

# Cytological and Histological Correlation of Medullary Carcinoma of Thyroid Based on Bethesda System for Reporting Thyroid Cytology: A Single Center Experience

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## ABSTRACT

**Background:** The cytological evaluation of thyroid carcinoma is a diagnostic tool with additional benefits of accuracy, being minimally invasive and cost-effective. However, a histological correlation is necessary to affirm its effectiveness in case of medullary carcinoma thyroid.

**Aim:** To evaluate the correlation between cytological and histological findings in cases of medullary carcinoma of the thyroid.

**Methods:** Retrospective study. Shaukat Khanum Memorial Cancer Hospital (SKMCH) from 1<sup>st</sup> December 2021 to 30<sup>th</sup> June, 2022. Thirty patients were sought who underwent cytology as well as histopathology after surgery. The data of fine needle aspiration cytology and the corresponding information were sought from the hospital archives. The Bethesda categorization was done according to the cytological appearance. Data after the histopathological analysis was also sought.

**Results:** Most of the patients 53.3% were females. Twenty out of these were diagnosed as medullary carcinoma. 93.3% of the Bethesda category IV were diagnosed as medullary carcinoma ( $p=0.000$ ). The majority of category IV FNAs were also adequate for performing tumor markers ( $p=0.00$ ).

**Practical Implication:** The study highlights the high incidence of medullary carcinoma in Bethesda Category IV thyroid nodules, with 93.3% being diagnosed. The majority of patients are female, highlighting the need for vigilance. The majority of fine needle aspirations (FNAs) for these nodules were adequate for performing tumor markers, indicating their reliability for diagnosis and treatment planning. The findings underscore the importance of thorough diagnostic procedures for this high malignancy rate.

**Conclusion:** The cytological categories of Bethesda have strong association with diagnosis of medullary carcinoma thyroid especially if category IV is diagnosed, there are high chances carcinoma may turn out to be medullary carcinoma.

**Keyword:** Bethesda system, Medullary carcinoma, Cytology, Histopathology

## INTRODUCTION

The commonest malignancy of the endocrine system is cancer of the thyroid gland, which has been on the rise for the past four decades<sup>1</sup>. One of the types of thyroid carcinoma is medullary thyroid carcinoma (MTC) arising from the parafollicular or C-cells. Its incidence is lesser than that of other differentiated tumors, i.e. around 1 to 2%<sup>2</sup>. It is linked with almost 13% of deaths due to cancer, with a 91% five-year survival, however, ten-year survival is a maximum of 75%. The 10-years survival decreases to 20% if disease is advanced and metastatic; being worse than other thyroid carcinomas<sup>3</sup>. Hence, the early diagnosis of this disease is very necessary to prevent its early dissemination.

The ultrasound scan and thyroid profiling help in directing the next investigation during the workup phase. The high-risk features, including an increase in size, hypo-echogenicity, irregular margins, a size taller than wide, microcalcifications, a solid internal structure, extra-thyroidal extension, and central vascularity, warrant fine needle aspiration<sup>4</sup>. FNAC helps in assessing the morphologic features of suspicious nodules, which provides important information when planning the therapeutic management of nodules<sup>5</sup>.

Introduced in 2007 and refined in 2017, the Bethesda System serves as a standardized tool for reporting thyroid cytology. This system, designed to create a universal language for medical practitioners, pathologists, and researchers, classifies thyroid aspirates into six distinct categories<sup>6</sup>. These categories range from I including non-diagnostic material to VI including malignancy<sup>7</sup>. According to an Indian study, 13% of patients in category VI of Bethesda suffered from medullary carcinoma, while the majority suffered from papillary carcinoma<sup>8</sup>. According to another study, the patients having Bethesda categories V and VI are diagnosed with medullary carcinoma thyroid other than papillary carcinoma<sup>9</sup>.

The purpose of this study is to elucidate the correlation between cytological and histological features in medullary carcinoma of the thyroid, employing the Bethesda System as a

diagnostic framework. Through this exploration, we aspire to enhance diagnostic precision and contribute valuable insights for tailored management strategies. No such study has been conducted previously comparing the Bethesda categories with histopathology.

## MATERIALS AND METHODS

This retrospective observational study was conducted at Shaukat Khanum Memorial Cancer Hospital (SKMCH) in Lahore from December, 2021 to June 2022. After approval from the ethics review board of the institution (EX-13-10-21-01), data from January 2009 to December 2019 present in the SKMCH archives was retrieved. All cytological reports from fine-needle aspiration (FNA) conducted at SKMCH, wherein the diagnosis of medullary carcinoma was explicitly given, or the report raised the possibility of medullary carcinoma or mentioned it in the differential diagnosis excluding cases lacking contact numbers, patients deceased before surgery or receiving the final histological report, and slides of cytology that were broken or deemed inadequate for analysis were retrieved. The cytological data was classified according to the Bethesda System for Reporting Thyroid Cytology. Histopathological data were retrieved from surgical pathology reports, with specimens assessed based on established histological criteria for medullary carcinoma. To ensure the accuracy and reliability of the assessments, all cases were meticulously reviewed by two consultants with significant experience in cyto-histopathology at SKMCH.

The FNA procedure was performed either by the palpation method or ultrasound assistance, ensuring a comprehensive approach to sample collection. Cytological smears comprised both wet-fixed and air-dried specimens. Air-dried smears were stained with Diff-Quik/Hemacolor, while wet-fixed smears utilized 95% ethyl alcohol as the fixative, and staining was done using Papanicolaou stain (Figs. 1-6). The Bethesda classification was done as I non-diagnostic, only cyst fluid or low cellularity, II consistent with benign nodule, III consistent with atypia of undetermined significance, IV consistent as follicular neoplasm, V as suspicious for malignancy or VI consistent with malignancy.10

Received on 25-11-2023

Accepted on 13-02-2024

In our study, the following features were considered according to the Bethesda categories:

IV - Follicular Neoplasm or Suspicion for a Follicular Neoplasm: This category included cases with microfollicular or trabecular arrangements and occasional spindled or plasmacytoid cells. Nuclei exhibited mild to moderate pleomorphism.

V - Suspicious for Malignancy: Cases in this category displayed highly cellular smears with marked nuclear atypia and pleomorphism. Prominent nucleoli, frequent mitotic figures, and a high nucleus-to-cytoplasm ratio were observed. The presence of large irregular clusters or sheets of cells and amyloid or amyloid-like material was noted.

VI - Malignant (Confirmed): This category encompassed cases with definitive evidence of medullary carcinoma, characterized by eccentric nuclei, finely granular chromatin, and cytoplasmic amyloid. Positive immunostaining for calcitonin or synaptophysin on cell block was a key feature. The sample is said to be adequate for cytological testing if there are six clusters containing at least ten follicular cells<sup>11</sup>.

Fig. 1: Round, ovoid, plasmacytoid and spindle cells forming small clusters (Diff Quik, 10x)

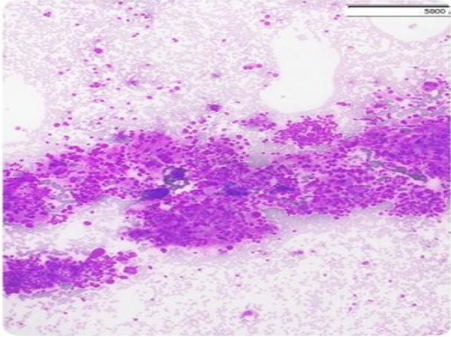


Fig. 2: Variable-sized cell with abundant cytoplasm and marked pleomorphism (Diff Quik, 100x)

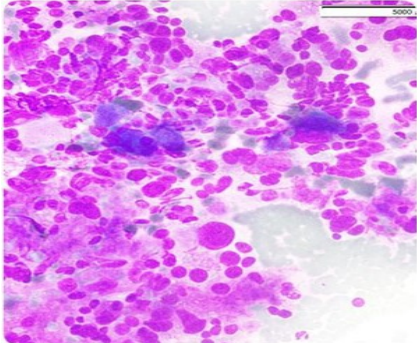


Fig. 3: Tumor cells with intranuclear inclusions and salt and pepper chromatin (Pap, 100x)

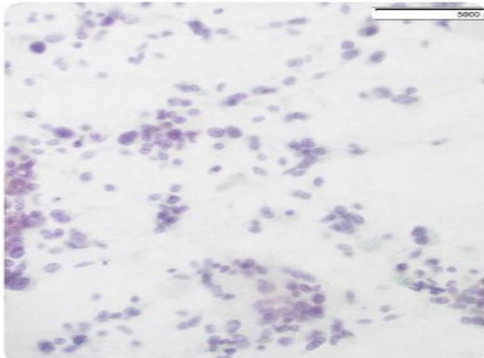


Fig. 4: Epithelioid to spindle cells with amyloid in the background (Cell Block, 10x)

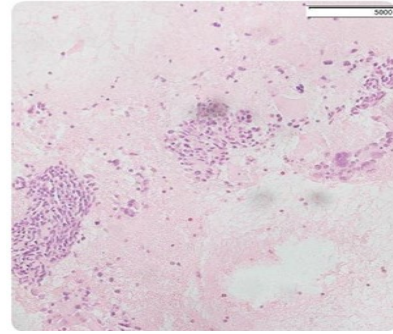


Fig. 5: Synaptophysin IHC shows Membranous and cytoplasmic positive staining

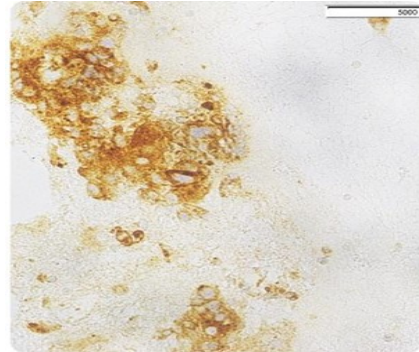
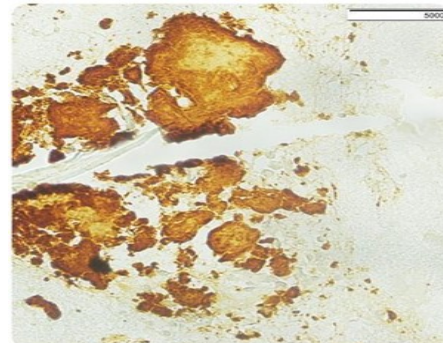


Fig. 6: Calcitonin IHC shows fine granular and cytoplasmic positive staining



The cytology material, when in excess, was mixed with fixative three times the volume of material and sent for preparation of the cell block. Then it was subjected to centrifugation for 10 minutes at 2000 rpm. The fluid at the top was wasted, and the cell bottom was mixed again with fixative and recentrifuged for 10 minutes at 3000 rpm. Then the tube was left for a quarter of a day. The cell button was then removed carefully and wrapped in filter paper. The cell button was processed as a routine biopsy specimen by cutting into a thickness of 3-4  $\mu\text{m}$ . Then staining was done and slides evaluated. Also, the histopathology of the surgical specimen was done, taking it as a gold standard.

Statistical analysis, including descriptive statistics and correlation analyses such as kappa statistics, was performed using SPSS version 21 to summarize patient demographics and assess the agreement between cytological and histological classifications.

**RESULTS**

There were 53.3% females and 46.7% males (Table 1). Medullary carcinoma was the predominant histological diagnosis, accounting for 66.7% of the cases. The distribution of cases across the

cytological categories was as Category IV: 22.2% of cases were histologically confirmed as medullary carcinoma, Category V: 66.7% of cases were histologically confirmed as medullary carcinoma and Category VI: A striking 93.3% of cases were histologically confirmed as medullary carcinoma (Table 2). Medullary carcinoma was the predominant histological diagnosis, accounting for 66.7% of the cases. The distribution of cases across the cytological categories was as Category IV: 22.2% of cases were histologically confirmed as medullary carcinoma, Category V:

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Table 1: Gender distribution of patients n=30

Gender	No.	%
Male	14	46.7
Female	16	53.3

Table 2: Correlation of histological and cytological findings

FNA Findings	Anaplastic Carcinoma	Follicular adenoma with Hurthle cell change	Hurthle cell adenoma	Hybrid thyroid carcinoma	IgG4 related sclerosis disease	Medullary carcinoma	Papillary thyroid carcinoma	Poorly differentiated carcinoma arising in background of follicular carcinoma	P value
IV	-	1 (11%)	2 (22%)	1 (11%)	-	2 (22%)	3 (33%)	-	0.000
V	-	-	-	-	1 (16.7%)	4 (66.7%)	-	1 (16.7%)	0.000
VI	6.7%	-	-	-	-	14 (93.3%)	-	-	0.000

Table 3: Cell block availability from cytology material

FNA Findings	Adequacy		P value
	Adequate	Less adequate	
IV	2 (22.3%)	7 (77.7%)	0.00
V	1 (16.7%)	5 (83.3%)	0.00
VI	14 (93.3%)	1 (6.7%)	0.00

between category IV (22%) and category V (66%) and the development of medullary carcinoma. An Indian study showed that half (50%) of patients in category V were later diagnosed as medullary carcinoma, while no patient was diagnosed as medullary carcinoma in category IV<sup>23</sup>. A large proportion of cases (25.5%) were suspicious for medullary carcinoma residing in Bethesda category V<sup>24</sup>.

Medullary carcinoma can be mistaken for papillary thyroid carcinoma, as amyloid present in medullary carcinoma can look like the thick colloid material seen in papillary carcinoma. The features of papillary cancer mimicking medullary carcinoma include single-cell pattern, mixed epithelial and spindle cell morphology, salt and pepper-like chromatin, binucleation, and plasmacytoid morphology. The medullary carcinoma expresses calcitonin, carcinoembryonic antigen, and neuroendocrine markers (synaptophysin, chromogranin, and INSM1). These also help to differentiate between medullary and papillary cancer of the thyroid. Hence, whenever available, the excess material should be made into cell blocks to be exposed to immunohistochemistry<sup>26</sup>.

One of the advantages of performing FNA of thyroid lesions is preventing unnecessary surgeries<sup>27</sup>. The only limitations in the use of FNAC are variation in method of sampling, clinical skills of the performer, the experience of a slide-reading cytologist, and even the vascularity of thyroid swelling<sup>28</sup>.

The results indicate a strong correlation between cytological findings and the histological diagnosis of medullary carcinoma, especially in category VI (malignant), which showed a high rate of confirmation through histopathology. The presence of characteristic cytological features, such as amyloid material and specific immunostaining markers, played a crucial role in the accurate diagnosis of medullary carcinoma. One of the markers helpful in detecting medullary carcinoma is Ki67. Its sensitivity in detecting the medullary carcinoma is quite low, however, if the Ki67 is more than 5% on the material of fine needle aspiration, it is highly predictive of medullary carcinoma of high grade upon resection. Some studies still opine that the cytological testing can still lead to undergrading of the tumor<sup>29-33</sup>.

The importance of cell block material and adequate sample size cannot be overstated in the accurate diagnosis of thyroid lesions. Cell block material provides additional tissue for histological examination and immunohistochemical staining, which is crucial for confirming the presence of specific markers like calcitonin and synaptophysin in medullary carcinoma. Adequate sample size ensures the representativeness of the lesion and increases the diagnostic accuracy of FNA cytology. The distribution of these histological diagnoses highlights the diversity of thyroid lesions encountered in clinical practice and underscores the importance of accurate cytological classification using the Bethesda System for effective patient management. Our study is a small sample retrospective study. Hence, a study with a larger cohort should be carried out for generalization on the population.

## DISCUSSION

Medullary carcinoma of the thyroid is a relatively rare disease accounting for 1% to 2% of all thyroid cancers. However, its mortality is high, mounting almost 8.6% of all deaths related to thyroid<sup>12</sup>. It is not well differentiated and has an infiltrative pattern consisting of solid sheets of discohesive cells in a fibrous stroma, which may contain amyloid<sup>13</sup>. The various investigations used to diagnose this deadly disease include ultrasonography, fine-needle aspiration (FNA), advanced technology, including computed tomography, magnetic resonance imaging, or positron emission tomography<sup>14</sup>. Out of these, fine-needle aspiration (FNA) is deemed the initial investigation of choice. Fine-needle aspiration (FNA) plays a pivotal role in the diagnosis of thyroid lesions, offering a minimally invasive, cost-effective, and accurate method for initial evaluation. The utility of FNA in guiding clinical management and surgical decision-making, especially in the context of thyroid malignancies, is well-established in the literature.

The Bethesda System is generally accepted grading of results of cytology worldwide for better communication between the clinicians.<sup>15</sup> The Bethesda grading is an adjunct to diagnose the thyroid lesions and hence help in proper management<sup>16</sup>. The previous studies show a sensitivity, specificity, and diagnostic accuracy of 97%, 50.7%, and 68.8%, respectively, of fine needle aspiration cytology. The study by Islam MS et al showed that 94% of FNACs were accurate and 97% were specific<sup>17</sup>. Our study uses this Bethesda system to find its association with histopathological evidence, especially in medullary carcinoma.

The medullary carcinoma patients included in our study showed more prevalence among females 16(53.3%). This result coincides with the results of Gogna et al<sup>18</sup> showed 1484(58.6%) females in their study group had medullary carcinoma of the thyroid in comparison to 1049(41%) males. The results were also consistent with that of a Dutch study showing that 52% patients (p=0.18) suffering from medullary carcinoma are females<sup>19</sup>. This study showed that 93% of patients in Bethesda category VI were diagnosed with medullary carcinoma later on histopathology. The rate of malignancy was 94% with Bethesda category VI in an American study<sup>20</sup>. In an Indian study, it was shown that 12.5% of patients in category VI had medullary carcinoma, which was next in line to papillary carcinoma (62.5%)<sup>21</sup>. A study by Kaur and Gupta<sup>22</sup> showed the same results: one out of five Bethesda category VI patients were later diagnosed with medullary carcinoma. The present study showed a significant relationship

## CONCLUSION

The Bethesda system categories IV, V and VI can be an indicator of medullary carcinoma thyroid. Hence, high level of suspicion should be there for attempts to make complete histological as early as possible.

**Authorship and contribution declaration:** Each author of this article fulfilled following Criteria of Authorship:

1. Conception and design of or acquisition of data or analysis and interpretation of data.
2. Drafting the manuscript or revising it critically for important intellectual content.
3. Final approval of the version for publication.

All authors agree to be responsible for all aspects of their research work.

**Funding:** None

**Conflict of interest:** The authors declare no conflict of interest in this study.

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**This article may be cited as:** Faiz MF, Loya A, Hameed MA, Syed M, Sheikh UN: Cytological and Histological Correlation of Medullary Carcinoma of Thyroid Based on Bethesda System for Reporting Thyroid Cytology: A Single Center Experience. *Pak J Med Health Sci*, 2024;18(4) 10-13.