ORIGINAL ARTICLE

Cell Pattern Analysis in Fine Needle Cytology of Solitary Thyroid Nodule with Histopathological Correlation

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Abstract:

Introduction:
Adequacy of thyroid aspirate comprise five to six groups of thyroid follicular epithelial cells with each group containing 10 or more cells on atleast two smeared slides. Thyroid fine needle cytology (FNC) can be used for cell pattern analysis.

Aims:
To study pattern analysis of solitary thyroid nodules (STN) and to assess diagnostic parameters for cyto diagnosis of thyroid lesions.

Materials and Methods:
Forty-seven cases of STN were subjected to cell pattern analysis and correlated with histopathological diagnosis. Aspirate smears were classified based on primary pattern and secondary pattern. Primary pattern was the most dominant pattern seen. The next co-dominant pattern was labelled as secondary pattern. The final Bathesda based cytological diagnosis was categorised based on these two patterns in each case. Histopathology report of these cases after surgery was correlated with the cytological diagnosis. For each correlation; sensitivity, specificity, positive predictive value, diagnostic accuracy was calculated by appropriate formula manually. Kappa measure of agreement was used for inter-observer variation.

Results:
Based on the cell pattern analysis of STN cases, the diagnostic parameters in our study were: Sensitivity- 71.43%, Specificity- 97.5%, Positive predictive value- 83.33%, Accuracy or Efficacy- 93.62%, False positive rate- 2.43%, False negative rate- 4.26%.

Conclusion:
The present study demonstrates the application of pattern analysis in diagnosing thyroid lesions on FNC which are easily reproducible. Also, such study can help beginners in pathology field to understand systematic arrival at a cytological diagnosis that helps triage neoplastic from non-neoplastic thyroid lesions.

Keywords: Cell pattern; Solitary nodule; Thyroid; Fine needle aspiration cytology

Introduction:
Cytopathology is the study of cells that have been exfoliated freely from the tissue surface or that have been collected by brushings, scraping, washingor by needle aspiration. Fine Needle Aspiration Cytology (FNAC) as a pre-operative, cost-effective, initial, minimally invasive investigation has been known world-wide[1].

FNAC is a method of cell investigation based on the removal of cell sample from a palpable lump or tumour with a fine needle attached to syringe by creating negative pressure (Frable WJ)[2].

Since the first ever report on FNAC by Kün M in 1847, this technique has indeed come a long way[3]. Diagnosis of thyroid nodules by needle biopsy was first described by Martin HE and Ellis EB in 1930 at New York, who used an 18-gauge needle aspiration technique[4]. Sweden based investigators introduced small-needle

Aspiration biopsy of the thyroid in the 1950s-1960s, and this technique came into wide spread use in North America in the 1980s[1,4]. Some workers modified the fine needle aspiration technique to increase the sample adequacy and reduce blood in the background of smears. Ultrasonographic guidance is useful in increasing cell material yield in thyroid lesions that werenon-palpable or small in size.

The technique of fine needle biopsy without aspiration (FNCB/FNC) was introduced in 1987 by Zajdela[5].The technique is based on the principle that the capillary pressure in a fine needle is sufficient to keep the detached cells inside the lumen of the needle. Jayaram G and Orell SR (2012) suggested that the cell yield by FNC is probably less than with aspiration technique (FNAC) whereas for highly vascular lesions, FNC yields better samples than FNAC technique. They suggested that FNC should be the method of choice for all superficial aspiration biopsies and highly vascular lesions except for cystic lesions, fibrotic or sclerotic hypo-cellular lesions, soft tissues, necrotic tumours and vague lumps[5].

There are a limited number of patterns observed in aspirated material of many lesion sites and many types of tumours. The variation, frequency and significance of every pattern vary with the lesion site of FNAC. Multiple researchers have used various methods to derive an inference based FNAC diagnosis of thyroid lesions to triage them into operative and non-operative groups[6].We conducted cell pattern analysis of solitary thyroid nodules (STN) based on a study by Lingegowda JB, et al (2010) to analyse and divide lesions into separate categories[6].

Materials and Methods:

The prospective, observational study was conducted for a period of two years in the Department of Pathology at Indira Gandhi Government Medical College (IGGMC), Nagpur in Central India.

Inclusion Criteria:

Patients with thyroid lesions, irrespective of their age And sex, referred for cytological study from ENT and Surgery OPD and admitted to ward were selected. Using clinical proforma, the clinical history was documented and clinical examination was conducted prior to obtaining cytological study samples.

Exclusion Criteria:

Patients not willing for USG-guided / unguided fine needle aspiration cytology of their thyroid lesions even after explaining the purpose, utility and consequence of the procedure were excluded from the study. Patients with psychiatric/ surgical illness or patients taking drugs that affect thyroid function tests were excluded.

Prior to aspiration, size, shape, mobility of thyroid swelling with swallowing, nodularity and clinical complaints were assessed. Fine needle cytology was done by non-aspiration technique (FNC) except for cystic lesions which were aspirated (FNAC). FNAC was done using 23-G needle attached to a 10 ml syringe which was further attached to syringe holder. The average number of FNC passes for adequate sampling of thyroid swelling was kept to two to three. In each case, haematoxylin and eosin (H&E), papanicolaou (PAP) and May-Grunwald-Giemsa (MGG)staining of smeared slides were done.

Cell Pattern approach[1,6]:

Out of the total 295 thyroid FNACs, the solitary thyroid nodules (STNs) diagnosed on ultrasonography were subjected to cell pattern analysis. Inadequately sampled cytological cases were excluded from the study. Two cytopathologists reviewed the cytological smears from STN cases. These two cytopathologists were not aware of routine cytological reporting and histopathological diagnosis of the cases. Pattern analysis was done according to criteria given by Lingegowda JB et al (2010)[6] (Refer Table 1).

The final provisional diagnosis on cytopathology was given out in the following manner[7].

1. Benign

2. Atypia of undetermined significance (AUS)

3. Neoplasm

4. Suspicious of malignancy

5. Malignant

The cytopathologists' were asked to identify the predominant pattern (primary) first, and then give out a diagnosis and then to identify the next dominant pattern (secondary) and give the combined pattern Bathesda based diagnosis. The variation between primary pattern, secondary pattern and final cytological diagnosis was matched with the final histopathological diagnosis for correlation and statistical data was prepared.

Statistical Analysis: Sensitivity, specificity, positive predictive value, negativepredictive value and diagnostic accuracy were calculated for each correlation. Kappa measure of agreement was used to calculate inter-observer variation.

Table No.1: List of various patterns given by Lingegowda JB et al[6]

Group	Primary pattern	Secondary pattern	Provisional diagnosis
I	Colloid-rich background	Hurthle cell rich Macrophage rich Microfollicle poor Microfollicle rich	Benign Benign Benign Atypia of undetermined significance (AUS)
II	Biphasic	Lymphocyte background Hurthle cell rich	Benign Neoplasm
III	Hurthle cell rich	Colloid-rich background Haemorrhagic background Lymphocytic background	Benign Neoplasm Benign
IV	Macrophage rich	Colloid-rich background Hurthle cell rich Monotonous crowding Microfollicle poor Microfollicle rich	Benign Neoplasm Malignant Benign Neoplasm
V	Monotonous crowding	Enlarged oval nucleus	Malignant
VI	Microfollicle poor	Colloid-rich background Hurthle cell rich Macrophage rich	Benign Neoplasm Benign
VII	Microfollicle rich	Colloid-rich background Haemorrhagic background Macrophage rich Enlarged oval nucleus	AUS Neoplasm AUS Malignant
VIII	Pleomorphic	Haemorrhagic background Amyloid background	Malignant Malignant

Results:

Out of the total FNACs conducted at our institution over a two year period, thyroid cytological reporting comprised 5.16%. 295 thyroid patients were included in the study. The mean age was 38.41 ± 14.49 years. The youngest patient was three years old which was cytologically diagnosed with thyroglossal cyst. The oldest patient was of 75 years with cytological diagnosis of nodular colloid goitre.

Out of 295 cases, 41 patients were males while 254 cases were females. Female to male ratio in our study was 6.02:1. 65.08% of thyroid patients yielded good cellularity on FNAC/FNC.

The most common clinical symptom in patients with thyroid lesions was swelling in the neck which was present in total 293 cases i.e., 98.98% incidence which moved with deglutition, followed by dysphagia in 53 (17.97%) cases. No thyroid swelling was palpated in two cases.

Out of 295 cases, the maximum number of patients (32.88%) presented with duration of symptoms being more than one year. Minimum number of cases-13.9% had the duration of symptoms between 6-12 months. The size of smallest thyroid swelling was 0.9 x 0.8 cm and the size of the largest swelling- 8 x 6 cm.

In our study in 293 out of 295 thyroid cases the swellings were palpable while in remaining two cases, only USG examination detected the thyroid swelling. Solitary thyroid lesions on palpation comprised 84.64% cases. 43.73% of thyroid lesions were solitary on USG thyroid examination. On comparison of nodularity on clinical examination with USG thyroid examination, the א2-value and p-value was highly significant showing that USG thyroid was better in diagnosing STN cases than clinical examination.

Out of 295 cases, solitary thyroid nodules (STN) were detected in 129 by USG of the neck. The 106 STN cases, out of these 129 cases were subjected to cytology. Out of 106 STN cases, 47 were subjected to surgery. Cyto-histopathology correlation after cell pattern analysis of these 47 cases is presented in Table No.3.

Inter-observer variability: Unanimous agreement was seen between the reviewing cytopathologists in 43 (91.49%) cases which included 27 benign, six AUS, five neoplasms and four malignancies and one suspicious for malignancy category.

One case diagnosed as benign by one reviewer and neoplasm by the other turned out to be benign lesion histologically.

AUS diagnosed cases by both the reviewers were six in number and all proved to be benign on histopathology. This suggests that AUS thyroid aspirates are usually benign and need to be followed-up with other follow-up FNAC, rather than resected promptly.

A single case diagnosed as neoplasm by reviewer 1 and benign by the other turned out to be malignant on histopathology. There was another discrepant case diagnosed as neoplasm by reviewer 1 and malignancy by reviewer 2, that was confirmed as malignancy on histopathology.

A single case diagnosed as malignant by reviewer 1 and neoplasm by the other, turned out to be benign (nodular colloid goitre) on histopathology.

By Muddegowda et al (2010), the unanimous agreement between the two reviewers was observed in 187 of 219 cases (85.38%)[7]. In our study, unanimous agreement between the two reviewers was observed in 43 of 47 cases (91.49%); including 27 benign, six AUS, five neoplasms and four malignancies and one suspicious for malignancy category. The reliable kappa score of agreement for cell pattern analysis of thyroid lesions was 0.95 between our reviewing cytopathologists. This score was better than 0.85 reported by Muddegowda et al (2010)[7].

In order to estimate the diagnostic parameters, the categories of Benign, AUS and Neoplasm on cytodiagnosis were clubbed under the group of 'Benign.' The cases with cytodiagnosis of suspicious of malignancy and malignancy were clubbed under the group of 'Malignancy.'

Table No.4 shows that out of 47 STN cases that were subjected to cyto-histopathogical correlation, malignancy was detected on pattern diagnosis in seven cases. On histopathology, the correctly correlated malignant cases were five in number (TP-True positive).

Two cases diagnosed as benign on FNAC turned out to be malignant on histopathology (FP-False negative) examination. Both these cases were cytologically diagnosed as follicular neoplasm but found to be follicular carcinoma on histopathological correlation.

The cases diagnosed on pattern analysis as benign were 40 out of the correlated 47 cases. The cases correctly correlated as benign (TN-True Negative) were 39 while there was a single case wrongly interpreted as benign on FNAC but found to be malignant (FP-False negative) on such correlation. This later discrepant case was cytologically diagnosed as papillary carcinoma and found on histopathology as nodular colloid goitre.

Diagnostic parameters obtained after usage of cell pattern analysis to detect thyroid malignancy:

Sensitivity- 71.43%
Specificity- 97.5%
Positive predictive value (PPV)- 83.33%
Accuracy or Efficacy- 93.62%
False positive rate- 2.43%
False negative rate- 4.26%

Table No.5 shows that Lingegowda PH, et al (2010) by their pattern analysis approach of STN cases had lower sensitivity but slightly higher specificity and efficacy as compared to our study in diagnosing thyroid lesions[6].Bommanahalli BP et al (2010) by their pattern analysis approach of goitre cases had higher sensitivity but slightly lower specificity than our study[8].

Table No.2: Comparison of nodularity on clinical examination with USG thyroid examination

Cases	On clinical examination		On USG neck examination	
	No. of cases	%	No. of cases	%
Single swelling	248	84.64	129	43.73
Multiple swellings	45	15.36	166	56.27
Total	293	100	295	100
א2-value	**36.71**			
p-value	**P<0.0001, Significant**			

Table No.3: Comparison of pattern diagnosis with histopathological diagnosis in 47 cases[6]

Group	Total no. of cases	Primary pattern	Secondary pattern	Pattern diagnosis		Histopathological diagnosis
				Category	No. of cases	
I	16	Colloid-rich background	Macrophage rich	Benign	8	Colloid goitre-3, MNG-2, Cystic colloid nodule-1, Thyroglossal cyst-1, Follicular adenoma-1
		Colloid-rich background	Microfollicle poor	Benign	6	Colloid goitre-2, Thyroglossal cyst-2, Nodular colloid goitre-2
		Colloid-rich background	Microfollicle rich	AUS	1	Granulomatous goitre-1
		Colloid-rich background	Lymphocytic background	Benign	1	Lymphocytic thyroiditis-1
II	7	Biphasic	Lymphocytic background	Benign	7	Follicular adenoma-1 Hashimoto's thyroiditis-5, Granulomatous Thyroiditis1
		Biphasic	Hurthle cell rich	Benign	0	-
III	1	Hurthle cell rich	Colloid-rich background	Benign	0	-
		Hurthle cell rich	Haemorrhagic background	Neoplasm	1	Nodular colloid goitre-1
		Hurthle cell rich	Lymphocytic background	Benign	0	-
IV	2	Macrophage rich	Colloid-rich background	Benign	2	Thyroglossal cyst-2
		Macrophage rich	Microfollicle poor	Benign	0	-
		Macrophage rich	Hurthle cell rich	Neoplasm	0	-
		Macrophage rich	Monotonous crowding	Malignant	0	-
		Macrophage rich		Benign	0	-
		Macrophage rich	Microfollicle poor Microfollicle rich	Neoplasm	0	-

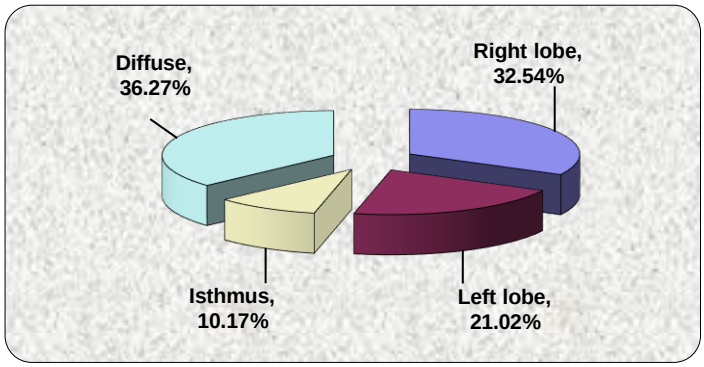
V	1	Monotonous crowding	Enlarged oval nucleus	Malignant	1	Papillary carcinoma-1
VI	6	Microfollicle poor Microfollicle poor Microfollicle poor	Colloid-rich background Lymphocytic background Hurthle cell rich Macrophage rich	AUS Benign Neoplasm Benign	5 1 0 0	Colloid goitre-1, Nodular colloid goitre-2, Follicular adenoma-2 Lymphocytic thyroiditis -1 - -
VII	11	Microfollicle rich Microfollicle rich Microfollicle rich Microfollicle rich	Colloid-rich background Haemorrhagic background Enlarged oval nucleus Macrophage rich	AUS Neoplasm Malignant AUS	3 6 2 0	Follicular adenoma-1, MNG -1, Colloid goitre-1 Follicular adenoma-3, Follicular carcinoma-2, MNG-1 Nodular colloid goitre-1, Follicular carcinoma-1 -
VIII	3	Pleomorphic Pleomorphic Pleomorphic	Haemorrhagic background Microfollicle rich Amyloid background	Malignant Suspicious Malignant	2 1 -	Medullary carcinoma-1 Anaplastic carcinoma-1 Medullary carcinoma-1 -

Table No.4: Comparison of Cell pattern diagnosis with Histo-pathological findings in 47 STN cases for diagnostic parameters

		Histopathology		
		Benign	**Malignant**	**Total Cytology**
FNAC	**Benign**	39 (TN)	2 (FN)	41
	Malignant	1 (FP)	5 (TP)	6
Total Histopathology		40	7	47

Table No.5: Comparison of diagnostic parameters in different studies after application of cell pattern analysis

Predictive values	**Lingegowda PH et al (2010)[6]**	**Bommanahalli BP et al (2010)[8]**	**Present study**
Sensitivity	66.7%	83.33%	71.43%
Specificity	98.9%	95.55%	97.5%
Efficacy	95.4%	-	93.62%
Clinical reliability	Reliable	Reliable	Reliable

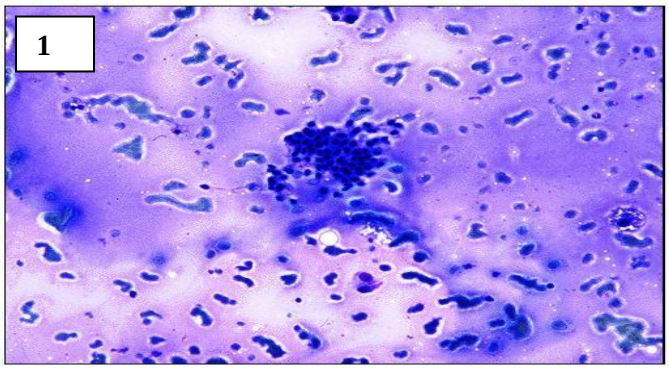


Pie chart / Figure 1 shows that bilateral / diffuse involvements of thyroid lesions were most common in 36.27% cases.

Conclusion:

Cell pattern analysis in STNcan offer an edge over routine cyto-diagnoses as it has high diagnostic parameters, thus reducing dilemma in patient management. Application of cell pattern analysis allows reproducible results among pathologists. We infer that cell pattern analysis is better suited for beginners in cytopathology for easier understanding.

Studies to evaluate various diagnostic parameters are necessary in all cytology centres to improve upon technical as well as interpretative errors. We suggest that cell pattern analysis can be applied to other organs too.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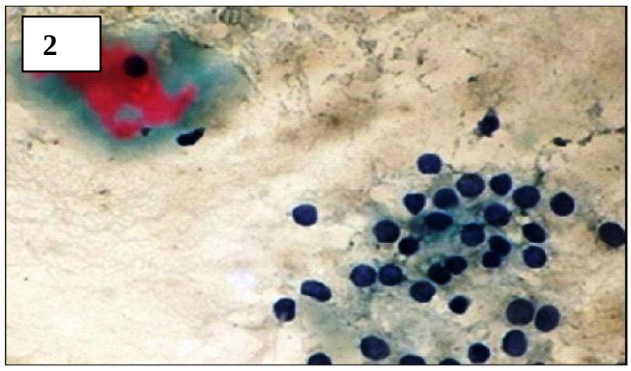
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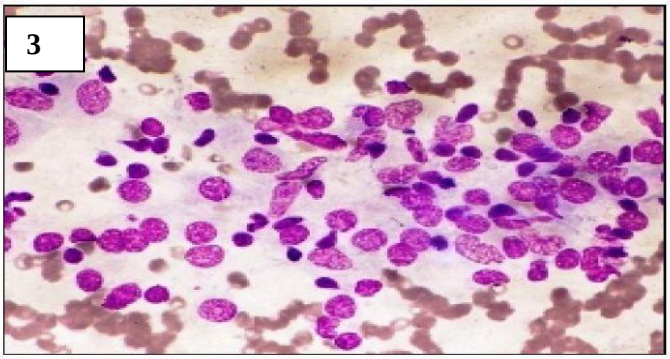
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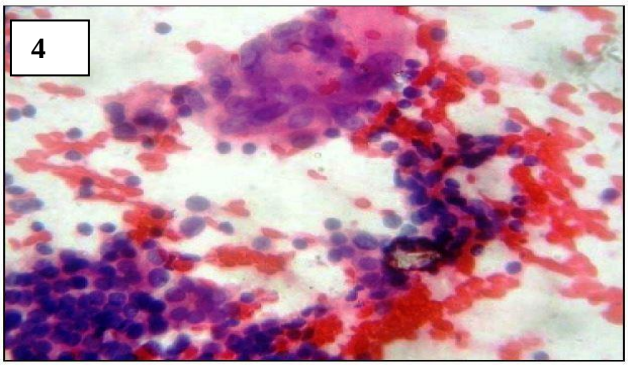
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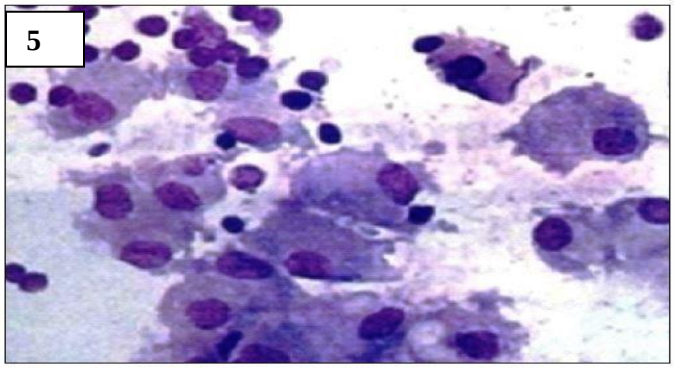
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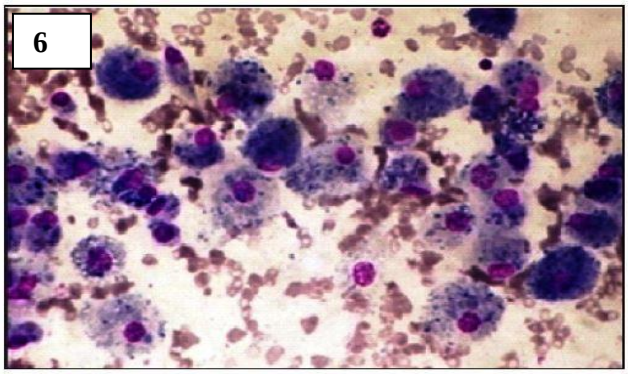
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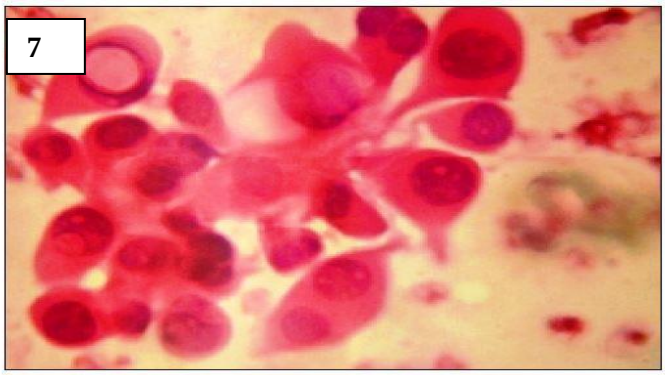
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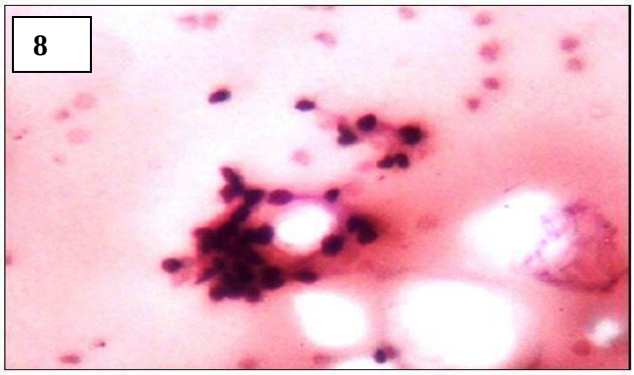
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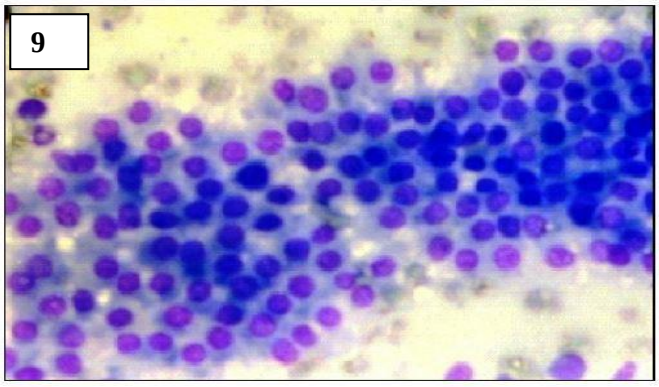

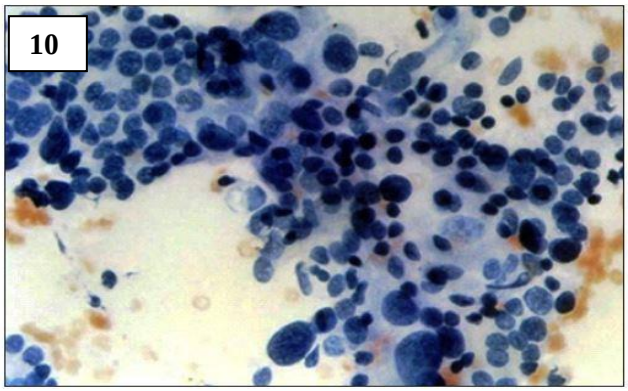

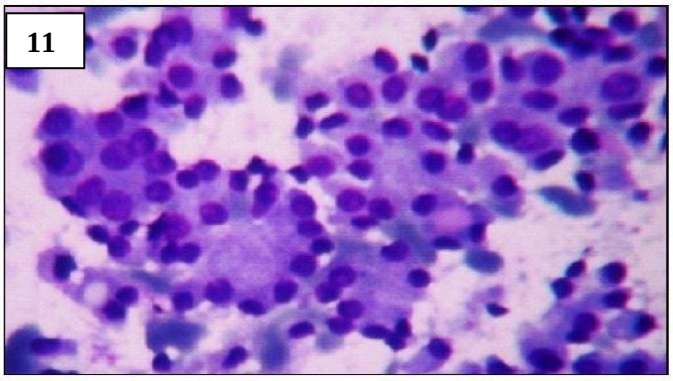

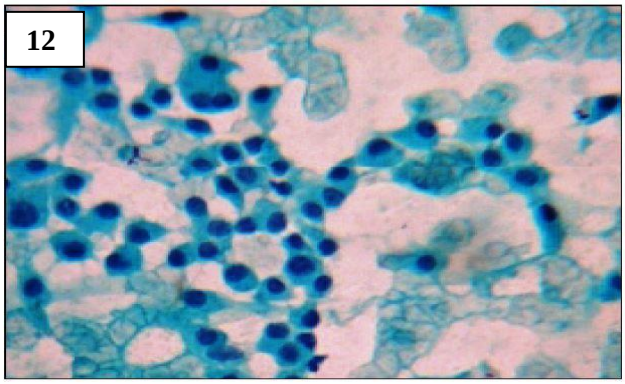

Figure. 1: Cytological microphotograph of Group I:Primary pattern as Colloid rich and secondary pattern as Microfollicle-poor(MGG, X 40).

Figure. 2: Cytological microphotograph of Group I:Primary pattern as Colloid rich and secondary pattern as Microfollicle-rich(PAP, X 400).

Figure. 3: Cytological microphotograph of Group II:Primary pattern as biphasic and secondary pattern as Lymphocyte rich background(MGG, X 400).

Figure. 4: Cytological microphotograph of Group II:Primary pattern as Biphasic and secondary pattern as Lymphocyte rich background(PAP, X 100).

Figure. 5: Cytological microphotograph of Group III:Primary pattern as Hurthle cell rich and secondary pattern as Colloid rich background(PAP, X 100).

Figure. 6: Cytological microphotograph of Group IV:Primary pattern as Hurthle cell rich and secondary pattern as Hemorrhagic background(PAP, X 100).

Figure.7: Cytological microphotograph of Group V:Primary pattern as Monotonous crowding and secondary pattern as Enlarged oval nucleus (H&E, X 400).

Figure.8: Cytological microphotograph of Group VI:Primary pattern as Microfollicle-poor and secondary pattern as colloid-rich background(H&E, X 100).

Figure. 9: Cytological microphotograph of Group VII:Primary pattern as Microfollicle-richand secondary pattern as Colloid-rich background(MGG, X 400).

Figure.10: Cytological microphotograph of Group VII:Primary pattern as Microfollicle-richand secondary pattern as Enlarged oval nucleus (PAP, X 400).

Figure.11: Cytological microphotograph of Group VII:Primary pattern as Microfollicle-richand secondary pattern as Hemorrhagic background(MGG, X 400).

Figure.12: Cytological microphotograph of Group VIII:Primary pattern as Pleomorphic and secondary pattern as Hemorrhagic background (PAP, X 400).

Conflict of Interests-Nil

Sources of Support- Nil

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