic agreement and provides non overlapping estimates of malignancy risks. Diagn Cytopathol. 2012; 40(1): E62-E68.
21. Bongiovanni M, Spitale A, Faquin WC, Mazzucchelli L, Baloch ZW. The Bethesda System for Reporting Thyroid Cytopathology: a meta-analysis. Acta Cytol. 2012; 56: 333-339.