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FNAC (Fine needle aspiration cytology) and histopathological correlation and reclassification of thyroid neoplasm in accordance with WHO classification 2022

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ABSTRACT

Background: While assessing whether a patient requires surgery or conservative treatment, the thyroid FNAC is crucial. “The Bethesda System of Reporting Thyroid Cytopathology” was introduced to resolve the problem of standardising terminology in Thyroid FNA diagnosis. This study aims to evaluate the histopathological correlation and to also assess the diagnostic accuracy of TBSRTC at an institutional level with respect to histopathology. Additionally, reclassification of these cases in accordance with new Update of WHO Classification of Thyroid tumors 2022.

Materials and Methods: This is a 3-year retrospective study of thyroid lesions from August 2019 to August 2022 comprising of 160 cases. Data was retrieved from institutional database and analysed. Fine needle aspiration Cytology- Histopathological correlation followed by determination of diagnostic accuracy of TBSRTC was done using Sensitivity, Specificity, PPV, NPV and accuracy. Reclassification was done on the 160 histopathological diagnosis based on the new updated classification.

Results: Study showed female predominance and largest cohort belonged to the age group of 21–40 years. Cytohistopathological correlation was done and 4 discordant cases were identified. The Sensitivity, Specificity, PPV, NPV and Accuracy was found to be 95.77, 98.87, 98.55%, 96.7% and 97.5% respectively. On reclassification, the tumors were now classified based on cell of origin and maximum no. of cases were identified under the category of Follicular- cell derived.

Conclusion: FNAC is a sensitive and specific method of evaluating thyroid lesions. TBSRTC shows excellent diagnostic accuracy in detecting malignant lesions and can be used for screening neoplasm. The new classification demonstrates classification based on tumour cell of origin. It is crucial for Clinicians and Pathologists to be informed of new terminologies and classification schemes to aid more therapeutic options for patients.

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1. Introduction

In India, 42 million people were estimated to be suffering from thyroid disease as of 2013,¹ according to statistics. While there are 8.7 cases of thyroid cancer per 100,000 people in India each year, there are 12.2% more palpable thyroid nodules than there are thyroid cancer cases.² Thyroid FNA plays main role in triaging patient for

either surgery or conservative management and is a valid first- line diagnostic method for palpable thyroid lesions.¹ FNAC, however, has limitations. The main drawbacks being false positive and false negative diagnoses resulting from overlap between cytological features of benign and malignant follicular neoplasms and additionally insufficient and inconclusive FNA.² Therefore, it is vital that cytopathologists give referring physicians concise and therapeutically helpful thyroid FNA findings. The NCI, Bethesda, Maryland introduced “The Bethesda System”

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to resolve the problem of standardising terminology and other thyroid FNA related difficulties and included 6 diagnostic categories with unique risk of malignancy and recommended clinical management for each category.¹ It was revised again in the year 2017.¹ This type of consistent reporting will allow efficient communication between various healthcare practitioners. It will also help with research into the molecular biology and epidemiology. Therefore, it is important to identify and learn from any errors, it is crucial to routinely analyse and maintain the credibility of such a system.

The 5th edition of the WHO's histologic classification of thyroid neoplasms, which was released in 2022,³ also includes newly recognised tumour types, subtypes, and a grading system. A better knowledge of cell of origin, pathologic characteristics (cytopathology and histopathology), molecular classification, and biological behaviour is now possible due to the revised classification, which has separated thyroid cancer into new groups.^{4,5} The new classification scheme emphasises that papillary thyroid cancer (PTC) should be subtyped based on HP markers independent of tumour size to avoid classifying all lesions less than one centimetre as low-risk disease.⁴ In the new classification, the follicular cell-derived tumours, which constitute the majority of the classification are now divided into benign, low-risk, and malignant neoplasms. The most notable change in the 2022 WHO classification, which has major implications for clinicians, is the two-tiered grading system that separates high-grade tumours from well-differentiated follicular cell-derived carcinomas and Medullary thyroid carcinoma. By Reviewing this, our goal was to achieve a clearer understanding of how reclassifying tumours according to the origin of the tumour cells can help patients at varying levels of risk obtain a personalised therapeutic approach.⁴

Along with cyto-histopathological correlation, this study aims to assess the diagnostic accuracy of The Bethesda System of Reporting Thyroid Cytopathology (TBSRTC). Additionally, by the revised WHO 2022 Classification of Thyroid Tumors, we are reclassifying thyroid lesions in line with the 2017 WHO classification of thyroid neoplasms.

2. Materials and Methods

Our study follows an observational retrospective study design of the thyroid lesions that came between the period of 3 years from August 2019 to August 2022 in the Central Diagnostic Laboratory of the department of Pathology at the AJ Institute of Medical Sciences & Research Centre in Mangaluru, Karnataka, India. Data was documented and reviewed from the institutional database. Sample size estimation was done considering $p = 21\%$ based on the study done by Sharma C et al² with 95% Confidence interval and allowable error, $l = 10\%$. The minimum sample size ($n = 4pq/l^2$) estimated for the study was 75. Our study used

sample size of 160 cases, of which both the FNA and the HP diagnosis were available and then evaluated. All cases of neoplastic thyroid lesions wherever Histopathology reports were available were considered as the Inclusion Criteria. Cases with Incomplete data, inadequate or poorly preserved samples, and cases wherever there were no corresponding histopathological diagnoses or vice versa was excluded from the study. FNA lesions were diagnosed based on TBSRTC. They were categorized into I–Non-diagnostic or unsatisfactory (ND) ; II- Benign including Benign follicular nodule, Lymphocytic (Hashimoto's) thyroiditis and others III- Atypia of undetermined significance (AUS) or Follicular lesion of undetermined significance (FLUS) ; IV : Follicular neoplasm (FN) or suspicious for a follicular neoplasm (SFN) or Suspicious for Hurthle cell neoplasm (SFH); which included Follicular adenoma (FA), Follicular carcinoma (FTC) V: Suspicious for malignancy (SFM), ; VI: Malignant category- included Papillary Carcinoma (PTC) Medullary Carcinoma(MC), Metastatic Carcinoma, Lymphoma. To avoid confusion, while assessing the diagnostic accuracy Category III was considered under Benign lesions whereas Category IV and Category V were considered as 'malignant' for statistical analysis as both lead to surgical management as far as the treatment is concerned. Category IV includes Follicular Neoplasm, which required surgical resections regardless of the nature of tumor i.e Benign or malignant. This facilitates an easier comparison and clearer final results. The thyroid lesions included non-neoplastic inflammatory lesions, both benign and malignant neoplasms of thyroid which were reported using the WHO Classification of thyroid neoplasms 2017. The FNA and HP diagnoses were compared after grouping the lesions as benign and malignant. Taking HP diagnosis as the gold standard, the patient's pre-operative FNA diagnosis using TBSRTC was compared to the post-operative HP diagnosis. The accuracy, sensitivity, specificity, positive and negative predictive values of FNA diagnosis by TBSRTC was determined. Further Statistical analysis was done using SPSS software 29.0. The following formulas were used to determine the diagnostic accuracy.

1. Sensitivity = True Positive (TP) / (True positive + False Negative(FN))
2. Specificity = True negative(TN) / (True negative + False positive(FP))
3. Positive predictive value (PPV) = True Positive / (True positive + False Positive).
4. Negative predictive value (NPV) = True negative / (True negative + False negative).
5. Diagnostic accuracy = (TP + TN) / (TP + TN + FP + FN).

As this study was conducted on the specimens and FNA samples received in the Central Diagnostic Laboratory, AJIMS & RC. Hence, consent was implied. Institutional

Ethical committee clearance (AJEC/REV/145/2023) was received.

To gain a better understanding of new taxonomy and terminologies, classification schemes introduced in the new WHO classification of thyroid tumors 2022, we have attempted to regroup the various histopathological diagnoses, based on their cell of origin, low risk or high-risk categories, and also the clinical outcomes in malignant thyroid neoplasms, which is in accordance with the new WHO classification 2022. The new terms were also used to rename the previous histopathological diagnosis.

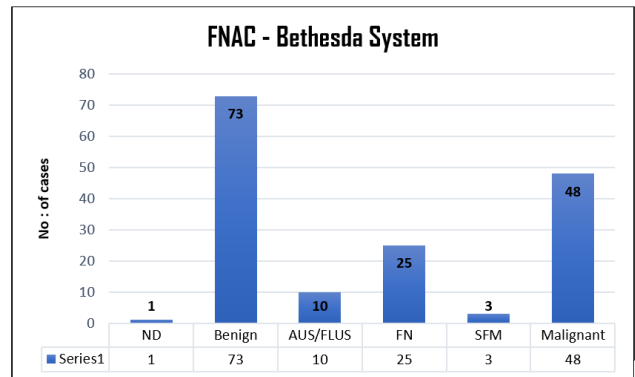
3. Results

The study was done on data 160 FNAC samples of thyroid lesions and corresponding HP specimens. The HP diagnosis which was taken as the gold standard, is kept as the reference to compare the accuracy of reporting the FNAC specimen of the corresponding thyroid lesions based on TBSRTC.

73 patients were in the age group of 21-40 years (45.6%) followed by 70 patients in the age group of 41-60 years of age group (43.6%), with the minimum age being 14 years and the maximum age being 81 years and mean age of 42.51 years. There were 82% of cases were females (131 cases) and 18% males (29 cases), with a male to female ratio of 1: 4.5.

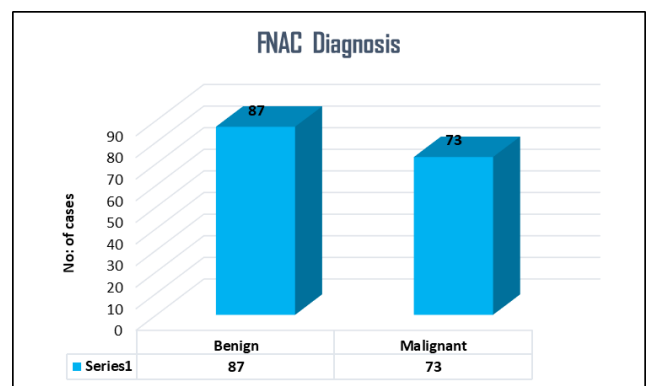
Among the 160 cases, (Graph 1) on Categorizing them according to TBSRTC maximum number of cases (73 cases) were diagnosed under Category II- Benign (45.6%) and it included 65 cases diagnosed as Benign follicular nodular disease (39.1%) – that comprised of adenomatoid nodule, Nodular colloid goitre(NCG), cystic colloid goitre. 4 cases were categorised as Consistent with Lymphocytic (Hashimoto’s) Thyroiditis (2.9%). One case of Infected thyroglossal cyst (0.6%) which is a developmental anomaly was also diagnosed. 10 cases (6.3%) were diagnosed as Category III- Atypia of undetermined significance (AUS)/Follicular lesion of undetermined significance (FLUS), in which only one case goitre (0.6%) was identified under FLUS which additionally had colloid. 25 cases (15.6%) were diagnosed under Category IV - Follicular neoplasm (FN). In the same category, 7 lesions (3.91%) that were suspicious for Hurthle cell neoplasm were included. 3 cases were categorised as Category V - Suspicious for malignancy. Category VI – Malignant, had the second highest number of cases (48 cases) and accounted for 30% of the total 160 cases in the study. It included 33 cases (20.6%) of Papillary thyroid carcinoma (PTC) and 7 cases (4.4%) of variants of PTC such as Follicular variant of papillary thyroid carcinoma (FVPTC). Other high grade tumors, such as 2 cases of Undifferentiated (Anaplastic) Carcinoma (1.3%), 1 case of medullary carcinoma (0.6%), 1 case of Squamous cell carcinoma (SCC) (0.6%), 3 cases of Metastatic Carcinoma from breast(2 cases) and kidney (1

case), 3 cases of Lymphoma. Only one case was categorised under Category 1 Non-diagnostic, this was included in the study as it was the only case with surgical follow-up and a histopathological diagnosis available.



Graph 1: FNAC diagnosis by The Bethesda System of Reporting Thyroid Cytopathology (TBSRTC)

To avoid confusion, categories like Category III (AUS/FLUS), Category IV (FN), and Category V (SFM) which had more ambiguous diagnoses had to be recategorized into Benign and Malignant categories in FNA cytology for correlating with histopathology. Hence, Category I & III was considered benign, whereas Category IV & V were considered “Malignant”, this also holds, because the usual mode of treatment for Category IV & V is surgical management. Following this under FNAC, 87 cases were considered Benign and 73 cases were considered malignant. (Graph 2)



Graph 2: Bar graph depicting Benign and malignant cases under FNAC

On reviewing the HP diagnosis of all 160 cases, 89 cases were diagnosed as benign lesions (55.6%) and 71 cases were diagnosed as malignant (44.4%) (Graph 3) was considered as Benign.

Results of cyto-histological correlation, is given in Graph 4 and discordant cases are listed in Table 1.

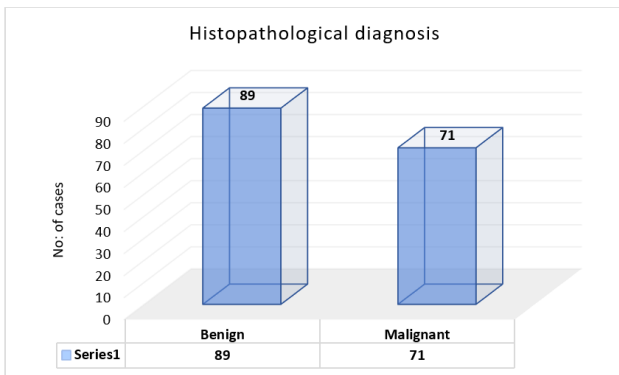
Table 1: Discordant cases in cyto-histopathological correlation (4 cases)

S. No.	Cytological diagnosis	Bethesda category	Histopathological diagnosis	Benign/malignant	Remarks
1.	Nodular Colloid Goitre (Figure 1)	2	Papillary thyroid microcarcinoma (Figure 2)	Malignant	False Negative
2.	Suspicious for Hurthle cell neoplasm (Figure 3)	4	Follicular Adenoma with Oncocytic change (Figure 4)	Benign	False Positive
3.	Nodular colloid goitre	2	Papillary thyroid microcarcinoma	Malignant	False Negative
4.	Blood with scant colloid (Figure 5)	1	Papillary thyroid microcarcinoma (Figures 6 and 7)	Malignant	False Negative

Table 2: 2 x 2 table for calculation of diagnostic accuracy of FNAC compared to Histopathology: True Positive (TP), False Positive (FP), True Negative (TN), False Negative (FN)

	Malignant (Histopathology)	Benign (Histopathology)
Malignant (Cytology)	68 cases (TP)	1 case (FP)
Benign (Cytology)	3 cases (FN)	88 cases (TN)

Total No: of Cases = 160



Graph 3: Bar graph depicting Benign and Malignant cases under histopathological diagnoses

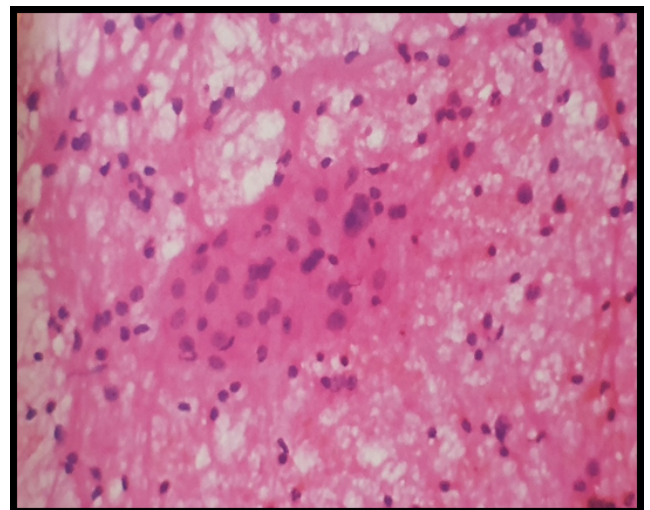
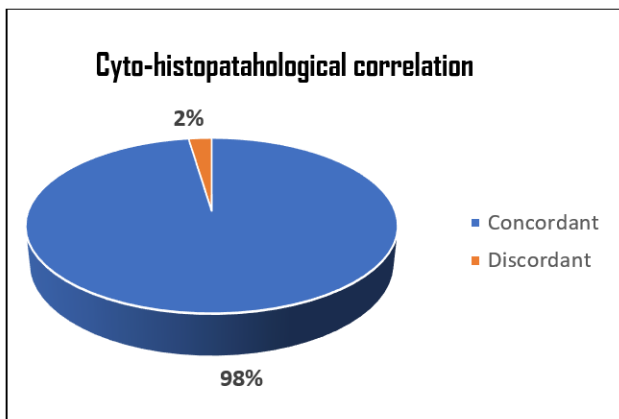


Fig. 1: Nodular colloid goitre - Category 2



Graph 4: Cyto-histological correlation- pie diagram depicting the concordant and discordant cases

The diagnostic accuracy was assessed, and the values were

1. Sensitivity = $TP / (TP + FN) = 68 / (68 + 3) * 100 = 95.77\%$.
2. Specificity = $TN / (TN + FP) = 88 / (88 + 1) * 100 = 98.87\%$.
3. Positive predictive value (PPV) = $TP / (TP + FP) * 100 = 68 / (68 + 1) = 98.55\%$.
4. Negative predictive value (NPV) = $TN / (TN + FN) * 100 = 88 / (88 + 3) = 96.7\%$.
5. Accuracy = $(TP + TN) / \text{Total number of cases} = (68 + 88) / 160 * 100 = 97.5\%$.

On reclassifying as per the new WHO classification 2022.⁴ The 160 cases were first divided based on the cell of origin

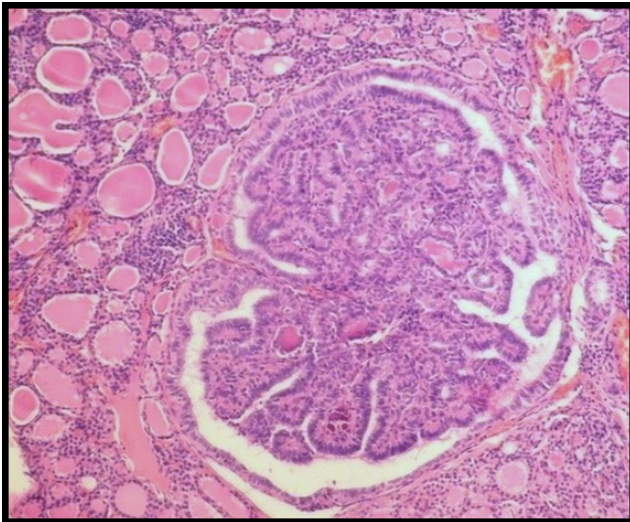


Fig. 2: A small cluster of papillary microcarcinoma was noted between the colloid goitre. This fragment was missed during FNA procedure

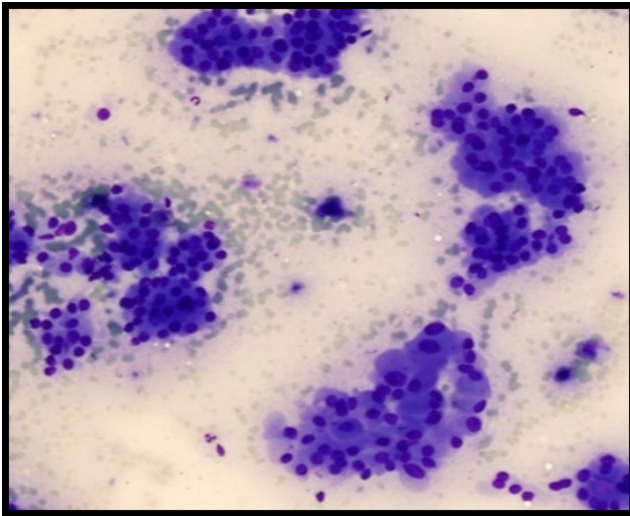


Fig. 3: Occasional clusters of follicular cells, with few of the cells showing Hurthle cell change

of tumor, in which 95% cases (152 cases) were classified under Follicular cell derived, which was the predominant category. The comparison of HP diagnosis under 2017 WHO Classification with new 2022 WHO classification, was done in brief for selected cases, where there were changes in nomenclature and reclassification in Table 3. Explanation for some of the revisions was also described wherever deemed necessary.

All 160 cases showed the same number of benign and malignant cases, and no case was reclassified to a malignant one or vice versa. As per the new WHO classification, in all the malignant cases, while categorising them based on Clinical outcome, 88.52% cases had

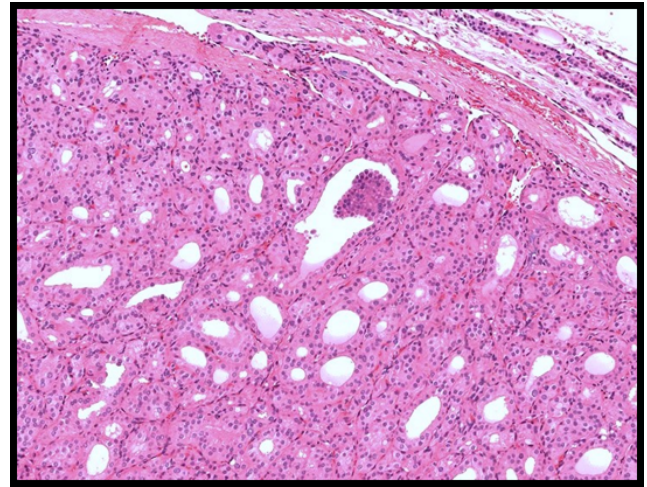


Fig. 4: Case of Follicular adenoma, with occasional oncocytic change of the follicular epithelium

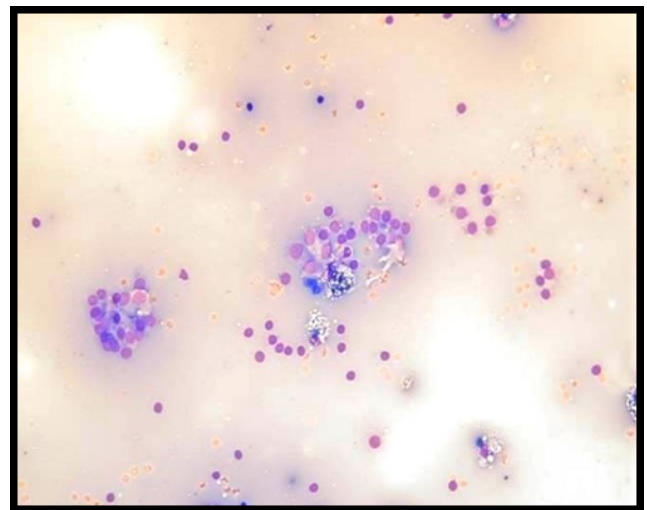


Fig. 5: Few scattered follicular cells, with scant colloid and RBCs-Category 1

favourable outcome, despite its HP diagnosis. 6.56% cases had Intermediate outcome, these included 2 cases each of poorly differentiated high grade carcinoma and differentiated high grade carcinoma. 4.92% of cases showed Poor clinical outcome and included all 2 cases of Anaplastic Carcinoma of Thyroid.

4. Discussion

As literature has shown that all endocrine lesions including thyroid shows female predominance, the present study shows female predilection with 80% cases in our study, this was consistent with the findings in the study done by Letha Padmom et al (85.7%)³ & Sharma C et al. (82%).² The male to female ratio was 1: 4.5 which is concordant with the study done by Prasanta kumar et al,⁶ who reported a

Table 3: Reclassification of tumors in our study: Comparing HP diagnoses under 2017 classification with HP diagnoses under 2022 classification - changes in nomenclature and classification of tumor Comparison of changes in nomenclature and classification of thyroid tumor w.r.t current study

Terms	HP diagnoses under 2017 WHO Classification	HP diagnoses under 2022 WHO Classification	Explanation (if any)
	Hurthle cell adenoma/ carcinoma	Oncocytic cell adenoma of thyroid/Oncocytic cell carcinoma of thyroid	
	Follicular variant of Papillary thyroid carcinoma	Follicular subtype of Papillary thyroid carcinoma	
Tumor type / Subtype	N/A - Multinodular goiter (Not included in 2017 classification)	Thyroid follicular nodular disease	Earlier MNG was and other benign follicular diseases were not included in WHO
	Invasive encapsulated Follicular variant of PTC	Invasive encapsulated Follicular variant Papillary carcinoma	Separated from other subtypes of PTC
	Papillary microcarcinoma variant	Further subtyping required	It is no longer an entity and regardless of the size, has to be further subtyped to its specific histology
	PTC, FTC with high grade features (Differentiated tumors with high grade features)	Differentiated high grade thyroid carcinoma	
	Papillary thyroid carcinoma-cribriform morular variant	Cribriform- morular thyroid carcinoma	Separate entity, separated from PTC
	Squamous cell Carcinoma	Anaplastic thyroid carcinoma, squamous cell pattern	
	Congenital anomalies- Thyroglossal cyst, Brachial cyst	Thyroglossal duct cyst-Classified under Development cell abnormality	Earlier not included in WHO classification, now included as new classification based on cell of origin
	N/A	NIFTP – classified under Follicular cell derive neoplasm- low risk neoplasm	A fairly new entity, which has good clinical outcome on excision, therefore categorized as Low risk neoplasm
	Medullary Carcinoma Thyroid	Classified under Thyroid C cell derived- medullary carcinoma thyroid- low grade	It classified into Low grade & high grade. In this case, after reviewing the mitotic rate it was $<5/2 \text{ mm}^2$ and had low Ki 67 values
	Metastatic Carcinoma – IDC & Clear Cell Renal Cell Carcinoma	N/A	Not described in the new classification, status of the diagnosis in thyroid classification remains unclear.
	Lymphomas	N/A	Not described in the new classification, status of the diagnosis in thyroid classification remains unclear.

female to male ratio 5.6:1. The most common age group noticed in our study was 21-40 years of age group (45.7%), this was in concordance with other studies done by Kumari S et al.⁷ and discordant with R Thakur et al⁸ (68.6%), were the commonest age group was the 4th and 5th decade.

In our study, the commonest lesion was NCG constituting about 36.9% of the total cases, this was in concordance with other National studies, such as Sreedevi et al.⁹

Mondal et al. found high incidence of category II lesions which was discordant with the findings in our study where there were 45. 2% of Category II lesions, which may be attributed to selections of only those cases which had a surgical follow up and histopathological diagnosis, Category II lesions are generally treated conservatively and are only operated on when they cause pressure symptoms, have high risk features or family history or due to much less common cosmetic purpose as well.¹⁰ The regional variation may also be an attributable cause for more occurrence of

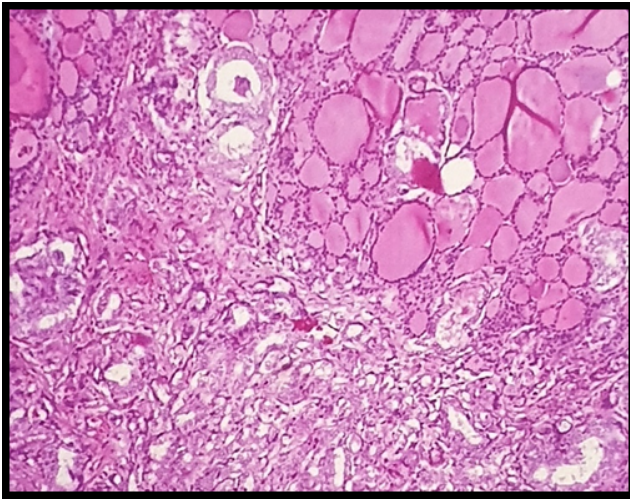


Fig. 6: A low power view of the tumor border shows neoplastic follicles infiltrating between the surrounding normal follicles and desmoplastic stromal reaction (H & E 200x)

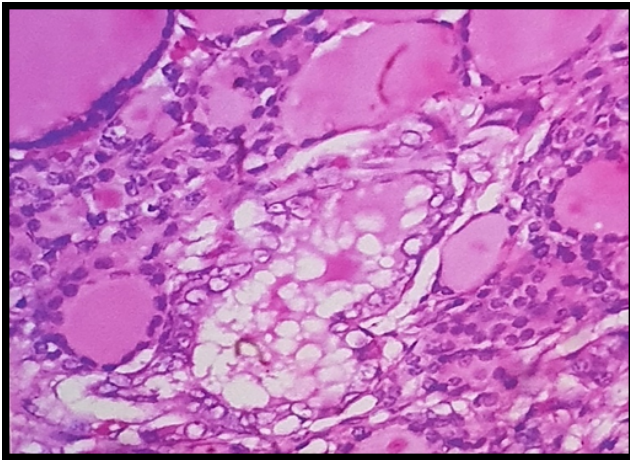


Fig. 7: Tumor borders shows tumor cells arranged in follicles infiltrating between normal follicles where they exhibit nuclear features of papillary carcinoma (H&E 400X)

thyroid lesions, as many people residing are natives of Coastal Karnataka region. Similarly, the number of cases diagnosed as Category VI – Malignant was 30%, which was similar to the studies done by Kumar Das P et al⁶ were 23.5% cases were neoplastic lesions, it was discordant with study done by Sharma C et al² where the total malignant cases included only 10% of the study, this was probably owing due to the larger sample size and inclusion of all benign FNA specimen, regardless of availability of HP diagnosis.

The sensitivity, specificity, PPV, NPV and accuracy were found to be 95.77, 98.87, 98.55%, 96.7% and 97.5% respectively. This indicated that the ability of FNA to detect malignancy in our series was quite high. The accuracy of

our study was 97.5% which reinforces that FNAC can be used as a reliable tool to detect thyroid malignancy. This was consistent with many studies in National and International literature. The comparison of Sensitivity, Specificity and accuracy of present study with previous studies in India is given in Table 4.

In our study there were 3 false negative cases, resulting in a false negative rate of 1.87%. The reasons for such a disparity was understood to be the possibility of insufficient representative material on FNA, due to sampling error. The HP diagnoses in all the 3 cases were Papillary thyroid microcarcinomas, where the tumor size is less than 1 cm, and these can be missed leading to a false negative diagnosis and in such cases one must attempt multiple passes or resort to ultrasound guided FNA, when there is strong clinical suspicion. According to a study done by Zhu et al,¹⁷ such false negative rates were found to be attributed to Specimen problems such as sampling errors and suboptimal material for which attempting multiple passes can help with decreasing the false negative rates. The false positive rate was 0.6% in our study and explains a scenario of over diagnosis. Zhu et al.¹⁷ also studied that increased rates of false -positive diagnosis were due to interpretational error while reporting hence it was noted based on their study that Cytopathologists should strengthen their criteria for the identification of adenomatous hyperplasia and cystic lesions to avoid false-positive diagnoses as it can lead to unnecessary thyroid surgery with a 2% - 10% risk for long-term postoperative morbidity.^{18,19}

Additionally, some studies in the literature estimate sensitivity and false negative rates of as low as 55.3% and 44.7%, respectively. The combination of operator variability, limited number, and the challenge of using FNAC for particular thyroid diseases in the diagnostic processes were the most plausible causes of the reduced sensitivity found in the study by Mistry et al.²⁰ The determinant factors for wide range of difference could be number of cases, the included diagnostic categories and how the cytopathologist classifies the suspicious lesions.

The literature available on the new classification scheme of WHO Classification of Thyroid tumors 2022⁴ were very few. Reclassifying the various tumours (Table 3) led us to the view that - new update places a stronger emphasis on understanding the molecular foundation of any disease and classifies them accordingly, which in turn aids in improving our understanding of the clinical outcome.²¹ To more aptly reflect, the clonal and non-clonal proliferations that clinically present as multinodular goitre, the previously heterogeneous group of benign neoplastic and non-neoplastic lesions is now referred to as "thyroid follicular nodular disease." There is a clear distinction between benign, low-risk, and malignant thyroid neoplasms that arise from follicular cells, which constitutes the predominant form of cell of origin in our study. The new

Table 4: Comparison of results of present study with the previous studies

Study	Year	Sensitivity (%)	Specificity (%)	Accuracy (%)
Gupta M ¹¹	2010	80	86.6	84
Sengupta A ¹²	2011	90	100	98.9
Esmail HA ¹³	2012	91.6	100	97
Ranjan Aggarwal ¹⁴	2015	96.7	100	97
Anand V ¹⁵	2017	99	100	99
Thakur R ⁸	2021	96	96	96
Verma S ¹⁶	2021	94.44	84.62	87.14
Present study	2023	95.77	98.87	97.5

WHO classification uses “subtype” instead of “variant” to avoid confusion with genetic variants and to standardize the terminology while reporting. This revision emphasises the need to categorise PTC microcarcinoma according to HP features rather than tumour size to avoid treating all small lesions as low-risk disease. Formerly known as the cribriform-morular variant of PTC is no longer considered PTC as it is listed under malignant ‘thyroid neoplasm of uncertain histogenesis’. Adoption of a grading system and high-grade thyroid carcinomas which requires counting mitotic rate by expressing the number of mitoses per mm², which is roughly equivalent to 10 high-power fields. A new diagnostic category called "differentiated high-grade thyroid carcinoma" includes PTCs, FTCs, and oncocytic carcinomas with high-grade characteristics linked to poorer prognoses, much like the previously recognised poorly differentiated thyroid carcinoma. Also, anaplastic thyroid carcinoma- squamous cell subtype is now recognised as a morphologic pattern rather than a primary occurrence in thyroid.^{4,21} However, there was some ambiguity in our study regarding the placement of non-thyroid cancers including metastatic thyroid carcinoma and non-Hodgkin's Lymphoma. This might be because the new classification separates tumours according to the cell of origin. Possibly, cancers that are not native to the thyroid may be addressed essentially as having aggressive biology.

5. Conclusion

FNAC is a sensitive and specific method of evaluating thyroid lesions, however, histopathology remains the gold standard test for diagnosing thyroid lesions. The Bethesda system is a standard scheme for reporting thyroid cytopathology and erases the ambiguity in reporting and helps with a more focused clinical management. This system shows excellent diagnostic accuracy in detecting malignant lesions and can be used for screening neoplasms. Our results are consistent with those available in the literature.

The new classification demonstrates more clearly to understand how thyroid malignancies are now categorised according to their molecular profile and tumour cell of origin. Clinicians and Pathologists must be informed of new

terminologies and classification schemes since the concept of low-risk neoplasms and histology-based grading systems aid in guiding tailored therapeutic options for patients at varying risk levels. The upcoming releases of clinical guidelines, cancer staging regimens, thyroid histopathology and cytology reporting systems will all be impacted further by the improved WHO classification and nomenclature.

6. Limitations of our Study

The low sample size for cyto-histopathological correlation, hence probable bias due to more malignant lesions, than seen in other studies.

7. Conflicts of Interest

The authors declare that they have no conflicts of interest.

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