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Original Research Article

Accuracy of different cytoarchitectural features in the diagnosis of papillary thyroid carcinoma by fine needle aspiration

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ABSTRACT

Introduction and Objectives: Fine needle aspiration (FNA) has about 90% diagnostic accuracy for papillary thyroid carcinoma (PTC) in an adequate sample. Ignorance of relative significance of individual cytologic features may lead to misdiagnosis due to reliance on a single or few features. Our objective was to determine the usefulness of individual and most appropriate combined cytologic features, for diagnosis of PTC.

Materials and Methods: H&E stained FNA smears of fifty cases each, of consecutive histologically confirmed PTC and benign thyroid cases (controls), reported over 3 years, were retrieved from the files. A total of 31 architectural, cytological and background features were assessed, blind to the final diagnosis and compared amongst the two groups. The statistical significance ($p<0.05$) of each parameter was determined by chi-square and odds ratio and combinations quality assessed by ROC curves.

Discussion: Twenty features were found to be statistically significant. Fourteen highly significant ($p<0.000$) features included flat syncytial sheets, anatomical borders, true papillae, cellular swirls, individually dispersed cells with eosinophilic cytoplasm, anisonucleosis, elongated oval nuclei, powdery chromatin, nuclear outline irregularity, thickened nuclear membranes, crescent shaped nuclei, intranuclear inclusions, histocytoid cells and soap bubble cytoplasmic vacuolation. The six statistically significant features ($p<0.05$) included micro-acinar structures, cellular crowding, nuclear overlapping, nuclear grooves, squamoid cells and bubble gum colloid. The control group also showed some of these single cytologic features. All the combinations of ≥ 4 features had a statistical significance of $p=0.000$ and specificity and PPV of 100% with ROC showing excellent results.

Conclusion: Using correct combination of cytologic features will increase accuracy of FNA diagnosis of PTC.

1. Introduction

Thyroid carcinoma accounts for approximately 1% of all malignancies.[1] It is the commonest endocrine malignancy of which papillary thyroid carcinoma (PTC) accounting for 80% of cases in adults[1–3] and 90% in children.[3] The peak incidence in adults is between 35-40 years and is commoner in females in the ratio of 3-4:1.[4] In Sri Lanka, thyroid gland malignancy is the third commonest malignancy in females with the highest incidence among 15-34 years.[4]

In the evaluation of a thyroid nodule, fine needle aspiration cytology (FNAC) is a well-established first line diagnostic test.[5–8] The diagnostic accuracy of PTC by FNA accounts for over 90% provided the sample is adequate.[8] The accepted criteria for cytodiagnosis of PTC includes true papillary tissue fragments together with nuclear features such as dusty pale enlarged nuclei, fine dusty powdery chromatin, chromatic ridge / bar (nuclear gsingle or multiple

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micro and macronucleoli, intranuclear cytoplasmic pseudo inclusions with dense stringy 'bubble-gum' colloid in the background.[9]

None of the aforementioned architectural, cytomorphological and background features however are unique to or diagnostic of PTC as they can be present in various other non-neoplastic and neoplastic thyroid lesions such as hyperplastic nodule, papillary hyperplasia, thyroiditis, Hurthle cell neoplasm and hyalinising trabecular adenoma.[9,10] It is challenging to distinguish reactive nuclear changes associated with lymphocytic thyroiditis from PTC since they share certain features like nuclear grooves and intranuclear inclusions.[11]

None of these features is diagnostic on their own, unless they occur in combination and are relatively widespread.[8,9] The commonest mistake in diagnosing PTC is when the cytopathologist places too much emphasis on a single cytological feature.[9]

Thus, unequivocal diagnosis of PTCFNA may be difficult to a inexperienced cytopathologists, when the only a few diagnostic features are present and/or if the features are the features are found in low frequency. Furthermore, variants of PTC like follicular variant may exhibit a very few nuclear features which may be present in only a few foci.[12] Therefore, it will be helpful to determine which individual cytoarchitecural features are most reliable for the diagnosis of PTC and also to determine which combination of features is more useful in the FNA diagnosis of PTC.

1.1. General objective

To determine the usefulness of individual cytoarchitectural features in FNAC smears for the diagnosis of PTC and its variants.

1.2. Specific objectives

To determine the most useful cytoarchitectural features for the diagnosis of PTC in cytology smears.

1. To determine the most useful cytoarchitectural features for the diagnosis of PTC in cytology smears.
2. To determine which combination of cytoarchitectural features is most reliable for diagnosis of papillary thyroid carcinoma in cytology smears.

2. Materials and Methods

2.1. Study design

Descriptive cross sectional study with an analytical component.

2.2. Study population and study setting

Fine needle aspiration smears from 50 consecutive histologically confirmed PTC cases, reported at the Departments of Pathology, Faculty of Medicine Colombo and at National Hospital, Sri Lanka during the period of January 2012 to September 2015 were retrieved from the archives. FNAs from 50 consecutive histologically confirmed cases of nodular goiter, chronic autoimmune thyroiditis or hyperplastic nodules reported at the same setting during the period from January 2014 to September 2015 were also retrieved to be used as controls.

2.3. Inclusion and exclusion criteria

Haematoxylin and eosin (H and E) stained slides of each case were selected consecutively.

The slides showing extensive drying artifact and slides which were broken or poorly staining were excluded.

2.4. Sample size calculation

Sample size was calculated according to the standard formula used to calculate the proportion, taking into consideration confidence interval (CI) of 70% (55-85%) of sensitivity and confidence level at 95%.

$$n = \frac{p\,(1-p)\,x\,Z^2}{c^2}$$

$$n = \frac{0.7(0.3) \times 1.96^2}{0.15^2}$$

n=36 each

n= Sample size

Z = Z value (1.96 for 95% confidence level).

p = Expected proportion in population based on previous studies

0.7 used for sample size needed

c = Confidence interval expressed as decimal (e.g., 0.15= ±15) the slides wa

According to the above formula minimum number of sample for the study is 36 in each group. Therefore, in our study we included 50 consecutive cases (papillary group) and 50 consecutive controls (non-papillary group).

2.5. Sampling technique and data collection

The cytology slides of the cases and the controls were mixed and selected randomly for the review.

The presence or absence of the following 31 architectural, cytological and background features were assessed on each H & E stained FNA smear.

2.5.1. Architectural features

1. Cellularity: defined by the number of cell clusters which is further divided into two groups: i. ≤20 ii. >20
2. Flat syncytial sheets: defined as flat sheets of follicular cells with lack of distinct cell borders.
3. Papillary structures with anatomical borders without fibro vascular cores: Anatomical border is defined as a well-defined sharp edge formed by a row of cuboidal or columnar cells.
4. True papillae with fibrovascular cores (Figure 1 A, B).

5. Micro acinar structures away from the sheets**:** defined by the micro acinar arrangement of follicular cells with well–defined lumina.
6. Cellular swirls: defined as concentrically organized aggregates of about 50-200 tumour cells. Majority of the peripherally situated cells have ovoid nuclei and their long axes are arranged perpendicular to the radius of the swirl (1) (Figure 1 C, D).
7. Individually dispersed bare nuclei.
8. Individually dispersed cells with eosinophilic cytoplasm (Figure 1 E).
9. Cellular crowding within nests and sheets**:** defined by overlapping of nuclei.

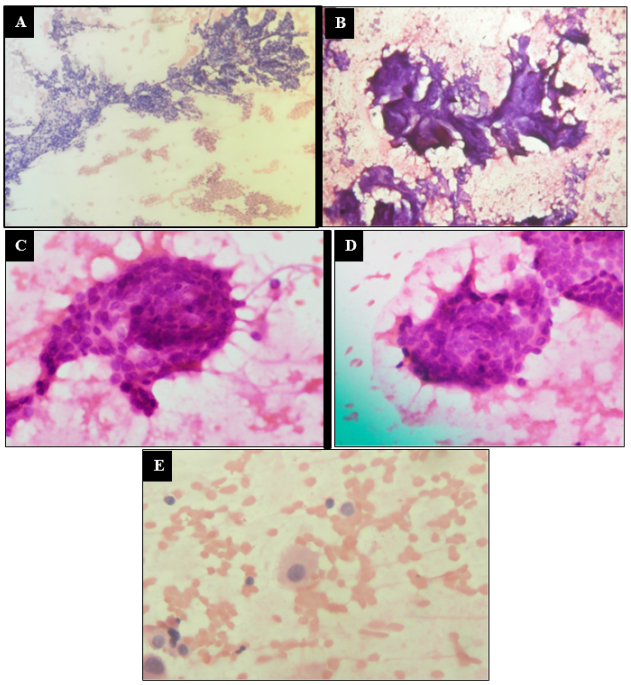

Fig. 1: Architectural features; **A,B**): True papillae with flat syncytial sheets (H&E x200); **C,D):** Cellular swirls (H&E x400); **E**): Individually dispersed cells with eosinophilic cytoplasm (H&E x400)

2.6. Cytological features

1. Anisonucleosis/Nuclear enlargement**:** Enlarged nucleus is defined as a nucleus > two times that of surrounding follicular epithelial cell (Figure 2 A).
2. Elongated, oval shaped nuclei or oblong nuclei (Figure 2 A)
3. Fine, powdery chromatin (Figure 2 A)
4. Longitudinal nuclear grooves/creases**:** defined as continuous groove or crease which is clearly defined (Figure 2 A).
5. Nuclear outline irregularity including notched nuclei (Figure 2 A)
6. Thickened nuclear membranes
7. Crescent shaped, collapsed nuclei with nuclear moulding (Figure 2 A)
8. Intranuclear cytoplasmic inclusions (INCI): defined as sharp, well- defined membrane like margin which is not optically clear but is similar to the colour and texture of the cytoplasm and occupies two-thirds of the nucleus.
9. Nucleoli: marginated and non-marginated
10. Histiocytoid cells**:** Cells with abundant and vacuolated cytoplasm and large irregular nuclei with occasional nucleoli, grainy chromatin which lack grooves and pseudo inclusions (Figure 2 B).
11. Cells with septate vacuoles in the cytoplasm: defined as small intracytoplasmic vacuoles which resemble soap bubbles.
12. Hurtheloid cells: Cells with abundant eosinophilic and finely granular cytoplasm.C
13. Metaplastic squamous cells: Cells with moderately abundant eosinophilic cytoplasm (Figure 2 C)s
14. Columnar cells: Cells with height two times more than their width showing oxyphilic featuress
15. Mitotic figures.

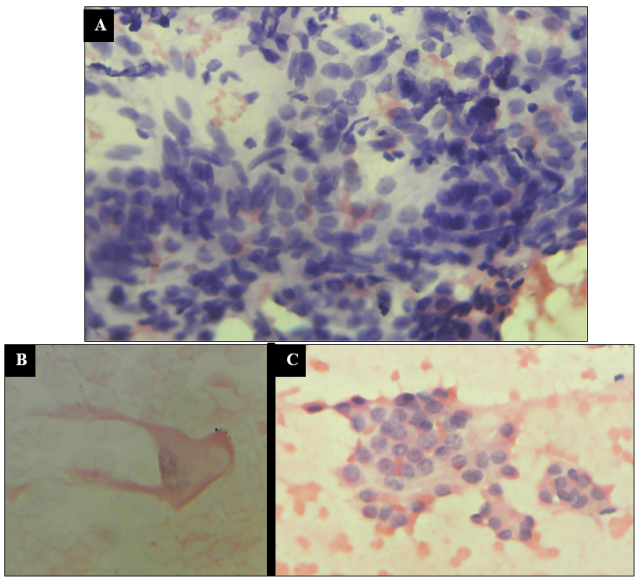

Fig. 2: Cytological features; **A):** Cellular crowding, anisonucleosis, oblong, grooved nuclei, powdery chromatin and occasional collapsed nuclei (H&E x400); **B**): Histiocytoid cells (H&E x400); **C**): Metaplastic squamous cells (H&E x400)

2.7. Background features

1. Bubble gum' or ropy colloid: strands and chunks of dense colloid intimately associated with the neoplastic cells.
2. Multinucleated giant cells

3. Psammoma bodies: Glassy, refractile, concentric calcified lamellated bodies
4. Cyst macrophages
5. Lymphocytes admixed with cell clusters
6. Plasma cells admixed with cell clusters
7. Neutrophils within the aspirates

For further assessment, the statistically significant cytological features were then combined into 10 groups as follows;

Combination 1: Flat syncytial sheets, true papillae, cellular swirls, anisonucleosis, elongated oval nuclei and INCI.[2,4,6,9,10,13]

Combination 2: Anatomical borders, true papillae, cellular swirls, elongated oval nuclei, fine powdery chromatin and crescent shaped collapsed nuclei.[3,4,6,10,11,14]

Combination 3: Flat syncytial sheets, true papillae, individually dispersed cells with eosinophilic cytoplasm, nuclear outline irregularity, thickened nuclear membranes and INCI.[2,4,8,13,15,16]

Combination 4: Anisonucleosis, fine powdery chromatin, nuclear outline irregularity, crescent shaped collapsed nuclei and INCI.[9,11,13–15]

Combination 5: Flat syncytial sheets, True papillae, cellular swirls, Anisonucleosis, elongated oval nuclei, nuclear grooves, thickened nuclear membranes, INCI and bubble gum colloid.[2–4,6,9,10,12,13,16]

Combination 6: True papillae and Cellular swirls.[3,4]

Combination 7: Nuclear overlapping and Fine powdery chromatin.[9,11]

Combination 8: Nuclear grooves an INCI.[10,13]

Combination 9: Flat syncytial sheets and Anatomical borders.[3,4]

Combination 10: Anisonucleosis and Nuclear grooves.[6,10]

2.8. Statistical analysis

The presence or absence of each cytoarchitectural feature was recorded and entered in a Microsoft excel sheet. The final data was then analyzed by SSPS software (version 23). The sensitivity, specificity, positive and negative predictive values (PPV and NPV) of the selected parameters were calculated after constructing two by two tables, considering the histological diagnosis as the gold standard (Table 1). The chi-square test and odds ratio were used to determine the statistical significance ($p<0.05$) of each cytological parameter. If the former two methods were not applicable like in instances where one or two of the values in 2x2 table is less than 5, the Fisher's exact test was used (Table 2).

Table 1: Comparison of the presence of cytoarchitectural features of FNAC amongst the patients with papillary carcinoma and non-papillary thyroid lesions (n=100)

Characteristc	Papillary (=50)	Non-papillary (=50)	Sensitivity%	Specificity %	PPV %	NPV %
Cellularity						
≤20 cells	14	20	72.0%	40.0%	54.5%	58.8%
>20 cells	36	30				
Flat syncytial sheets						
Present	43	12	86.0%	76.0%	78.2%	84.4%
Absent	7	38				
Anatomical borders						
Present	37	08	74.0%	84.0%	82.2%	76.4%
Absent	13	42				
True Papillae						
Present	28	0	56.0%	100.0%	100.0%	69.4%
Absent	22	50				
Microacinar structures away from the sheets						
Present	40	24	80.0%	52.0%	62.5%	72.2%
Absent	10	26				
Cellular swirls						
Present	29	5	58.0%	90.0%	85.3%	68.2%
Absent	21	45				
Individually dispersed bare nuclei						
Present	48	49	96.0%	2.0%	49.5%	33.3%
Absent	02	01				
Individually dispersed cells with eosinophilic cytoplasm						
Present	36	0	72.0%	100.0%	100.0%	78.1%
Absent	14	50				
Anisonucleosis/ nuclear enlargement						
Present	47	28	94.0%	44.0%	62.7%	88.0%
Absent	03	22				
Cellular crowding with nuclear overlapping						
Present	47	34	94.0%	32.0%	58.0%	84.2%
Absent	03	16				
Elongated oval nuclei						
Present	47	16	94.0%	68.0%	74.6%	91.9%
Absent	03	34				
Fine powdery chromatin						
Present	47	08	94.0%	84.0%	85.5%	93.3%
Absent	03	42				
Nuclear grooves						
Present	49	43	98.0%	14.0%	53.3%	87.5%
Absent	01	07				
Nuclear outline irregularity including notched nuclei						
Present	44	02	88.0%	96.0%	95.7%	88.9%
Absent	06	48				
Thickened nuclear membranes						
Present	45	08	90.0%	84.0%	84.9%	89.4%
Absent	05	42				
Crescent shaped collapsed nuclei with nuclear moulding						
Present	43	12	86.0%	76.0%	78.2%	84.4%
Absent	07	38				

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Table 1 continued

INCI						
Present	42	20	84.0%	60.0%	67.7%	78.9%
Absent	08	30				
Nucleoli: Marginated and non-marginated						
Present	47	42	94.0%	16.0%	52.8%	72.7%
Absent	03	08				
Histiocytoid cells						
Present	25	0	50.0%	100.0%	100.0%	66.7%
Absent	25	50				
Cells with septate vacuoles in the cytoplasm						
Present	14	0	28.0%	100.0%	100.0%	58.1%
Absent	36	50				
Hurtheloid cells						
Present	08	20	16.0%	60.0%	28.6%	41.7%
Absent	42	30				
Columnar cells						
Present	03	0	6.0%	100.0%	100.0%	51.5%
Absent	47	50				
Mitoses						
Present	06	01	12.0%	98.0%	85.7%	52.7%
Absent	44	49				
Metaplastic squamous cells						
Present	06	0	12.0%	100.0%	100.0%	53.2%
Absent	44	50				
Bubble gum or ropy colloid						
Present	24	11	48.0%	78.0%	68.6%	60%
Absent	26	39				
Multinucleated giant cells						
Present	41	38	82.0%	24.0%	51.9%	57.1%
Absent	09	12				
Psammoma bodies						
Present	05	0	10.0%	100.0%	100.0%	52.6%
Absent	45	50				
Cyst macrophages						
Present	18	27	36.0%	46.0%	40.0%	41.8%
Absent	32	23				
Lymphocytes admixed with cell clusters						
Present	46	38	92.0%	24.0%	54.8%	75.0%
Absent	04	12				
Plasma cells admixed with cell clusters						
Present	36	25	72.0%	50.0%	59.0%	64.1%
Absent	14	25				
Neutrophils within the aspirate						
Present	10	09	20.0%	82.0%	52.6%	50.6%
Absent	40	41				

PPV – Positive predictive value; NPV – Negative predictive value

Table 2: Detection of significance of each characteristic by chi square and Fisher's exact test where applicable

Characteristc	Papillary (=50)	%	Non-papillary (=50)	%	Significance
Cellularity					
≤20 cells	14	28.0%	20	40.0%	X2 = 1.604, df = 1, p = >0.05
>20 cells	36	72.0%	30	60.0%	Odds ratio 0.583 (CI 0.252—1.348)
Flat syncytial sheets					
Present	43	86.0%	12	24.0%	X2 = 38.828, df=1, p=0.000
Absent	7	14.0%	38	76.0%	Odds ratio 19.452 (CI 6.950 – 54.446)
Anatomical borders					
Present	37	74.0%	08	16.0%	
Absent	13	26.0%	42	84.0%	
True Papillae					
Present	28	56.0%	0	0%	
Absent	22	44.0%	50	100.0%	
Microacinar structures away from the sheets					
Present	40	80.0%	24	48.0%	X2 = 11.111, df=1, P < 0.05
Absent	10	20.0%	26	52.0%	Odds ratio 4.333 (CI 1.784—10.528)
Cellular swirls					
Present	29	58.0%	5	10.0%	X2 =25.668, df=1, P= 0.000
Absent	21	42.0%	45	90.0%	Odds ratio 12.429 (CI 4.216-36.643)
Individually dispersed bare nuclei					
Present	48	96.0%	49	98.0%	Two values are less than 5 hence the statistical tests cannot be used for significance
Absent	02	4.0%	01	2.0%	
Individually dispersed cells with eosinophilic cytoplasm					
Present	36	72.0%	0	0%	Fisher's exact test p=0.000
Absent	14	18.0%	50	100.0%	
Anisonucleosis/ nuclear enlargement					
Present	47	94.0%	28	56.0%	Fisher's exact test p=0.000
Absent	03	6.0%	22	44.0%	
Cellular crowding with nuclear overlapping					
Present	47	94.0%	34	68.0%	Fisher's exact test p<0.05
Absent	03	6.0%	16	32.0%	
Elongated oval nuclei					
Present	47	94.0%	16	32.0%	Fisher's exact test p=0.000
Absent	03	6.0%	34	68.0%	
Fine powdery chromatin					
Present	47	94.0%	08	16.0%	Fisher's exact test p=0.000
Absent	03	6.0%	42	84.0%	
Nuclear grooves					
Present	49	98.0%	43	86.0%	
Absent	01	2.0%	07	14.0%	
Nuclear outline irregularity including notched nuclei					
Present	44	88.0%	02	4.0%	
Absent	06	12.0%	48	96.0%	

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Table 2 continued

Thickened nuclear membranes					
Present	45	08	90.0%	84.0%	X2 =54.958, df=1, p=0.000
Absent	05	42			Odds ratio 47.250 (CI 14.319-155.915)
Crescent shaped collapsed nuclei with nuclear moulding					
Present	43	86.0%	12	24.0%	X2=38.828, df=1, p=0.000
Absent	07	14.0%	38	76.0%	Odds ratio 19.452 (CI 6.950-54.446)
INCI					
Present	42	84.0%	20	40.0%	X2=20.543, df=1, p=0.000
Absent	08	16.0%	30	60.0%	Odds ratio 7.875 (CI 3.063—20.247)
Nucleoli: Marginated and non-marginated					
Present	47	94.0%	42	84.0%	Fisher's exact test p>0.05
Absent	03	6.0%	08	16.0%	
Histiocytoid cells					
Present	25	50.0%	0	0%	
Absent	25	50.0%	50	100.0%	
Cells with septate vacuoles in the cytoplasm					
Present	14	28.0%	0	0%	
Absent	36	72.0%	50	100.0%	
Hurtheloid cells					
Present	08	20	16.0%	60.0%	X2 =7.143, df=1, p>0.05
Absent	42	30			Odds ratio 0.286 (CI 0.111-0.735)
Columnar cells					
Present	03	0	6.0%	100.0%	Two values are less than 5 hence the statistical tests cannot be used for significance
Absent	47	50			
Mitoses					
Present	06	01	12.0%	98.0%	Fisher's exact test p>0.05
Absent	44	49			
Metaplastic squamous cells					
Present	06	0	12.0%	100.0%	Fisher's exact test p<0.05
Absent	44	50			
Bubble gum or ropy colloid					
Present	24	11	48.0%	78.0%	X2 =9.653, df=1, p<0.05
Absent	26	39			Odds ratio 0.260 (CI 0.109-0.621)
Multinucleated giant cells					
Present	41	38	82.0%	24.0%	X2 =0.542, df=1, p>0.05
Absent	09	12			Odds ratio 1.439 (CI 0.545-3.797)
Psammoma bodies					
Present	05	10.0%	0	0%	
Absent	45	90.0%	50	100.0%	
Cyst macrophages					
Present	18	36.0%	27	54.0%	X2=3.273, df=1, p>0.05
Absent	32	64.0%	23	46.0%	Odds ratio 0.479 (CI 0.215-1.068)

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Table 2 continued

Lymphocytes admixed with cell clusters					
Present	46	92.0%	38	76.0%	Fisher's exact test p=>0.05
Absent	04	8.0%	12	24.0%	
Plasma cells admixed with cell clusters					
Present	36	72.0%	25	50.0%	X2 =5.086, df=1, p<0.05
Absent	14	18.0%	25	50.0%	Odds ratio 2.571 (CI 1.122-5.895)
Neutrophils within the aspirate					
Present	10	20.0%	09	18.0%	X2 =.065, df=1, p>0.05
Absent	40	80.0%	41	82.0%	Odds ratio 1.139 (CI 0.419-3.097)

Then the sensitivity, specificity, PPV and NPV of the above combinations were calculated (Table 3). The 2x2 table is formed in consideration of the presence or absence of all the features.

Then the combinations of the cytoarchitectural features were further analyzed by receiver operating characteristic (ROC) curves to determine the correlation of the combined features with PTC and also to conclude whether the combination of more than two features were more significant than the combination of only two features (Figure 3 and Table 4).

According to the ROC curves the results were interpreted as follows:

Area under the curve	Quality of the test
0.9-1	Excellent
0.8-0.9	Good
0.7-0.8	Fair
0.6-0.7	Poor
0.5-0.6	Fail

The cytological features were categorized according to the specificity and the PPV into the following three groups and the sensitivity of each feature was compared (Table 5).

1. 100% Specificity and 100% PPV
2. 90% Specificity and 90% PPV
3. 80% - 90% Specificity and 80% PPV

3. Results

A total of 100 FNAs were evaluated. In the papillary group 31 cases had been diagnosed as PTC (Thy 5, Bethesda 6) whilst 19 cases had been diagnosed suspicious for PTC (Thy4, Bethesda 5). All FNA cases diagnosed as diagnostic of and suspicious for PTC were confirmed as PTC by histology. In the control group, 2 cases had been diagnosed as follicular proliferation (Thy 3). Rest of the cases were diagnosed as benign (Thy2, Bethesda 2) of which 28 cases had been diagnosed as colloid storing goiter and 20 cases as chronic autoimmune thyroiditis on cytological evaluation. The two cases diagnosed as follicular proliferation were confirmed as hyperplastic nodules by histology. The remaining 48 cases were confirmed histologically as colloid storing goiter (28 cases) or chronic autoimmune thyroiditis (20 cases).

According to the statistical analysis, the highly statistically significant (p=0.000) cytoarchitectural features are flat syncytial sheets, anatomical borders, true papillae, cellular swirls, individually dispersed cells with eosinophilic cytoplasm, anisonucleosis, elongated oval nuclei, powdery chromatin, nuclear outline irregularity, thickened nuclear membranes, crescent shaped collapsed nuclei, intranuclear inclusions, histocytoid cells and cells with soap bubble cytoplasmic vacuolation. Most of these features also had high sensitivity, specificity, PPV and NPV (>70%). However, features like true papillae, cellular swirls, histocytoid cells and cells with soap bubble cytoplasmic vacuolation had low sensitivity but very high specificity whereas features like intranuclear inclusions, anisonucleosis and elongated nuclei had high sensitivity but slightly low specificity.

The other architectural, cytological and background features which were found to be statistically significant ($p<0.05$) were microacinar structures away from the sheets, cellular crowding with nuclear overlapping, nuclear grooves, squamoid cells and bubble gum colloid. All these features except the latter two had high sensitivity and low specificity.

Squamoid cells and bubble gum colloid were more specific (100% and 78% respectively) than sensitive (12% and 48% respectively). Presence of plasma cells admixed with cell clusters also showed statistical significance ($p<0.05$) in this study with a sensitivity of 72% and a specificity of 50%. Rest of the features (cellularity, marginated and non-marginated nucleoli, mitosis, multinucleated giant cells, hurtheloid cells, cyst macrophages, lymphocytes and neutrophils within the aspirate) were statistically insignificant ($p>0.05$). Although psammoma bodies which are generally considered to be significant, failed to prove so in our study. The statistical studies could not be applied on two features; individually dispersed bare nuclei and columnar cells, as two of the four values were less than five. It was not possible to analyze individual cytoarchitectural parameters by ROC since the variables were categorical and not continuous.

All the combinations of more than two features were found to be statistically highly significant in differentiating between PTC and other thyroid pathologies with a statistical significance of p=0.000 with specificity and PPV of 100% in all five combinations. Combination 3 which included flat syncytial sheets, true papillae, individually dispersed cells with eosinophilic cytoplasm, nuclear outline irregularity, thickened nuclear membranes and showed 100% sensitivity and specificity.

Our study also showed that all the combinations of more than two features to be in the first category of 0.9-1 so the test quality indicated as being excellent. All the five combinations are more or less similar with the highest area under the curve to be for combination 2 which is 0.936. The ROC for combinations of only two features completely failed the test thus highlighting the importance of having more than two cytological features for the combinations to be significant.

Although the single cytological parameters could not be compared to the combination parameters in the ROC, it was shown that certain statistically significant single cytological parameters were present in some proportion in the non-papillary group as well. However, when more than

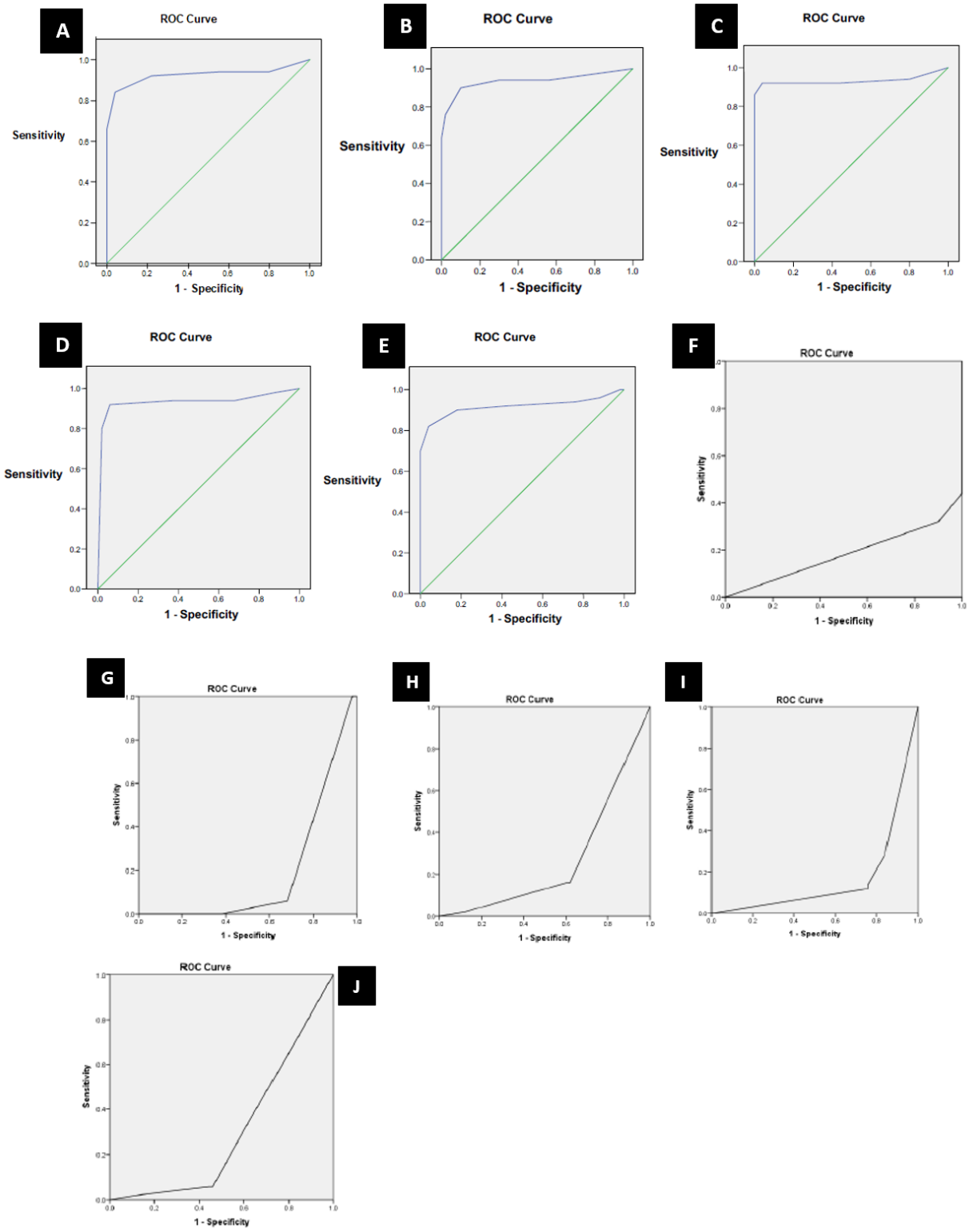

Fig. 3: ROC curves of the combination; **A-J:** Combination 1 -10

Table 3: Calculating sensitivity, specificity, PPV, NPV and significance of combinations of statistically important selected cytoarchitectural features

Characteristc	Papillary (=50)	Non-papillary (=50)	Sensitivity %	Specificity %	PPV %	NPV %	Significance
Combination 1							
Present	20	0	40%	100%	100%	62.5%	Fisher's exact test p=0.000
Absent	30	50					
Combination 2							
Present	22	0	44%	100%	100%	64%	Fisher's exact test p=0.000
Absent	28	50					
Combination 3							
Present	25	0	100%	100%	100%	66.6%	Fisher's exact test p=0.000
Absent	25	50					
Combination 4							
Present	40	0	80%	100%	100%	83.3%	Fisher's exact test p=0.000
Absent	10	50					
Combination 5							
Present	41	0	18%	100%	100%	54.9%	Fisher's exact test p=0.000
Absent	09	50					

CI – Confidence interval

Table 4: ROC of the combinations: area under the curve

Combination	Area	Standard error[a]	Asymptotic significance[b]	Asymptotic 95% confidence interval	
				Lower bound	Upper bound
1	0.924	0.032	0.000	0.862	0.987
2	0.936	0.028	0.000	0.861	0.990
3	0.932	0.033	0.000	0.869	0.996
4	0.933	0.031	0.000	0.873	0.993
5	0.917	0.033	0.000	0.853	0.982
6	0.182	–	–	–	–
7	0.188	–	–	–	–
8	0.268	–	–	–	–
9	0.165	–	–	–	–
10	0.301	–	–	–	–

Thetest result variable(s): Total 2 has at least one tie between the positiveactual state group and the negative actual state group. Statistics may bebiased.
a. Under the nonparametric assumption
b. Null hypothesis: true area = 0.5

two statistically significant cytoarchitectural features were combined, all had 100% specificity and none were present in the non-papillary group as a total. This signifies the use of combination of cytoarchitectural features in the diagnosis of PTC on FNA.

As indicated in Table 5, many individual cytological features with 100% specificity and 100% PPV {except for individually dispersed cells with eosinophilic cytoplasm (72%) and true papillae (56%)} had sensitivity of 50% or less. This highlights the problem in cytological diagnosis of PTC, where individual cytological features with a high specificity and positive predictive value may not always be reliable due to low sensitivity. Nuclear outline irregularity in the category of 90% specificity and 90% PPV also had a high sensitivity (88%). In the category of 80-90% specificity and 80% PPV, most of the features had a high sensitivity except for the mitoses (12%).

4. Discussion

The main aim of our study was to determine the usefulness of individual cytoarchitectural features in FNA smears for the diagnosis of PTC and its variants and to further determine not only the most useful cytoarchitectural features but also the most reliable combination of cytoarchitectural features for the diagnosis of PTC in cytology smears.

The rationale behind this was that in spite of the well-defined cytological features described in numerous studies the diagnosis of PTC on cytology is often quite difficult and as of date, there has been no international standard which exists for the cytological diagnosis of PTC.

Twenty out of the 31 cytological parameters analyzed in our study were found to be statistically significant in the diagnosis of PTC when compared to the control group.

Table 5: Categorization of cytologic features according to the specificity and the PPV. 100% Specificity and 100% PPV

100% Specificity and 100% PPV	
Characteristics	**Sensitivity %**
Individually dispersed cells with eosinophilic cytoplasm	72
True Papillae	56
Histiocytoid cells	50
Cells with septate vacuoles in the cytoplasm	28
Metaplastic squamous cells	12
Psammoma bodies	10
Columnar cells	6
90% Specificity and 90% PPV	
Characteristics	**Sensitivity %**
Nuclear outline Irregularity	88
80-90% Specificity and 80% PPV	
Characteristics	**Sensitivity %**
Fine powdery chromatin	94
Thickened nuclear membranes	90
Anatomical borders	74
Cellular swirls	58
Mitoses	12

Of these, 14 features had a significance of p=0.000 whilst six features had a significance of p<0.05. The 14 features included flat syncytial sheets, anatomical borders, true papillae, cellular swirls, individual cells with eosinophilic cytoplasm, anisonucleosis, elongated oval nuclei, fine powdery chromatin, nuclear outline irregularity, thickened nuclear membranes, crescent shaped collapsed nuclei with or without nuclear moulding, INCI, histiocytoid cells and cells with septate vacuolation. There was a significant correlation between the histologic diagnosis of PTC and the cytological findings mentioned above.

However, when the statistically significant cytoarchitectural features were combined, all the five combinations of more than four features had 100% specificity and none of the features when combined were all present in the non-papillary group. According to the ROC, combination of more than four cytological features was more significant than combining two features. Conclusion

This study signifies the importance of the use of combination of a larger number of cytoarchitectural features in the diagnosis of PTC on FNA. Further recommendation is to include a larger study group to devise a scoring system which can then be applied to classify the FNA as diagnostic for PTC.

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None.

6. Conflict of Interest

None.

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