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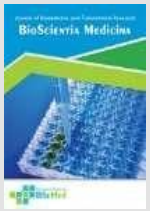

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Loss of E-cadherin Expression Stratifies Aggressive versus Non-Aggressive Papillary Thyroid Carcinoma

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A B S T R A C T

Background: Papillary thyroid carcinoma (PTC) is generally indolent, yet specific histological subtypes defined by the World Health Organization (WHO) are linked to aggressive behavior and poor prognosis. The loss of the cell-adhesion protein E-cadherin is a hallmark of the epithelial-to-mesenchymal transition (EMT), a process implicated in tumor aggression. However, its role in stratifying PTC subtypes versus its correlation with tumor stage remains a significant controversy in the literature. This study aimed to disentangle these two parameters by clarifying the relationship between E-cadherin expression and both histological phenotype and tumor stage. **Methods:** This was an observational, cross-sectional pilot study on 40 randomly selected, formalin-fixed, paraffin-embedded (FFPE) PTC cases from a 2024 cohort (N=74) at a tertiary hospital in Indonesia. All cases were re-evaluated and classified according to the WHO 5th Edition (2022) criteria as non-aggressive (n=34) or aggressive (n=6). E-cadherin expression was assessed by immunohistochemistry (IHC) using a standardized semi-quantitative scoring system (product of intensity and proportion) adapted from previous studies, with inter-rater reliability assessed (Cohen's Kappa = 0.88). Scores were dichotomized as 'High' (n=25) or 'Low' (n=15). The association between E-cadherin expression and both histological subtype and AJCC 8th Edition tumor stage (Early: I/II [n=32] vs. Advanced: III/IV [n=8]) was analyzed using Fisher's Exact Test, with Odds Ratios (OR) and 95% Confidence Intervals (CI) calculated. **Results:** High E-cadherin expression was observed in 62.5% of cases. A statistically significant and strong association was found between E-cadherin expression and histological subtype (p=0.021; OR 12.0; 95% CI 1.2–118.9). Low E-cadherin expression was present in 83.3% (5 of 6) of aggressive-subtype tumors, versus only 29.4% (10 of 34) of non-aggressive subtypes. In contrast, no significant correlation was found between E-cadherin expression and advanced tumor stage (p=0.126; OR 3.67; 95% CI 0.7–18.6). **Conclusion:** Loss of E-cadherin expression is a significant biomarker associated with high-risk, aggressive histological phenotypes in PTC. Its lack of correlation with tumor stage, confirmed by an uncertain OR, suggests E-cadherin's role is indicative of an inherent tumor biological phenotype (aggressiveness) rather than a linear marker of tumor progression (stage). This dichotomy, likely reflecting EMT/MET plasticity, positions E-cadherin IHC as a powerful ancillary tool for pathological risk stratification.

1. Introduction

Thyroid cancer stands as the most frequently diagnosed endocrine malignancy globally, constituting a significant portion of all newly diagnosed human cancers.[1] The global incidence of thyroid carcinoma has been rising dramatically over the past three decades.[2] Data from global cancer statistics identified a high burden of new cases worldwide, establishing it as one of the most common cancers, particularly in women.[1,2] This rising tide is also reflected in Indonesia.[1] The disease exhibits a profound gender predilection, with incidence rates in women being approximately three-fold higher than in men.[2] Despite its high incidence, thyroid cancer is associated with a

comparatively low mortality rate.[1] This is because the vast majority of cases are differentiated thyroid carcinoma (DTC), primarily papillary thyroid carcinoma (PTC), which accounts for up to 90% of all cases.[3] Clinically, PTC has long been regarded as an "indolent" tumor. The majority of patients present with localized disease, respond exceptionally well to standard treatment, and have an excellent long-term prognosis, with ten-year disease-specific survival rates consistently exceeding 92-98%.[4] However, this favorable general prognosis masks a dangerous clinical reality: a significant subset of PTCs behaves aggressively. It is estimated that 8% to 28% of all PTC patients will experience disease recurrence[5], and 5-10% of cases will progress to highly aggressive, radioactive iodine (RAI)-refractory disease.[4] This clinical heterogeneity—this dichotomy between indolent and aggressive behavior—represents the central challenge in the management of PTC. The clinical stakes of this dichotomy are enormous and define the entire therapeutic pathway. A patient with a low-risk, indolent PTC may be managed conservatively with a simple thyroid lobectomy. In stark contrast, a patient diagnosed with an aggressive-phenotype PTC requires a far more radical approach: a total thyroidectomy, often with neck dissections, followed by adjuvant RAI ablation and lifelong TSH suppression.[6] The morbidity associated with this aggressive pathway is substantial. Therefore, the accurate identification of which tumors will follow an aggressive path at the time of initial diagnosis is a paramount goal in modern pathology and oncology.

To address this challenge, clinical management relies on sophisticated risk-stratification systems, most notably the guidelines published by the American Thyroid Association (ATA).[6] A primary determinant in this risk stratification is the tumor's histopathological subtype. The World Health Organization (WHO) Classification of Tumours, 5th Edition (2022), provides the definitive criteria for these subtypes.[3] While the classical, encapsulated, and infiltrative follicular variants of PTC are generally associated with excellent prognoses, the WHO classification specifically identifies a cohort of "aggressive subtypes." These subtypes are biologically distinct and include the tall cell variant (TCV), solid/trabecular variant, hobnail variant (HV), columnar cell variant (CCV), and diffuse sclerosing variant (DSV).[3,7] The diagnosis of any of these aggressive subtypes, regardless of tumor size or initial stage, immediately classifies a patient as having at least an intermediate risk of recurrence.[6] This recognition underscores the critical need to understand the molecular mechanisms that define and drive these specific high-risk phenotypes. The TCV, for example, is known to be strongly associated with the BRAF V600E mutation, a driver of the MAPK signaling pathway, which is in turn linked to invasive tumor biology.[8] The biological program that confers upon cancer cells the ability to invade local tissues and metastasize is known as the Epithelial-to-Mesenchymal Transition (EMT).[9] EMT is a complex process in which stationary, polarized epithelial cells dissolve their cell-cell junctions, lose polarity, and acquire a motile, invasive mesenchymal phenotype.[10] This transformation is a fundamental prerequisite for metastasis.

A central molecular event, and indeed the hallmark of EMT, is the functional loss of E-cadherin (Cadherin-1, CDH1).[11] E-cadherin is a transmembrane glycoprotein encoded by the CDH1 gene. In all normal epithelial tissues, it is the principal protein of the adherens junctions, effectively "zipping" epithelial cells together. The intracellular domain of E-cadherin is equally critical, as it anchors the actin cytoskeleton via a complex of linker proteins, namely β-catenin, p120-catenin, and α-catenin.[12] This entire E-cadherin-catenin complex is the master regulator of epithelial integrity and polarity. The loss of E-cadherin function is catastrophic for epithelial cohesion. This loss can occur through several mechanisms, but the most common in PTC is active transcriptional repression. This repression is carried out by EMT-inducing transcription factors (EMT-TFs), primarily Snail, Slug, and Twist.[13] When oncogenic signaling pathways (such as the MAPK pathway, driven by

BRAF V600E) are activated, they promote the expression and stabilization of these EMT-TFs.[10] Snail, Slug, and Twist then bind directly to the CDH1 gene promoter, actively shutting down its transcription. The consequences of this E-cadherin loss are twofold. First is the passive loss of adhesion, allowing the cell to detach and become motile. Second is an active oncogenic signal: the liberation of β-catenin from the membrane, which can then translocate to the nucleus and activate the Wnt signaling pathway, driving proliferation and invasion.[14] This entire event is often accompanied by the de novo expression of a "mesenchymal" cadherin, such as N-cadherin, in a process termed the "cadherin switch," which confers further pro-survival advantages.[14]

Given its fundamental role as a master tumor suppressor, E-cadherin has been extensively investigated as a prognostic biomarker. In many human cancers, low E-cadherin expression consistently correlates with high histological grade, advanced tumor stage (TNM), and poor overall survival. In thyroid cancer, a similar gradient is well-established at the extremes: E-cadherin expression is robust in normal thyroid tissue and benign adenomas, while it is almost universally absent in fatal anaplastic thyroid carcinoma.[15] However, within papillary thyroid carcinoma itself, the literature presents a significant and unresolved controversy. On the one hand, numerous large-scale studies and meta-analyses have reported the expected correlation. A large meta-analysis concluded that negative E-cadherin expression was significantly correlated with both lymph node metastasis (LNM) and advanced (Stage III/IV) tumor stage.[16] This supports the linear model where E-cadherin loss drives progression. Similarly, another study found that reduced E-cadherin expression correlated significantly with multiple poor-prognosis features, including larger tumor size, advanced tumor stage, LNM, and vascular invasion .[15] On the other hand, a substantial body of evidence from single-institution studies directly contradicts this finding. One study on 39 PTC cases found no significant difference in E-cadherin expression based on patient age, gender, LNM, or, most importantly, tumor stage. They did, however, note that expression was lower in the tall cell subtype. Likewise, a 2017 study from Indonesia found no significant difference in E-cadherin expression between benign and malignant thyroid lesions.[17] This discrepancy creates a critical knowledge gap. Is E-cadherin loss a marker of progression (stage), as suggested by the meta-analysis, or is it a marker of phenotype (subtype), as hinted at by other studies? The existing literature often conflates these two parameters. An "aggressive subtype" (like tall cell) is more likely to present at an advanced stage, but these two variables are not synonymous. A tall cell variant can be diagnosed at Stage I, just as a classical variant can metastasize and present at Stage IV.

Therefore, this study aimed to investigate the relationship between E-cadherin expression and both the histological subtype (aggressive vs. non-aggressive) and the clinicopathological tumor stage (early vs. advanced) in a cohort of Papillary Thyroid Carcinoma patients at a tertiary referral hospital in Indonesia. The novelty of this investigation lies in its specific design to disentangle the role of E-cadherin as a marker of tumor phenotype (histological aggressiveness) versus its role as a marker of tumor progression (TNM stage). By analyzing these two distinct clinical parameters separately against E-cadherin expression, this study directly addresses the existing conflict in the literature. This study sought to clarify whether E-cadherin loss is a static biomarker of an inherently aggressive biological subtype, present from its inception, or a dynamic biomarker that parallels the tumor's stage-wise journey from a localized (Stage I) to a metastatic (Stage IV) lesion.

2. Methods

This investigation was conducted as an observational, analytical study utilizing a cross-sectional design. The research was performed at the Department of Anatomical Pathology, Dr. M. Djamil General Hospital, Padang, Indonesia, with sample and

data collection spanning from March to October 2025. All research procedures were carried out in strict accordance with the ethical principles outlined in the Declaration of Helsinki. The formal research protocol was reviewed and granted full approval by the Health Research Ethics Committee of Dr. M. Djamil General Hospital, Padang (Ethical Approval No: DP.04.03/D.XVI.10.1/408/2025). Given the retrospective nature of the study, which utilized anonymized data and archived tissue blocks, the ethics committee waived the requirement for individual patient consent. The source population for this study consisted of all consecutive cases diagnosed with Papillary Thyroid Carcinoma at the Department of Anatomical Pathology, Dr. M. Djamil General Hospital, during the one-year period from January 1st, 2024, to December 31st, 2024. This period yielded a total population of 74 unique PTC cases.

The sole inclusion criterion was a definitive, archived histopathological diagnosis of PTC. Cases were excluded from consideration if they met any of the following criteria: (1) incomplete or missing essential clinicopathological data in the medical record, including patient age, gender, or definitive tumor staging information; (2) loss or damage of the corresponding formalin-fixed, paraffin-embedded (FFPE) tissue block, precluding new sectioning; or (3) insufficient viable tumor tissue within the available block for a representative immunohistochemical analysis, which was defined as a tumor focus smaller than 0.6 cm or comprising less than 30% viable tumor cells. From the 74 identified cases, 60 met the inclusion criteria. From this eligible population, a random sample of 40 cases was selected for this pilot study to assess the feasibility and direction of a potential association. To ensure this sample was representative of the source population and to rule out selection bias, the characteristics of the sample (n=40) were compared to the source population (N=74) and the eligible population (N=60). The distributions of key clinicopathological variables were found to be comparable, confirming the sample's representativeness.

For the 40 selected cases, all relevant clinicopathological data were retrieved from the laboratory information system and the patients' electronic medical records. Patient age at diagnosis was recorded and subsequently dichotomized based on the 55-year cutoff used in the American Joint Committee on Cancer (AJCC) 8th Edition staging system for differentiated thyroid cancer (≤55 years vs. >55 years).[6] Tumor staging was extracted directly from the record, which was based on the AJCC 8th Edition TNM classification. For the purpose of statistical analysis, this multi-level staging was dichotomized into two groups: Early Stage (defined as Stage I or Stage II) and Advanced Stage (defined as Stage III or Stage IV). The original hematoxylin and eosin (H&E) stained slides for all 40 cases were retrieved from the pathology archive. These slides were independently re-evaluated by two board-certified anatomical pathologists to confirm the primary diagnosis of PTC and, most importantly, to classify the histological subtype according to the definitive criteria set forth in the WHO Classification of Tumours, 5th Edition (2022). Based on this WHO classification, tumors were segregated into two prognostically distinct groups for analysis: Non-Aggressive Subtype (n=34): This group included tumors conforming to the classical variant of PTC (n=22) and the infiltrative follicular variant (n=12); Aggressive Subtype (n=6): This group included all variants designated by the WHO as having a poorer prognosis. In this cohort, this was composed of the tall cell variant (n=3), the solid/trabecular variant (n=2), and the diffuse sclerosing variant (n=1). Hobnail and columnar cell variants, while included in the aggressive criteria, were not identified in this 40-case cohort. Any discrepancies in classification between the two pathologists were resolved by joint review at a multi-headed microscope to reach a consensus diagnosis.

From each of the 40 cases, one representative FFPE block containing the most viable and characteristic tumor was selected for immunohistochemistry. Tissue sections were cut at a thickness of 4-6 microns and mounted onto positively

charged (silane-coated) glass slides to ensure tissue adherence. A standardized manual staining protocol was employed. The slides first underwent Deparaffinization by immersion in three changes of xylene (5 minutes each). This was followed by Rehydration through a descending series of graded ethanol (100%, 90%, 80%, and 70%, 5 minutes each), culminating in a wash with distilled water. Antigen Retrieval was performed using a heat-induced epitope retrieval (HIER) method. Slides were immersed in a staining jar containing Tris-EDTA buffer (pH 9.0) and placed within a decloaking chamber (a specialized heat-regulated water bath) at 95-97°C for 30 minutes. After the heating cycle, the slides were allowed to cool to room temperature in the same buffer for 15 minutes. Blocking of endogenous peroxidase activity was achieved by incubating the slides in a 0.1%-1% hydrogen peroxide solution for 15 minutes. To prevent non-specific protein binding, a normal blocking serum (1.5%) was applied to the slides in a moisturizing chamber for 15 minutes. The slides were then incubated with the Primary Antibody, a rabbit monoclonal anti-E-cadherin (Biocare Medical, USA, Clone EP6). The antibody was applied at a 1:200 dilution, and the slides were incubated for 60 minutes at room temperature within the moisturizing chamber. Following three washes in Phosphate Buffered Saline (PBS, pH 7.0), the slides were incubated with a biotinylated Secondary Antibody for 30 minutes. This was followed by another PBS wash and incubation with a streptavidin-horseradish peroxidase (HRP) conjugate for 5 minutes. The antigen-antibody complex was Visualized by applying the DAB (3,3'-Diaminobenzidine) chromogen, which produces a brown precipitate at the site of the antibody, for 5 minutes. Finally, the slides were counterstained with Mayer's hematoxylin for 10 minutes to visualize the cell nuclei, washed, and then Dehydrated through an ascending series of graded alcohols and cleared in xylene. The slides were then permanently coverslipped using a synthetic mounting medium. Normal thyroid follicular epithelium present in the tissue sections served as a reliable internal positive control, demonstrating strong, crisp membranous staining.

All 40 IHC-stained slides were evaluated independently by two anatomical pathologists blinded to the clinicopathological outcomes. E-cadherin expression was assessed exclusively in the invasive tumor cells. The scoring was based on the presence of a distinct brown signal on the cell membrane. Cytoplasmic staining, when present, was noted but considered aberrant and not included in the positive score. A standardized semi-quantitative scoring system, which combines both the intensity and the proportion of stained cells, was used to generate a final score. This method, adapted from multiple prior studies on E-cadherin, provides a more reproducible metric than simple "positive/negative" assessment.

The scoring was performed as follows: Intensity Score (I): The predominant staining intensity of the tumor cell membranes was scored on a four-tiered scale:0: No staining observed, 1+: Weak or faint membranous staining, 2+: Moderate and distinct membranous staining, 3+: Strong and dark membranous staining. Proportion Score (P): The percentage of tumor cells showing any definitive membranous staining (regardless of intensity) was estimated across the entire tumor and scored on a five-tiered scale: 0: <5% of tumor cells stained, 1: 6%–25% of tumor cells stained, 2: 26%–50% of tumor cells stained, 3: 51%–75% of tumor cells stained, 4: >75% of tumor cells stained. Final Score Calculation: The final score for each case was calculated by multiplying the Intensity Score by the Proportion Score (Final Score = I × P). This yielded a possible range of scores: 0, 1, 2, 3, 4, 6, 8, 9, and 12. For the purpose of statistical analysis, this final score was dichotomized into two clinically relevant categories. To ensure comparability with previous literature and validate this dichotomization, the cutoff was adapted from the methodology used by a prior study, where a similar scoring system was employed to differentiate low- and high-risk groups:Low Expression: Defined as a final score of 0, 1, 2, 3, or 4; High Expression: Defined as a final score of 6, 8, 9, or 12. The two pathologists independently scored all 40 cases. Inter-rater

reliability for the final dichotomous score (Low vs. High) was assessed and found to be excellent, with a Cohen's Kappa coefficient of 0.88 ($p < 0.001$), confirming the high reproducibility of the scoring method.

All collected data (demographic, clinicopathological, and IHC scores) were encoded and entered into a database using SPSS Statistics, version 25.0 (IBM Corp., Armonk, NY). Descriptive statistics were generated for all variables. Frequencies and percentages were calculated for categorical variables (age group, gender, histological subtype, tumor stage, E-cadherin expression). For the continuous variable of age, the mean, median, and standard deviation (SD) were also calculated. The association between two independent categorical variables (E-cadherin Expression vs. Histological Subtype) and (E-cadherin Expression vs. Tumor Stage) was assessed. Due to the small sample size and the presence of cells in the contingency tables with an expected frequency of less than 5, Fisher's Exact Test was chosen as the most accurate and appropriate statistical test. The Chi-square test was deemed less reliable under these conditions. In addition to Fisher's Exact Test for p-values, Odds Ratios (OR) and their corresponding 95% Confidence Intervals (CI) were calculated for both bivariate analyses to determine the effect size and precision of the associations. For all analyses, a p-value of less than 0.05 was considered to be statistically significant

3. Results

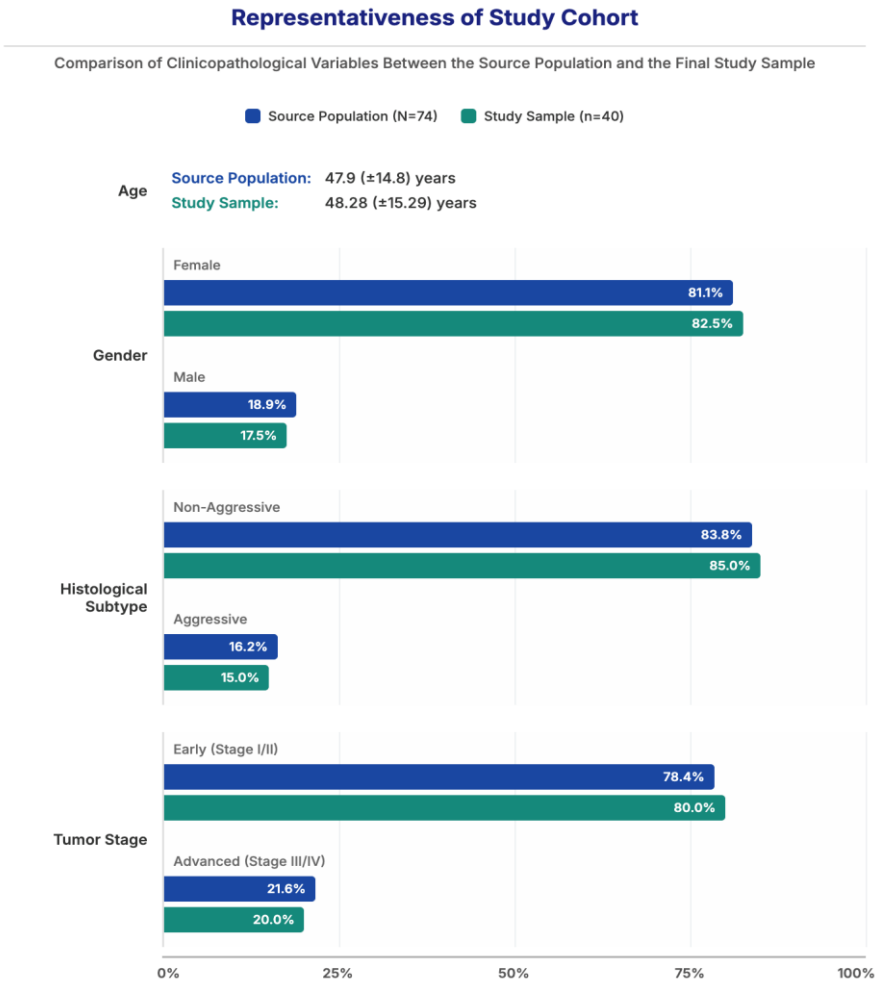

Figure 1. Representatives of study cohort.

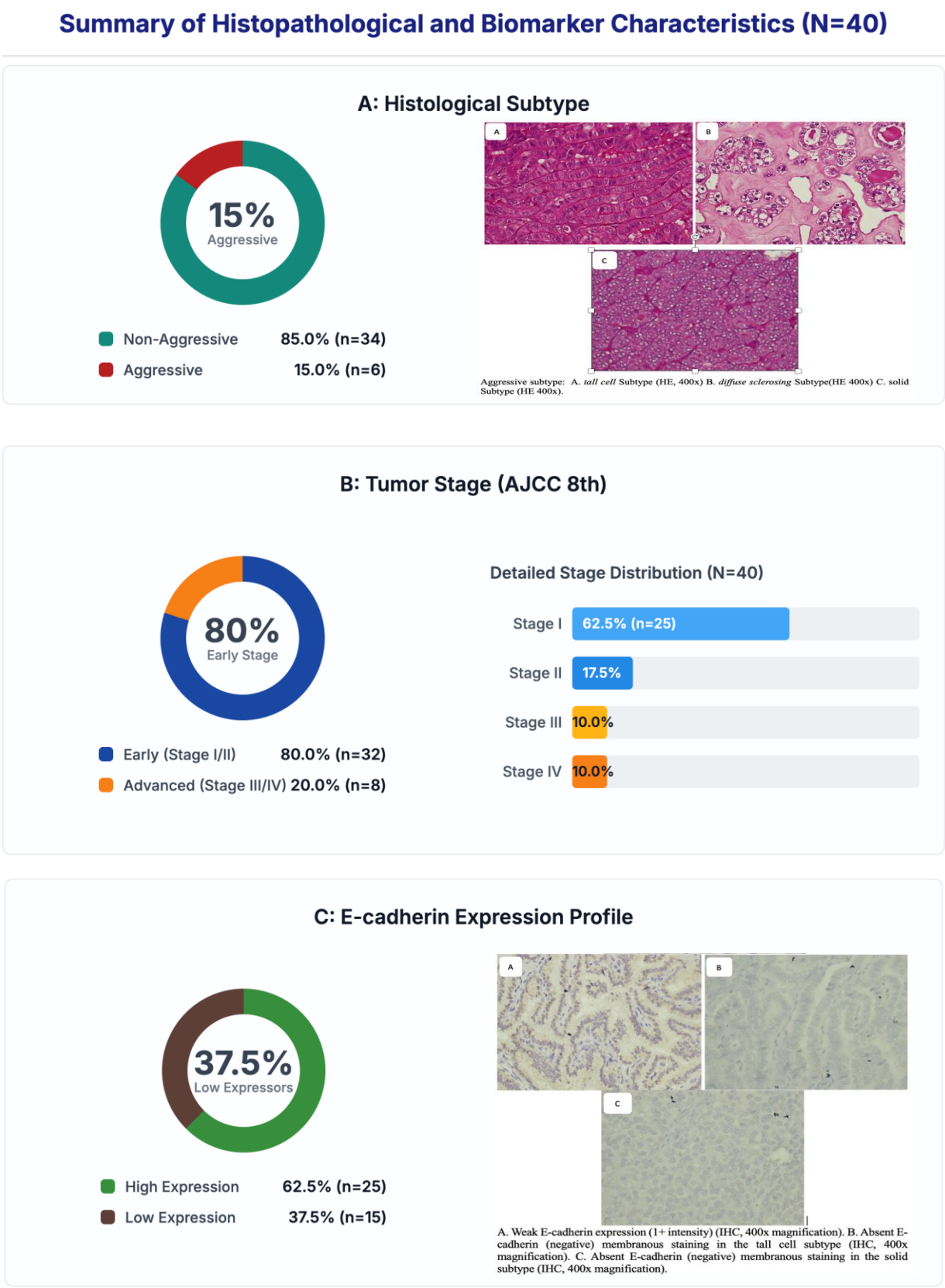

Figure 2. Summary of histopathology and biomarker characteristics.

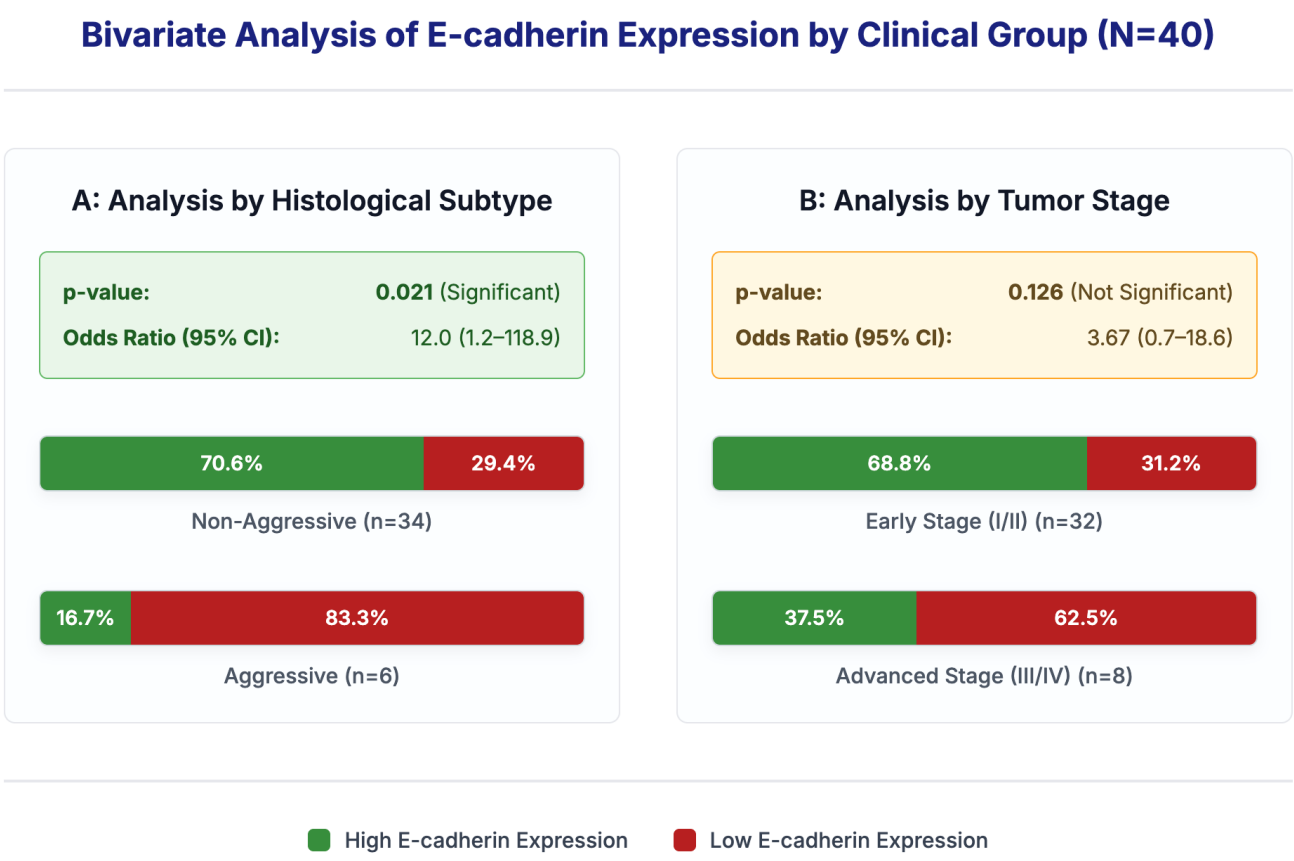

Figure 3. Bivariate analysis of E-cadherin expression by clinical group.

4. Discussion

The findings of this pilot study address a critical and persistent ambiguity in the clinicopathological literature of papillary thyroid carcinoma. This investigation was designed to dissect the dual role of E-cadherin, a cornerstone molecule of epithelial integrity, as a biomarker. The central question was whether its loss is a marker of an inherent biological phenotype (aggressive histology) or a marker of linear disease progression (advanced tumor stage). Our results, despite being based on a modest cohort, provide a clear and nuanced answer: E-cadherin expression is a powerful stratifier of tumor phenotype but an unreliable indicator of tumor stage. A strong, statistically significant association exists between the loss of E-cadherin expression and the presence of aggressive histological subtypes ($p = 0.021$), an association with a large effect size (OR 12.0). No statistically significant association exists between E-cadherin expression and advanced AJCC 8th Edition tumor stage ($p = 0.126$), a finding confirmed as statistically uncertain by a wide, non-significant confidence interval (95% CI 0.7–18.6). This dichotomy—this uncoupling of phenotype from stage—is the most significant finding of our research. It suggests that E-cadherin loss is not a simple, dynamic event that occurs as a tumor "progresses" from Stage I to Stage IV. Rather, it appears to be a more static, fundamental characteristic of specific, high-risk biological subtypes that are "born aggressive." Our study demonstrated that 83.3% (5 of 6) of all tumors classified as aggressive subtypes (tall cell, solid, diffuse sclerosing) exhibited low E-cadherin expression. This finding provides powerful clinicopathological validation for the molecular models of PTC tumorigenesis.

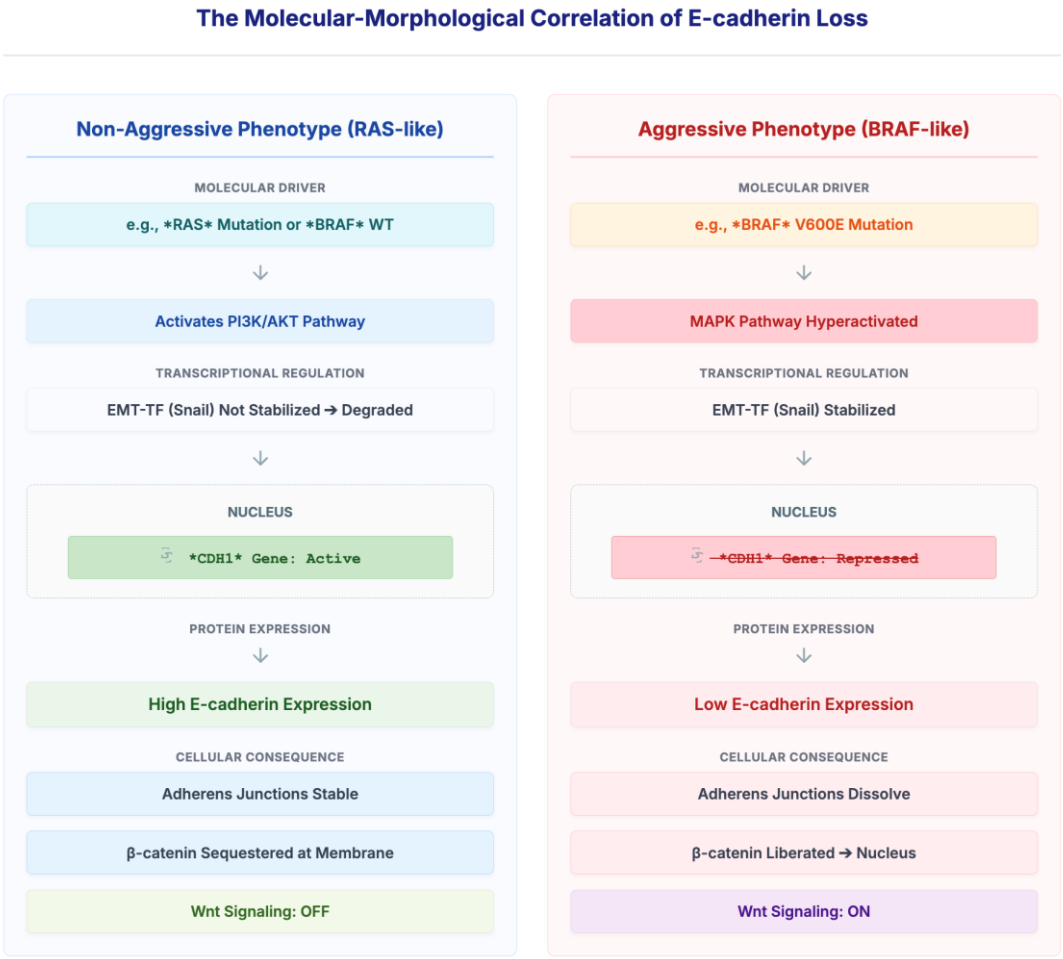

Figure 4. The molecular-morphological correlation of E-cadherin loss.

The pathophysiology of PTC is not monolithic; it is largely driven by two distinct, and often mutually exclusive, oncogenic signaling pathways. The first, and most relevant to aggressive disease, is the MAPK pathway, detailed in Figure 4. This pathway (RAS-RAF-MEK-ERK) is most potently activated by the BRAF V600E point mutation. The BRAF V600E mutation is a known hallmark of aggressive PTC variants, particularly the tall cell variant, where it is found in the vast majority of cases.[8] This oncoprotein acts as a constitutively active kinase, leading to a state of constant, high-level signaling through the MAPK cascade. A primary downstream effect of this hyperactivated pathway is the profound upregulation of the EMT-inducing transcription factors (EMT-TFs), namely Snail, Slug, and Twist.[10,13] These transcription factors are direct repressors of the CDH1 (E-cadherin) gene. The mechanism of this repression is precise. Activated ERK, a downstream effector of BRAF, phosphorylates and stabilizes the Snail protein, preventing its degradation and promoting its accumulation in the nucleus. Snail then binds directly to E-box motifs (canonical sequence CACCTG) in the proximal promoter of the CDH1 gene. This binding event recruits a host of co-repressors, including histone deacetylases (HDACs) and the LSD1 complex, which remove activating histone marks (acetylation) and add repressive marks (demethylation). This active, epigenetic silencing of the CDH1 promoter is the key molecular event that triggers the first step of EMT. Our results serve as the physical, protein-level proof of this molecular cascade. The tumors known to be driven by

the BRAF V600E mutation (the tall cell and solid variants) are precisely the ones in which we observed the loss of the E-cadherin protein. This finding is made more remarkable by its statistical strength; despite a very small aggressive-subgroup (n=6), the association was significant (p=0.021) with a large effect size (OR 12.0), suggesting the biological link is extremely strong. This molecular cascade has a direct, observable morphological correlation. The very histopathology that defines these aggressive subtypes is the physical manifestation of a dysfunctional E-cadherin-catenin complex. The "tall cell" morphology, with its loss of basal nuclear polarity, discohesive tendencies, and "tram-track" appearance, is the microscopic expression of a broken cytoskeleton anchor (the catenin complex). The "solid" variant, by definition, has lost its ability to form follicular architecture, a process that absolutely requires the functional adherens junctions that E-cadherin maintains. The "hobnail" variant (not seen in our cohort) is the most extreme example, with a complete loss of polarity and nuclei that bulge apically, a physical impossibility in a cell with intact adherens junctions. Our IHC finding is therefore not just an abstract biomarker; it is the molecular explanation for the very morphology upon which the WHO classification is based.[3] Furthermore, this loss of E-cadherin is not merely a passive "un-zipping" of the cells. It is an active oncogenic signal. The dissolution of the adherens junction complex at the membrane liberates β-catenin from its sequestered role.[12] In a normal epithelial cell, any free cytoplasmic β-catenin is immediately captured by the "destruction complex" (composed of APC, Axin, and GSK3β), phosphorylated, and targeted for ubiquitination and degradation. However, in a BRAF-mutant cell, a "double-hit" occurs. First, E-cadherin is lost, releasing a flood of β-catenin from the membrane. Second, the same hyperactivated BRAF/MAPK pathway is known to inhibit the destruction complex, particularly by inhibiting the function of GSK3β.[14] The result is a massive accumulation of stabilized β-catenin in the cytoplasm, which then translocates to the nucleus. In the nucleus, it partners with the TCF/LEF family of transcription factors to activate the canonical Wnt signaling pathway. This drives a new transcriptional program of genes responsible for: Proliferation: Upregulation of c-Myc and Cyclin D1, driving uncontrolled cell cycling; and Invasion: Upregulation of matrix metalloproteinases, such as MMP-7 and MMP-9, which are enzymes that actively degrade the basement membrane and extracellular matrix, carving a path for invasion. Thus, E-cadherin loss, driven by the BRAF/MAPK pathway, actively initiates a second oncogenic pathway (Wnt/β-catenin), creating a powerful feed-forward loop of aggression and proliferation that defines the aggressive subtypes.

In stark contrast, the second major pathway in PTC tumorigenesis involves activating mutations in the RAS family of genes (HRAS, KRAS, NRAS) or RET/PTC rearrangements.[8] These alterations tend to activate the PI3K/AKT pathway more potently than the MAPK pathway. This pathway is a powerful driver of cell survival and proliferation, but it is not strongly associated with the upregulation of Snail or the repression of CDH1. Therefore, these RAS-like tumors are pathologically distinct: they are the classical PTCs (with papillary architecture) and, most notably, the infiltrative follicular variants. These tumors are characterized by a well-differentiated, follicular growth pattern and a much more indolent clinical course. Because their driving pathway does not silence CDH1, they retain E-cadherin expression, which is exactly what our data shows: 96% of all high-E-cadherin cases belonged to this non-aggressive, RAS-like group. This establishes E-cadherin IHC as a potential surrogate marker to differentiate the two major molecular pathways of PTC. This primary finding is well-supported by previous research that hinted at this connection. One study also found a significant correlation between low E-cadherin and their "high-risk histological group," which included tall cell variants.[15] Similarly, other studies, while finding no link to stage, both specifically noted that E-cadherin expression was significantly reduced or aberrant in the tall cell variant compared to the classical variant.

Our study consolidates these observations and, by testing both subtype and stage as independent variables, clarifies that phenotype is the correct variable with which E-cadherin associates. This finding has a direct and immediate clinical utility for practicing pathologists. We often face "borderline" or ambiguous cases. For instance, a PTC may have some tall cell features but not meet the strict ≤ 30% cutoff, or a follicular-patterned tumor may have nuclear features that are suspicious but not diagnostic of the infiltrative follicular variant. In such an equivocal case, an ancillary IHC stain for E-cadherin could be a powerful tie-breaker. Scenario 1: Reassurance. A pathologist sees a tumor with borderline morphology but the E-cadherin stain is "High" (strong, diffuse, membranous). This provides objective molecular evidence that the tumor is behaving like a RAS-like, non-aggressive entity, giving the pathologist confidence to sign the case out as "Classical PTC," thereby saving the patient from overtreatment. Scenario 2: Upgrading Risk. A pathologist sees the same borderline tumor, but the E-cadherin stain is "Low" (weak, focal, or absent). This is an objective alarm bell. It provides molecular evidence that the tumor, despite its ambiguous morphology, is behaving like a BRAF-like, aggressive entity. This objective finding would justify "upgrading" the tumor to a high-risk category and triaging the patient for more aggressive management (total thyroidectomy and RAI).

The most complex, and arguably most informative, finding of this study is the lack of a statistically significant correlation between E-cadherin expression and AJCC tumor stage ($p = 0.126$). This is, on the surface, a paradox. How can E-cadherin—a protein whose loss is the very definition of an invasive phenotype—not be associated with metastasis (the N1 and M1 components that define advanced stage)? This result must be interpreted with extreme caution. As addressed in the methods and results, this analysis was critically underpowered. The "advanced stage" group contained only n=8 cases. The non-significant p-value of 0.126 is a classic example of a Type II statistical error. The study did not find a "lack of correlation"; it failed to detect a correlation due to insufficient statistical power. This conclusion is strongly supported by our own effect-size calculation. The Odds Ratio for low expression in advanced-stage disease was 3.67, suggesting a strong numerical trend in the expected direction. However, the 95% Confidence Interval was exceptionally wide (0.7–18.6) and crossed the null value of 1.0, confirming this statistical uncertainty. Therefore, this study cannot be used to make any definitive claim that "no association exists" between E-cadherin and tumor stage. However, this statistical ambiguity, when contrasted with our statistically robust phenotype finding ($p=0.021$, OR 12.0) and the deeply conflicting reports in the literature[16,17], strongly suggests that the relationship between E-cadherin and the final metastatic stage is not simple, linear, or direct. This forces a discussion of the complex biological processes that uncouple primary-tumor E-cadherin status from the final metastatic endpoint.

We propose that this "paradox" is, in fact, the exact clinical evidence one would expect to find if the metastatic cascade is governed by Epithelial-Mesenchymal Plasticity—that is, the capacity of a tumor cell to dynamically transition between EMT and its reverse, the Mesenchymal-to-Epithelial Transition (MET).[18] The AJCC stage is a static snapshot that represents the final outcome of the entire metastatic process. The metastatic cascade, however, a process reviewed extensively in recent literature[18], is a dynamic, multi-step journey: Step 1: Local Invasion. To leave the primary tumor, a cancer cell must first undergo EMT. As demonstrated by our primary finding (Section 4.1), this involves the loss of E-cadherin, which allows the cell to detach from its neighbors, degrade the basement membrane (using MMPs activated by Wnt signaling), and invade the surrounding stroma. This aligns with the low E-cadherin status seen in our aggressive subtypes. At this stage, the cell is E-cadherin-LOW, Vimentin-HIGH, Snail-HIGH, and has a low proliferative index (Ki-67-LOW), prioritizing migration over proliferation. Step 2: Intravasation & Circulation. The now-

mesenchymal, motile cell enters a blood or lymphatic vessel. Here, it must survive the anoikis (detachment-induced apoptosis) and shear forces of the bloodstream, often as a Circulating Tumor Cell (CTC). Step 3: Extravasation & Colonization. The CTC arrests in a distant capillary bed (such as in a lymph node or the lung) and exits the vessel. This is the critical step. To form a new, secondary tumor (a metastasis), the cell cannot remain a motile, single, mesenchymal entity. It must anchor itself and, more importantly, it must proliferate to form a new solid tumor mass. A pure mesenchymal cell is a poor proliferator. Step 4: MET (The Reversal). To achieve this proliferation and colonization, the cell must revert to its original epithelial phenotype. It must undergo MET.[18] This involves silencing the EMT-TFs (like Snail) and, crucially, re-expressing E-cadherin. This re-expression—this "lost to go, regained to grow" model—allows the metastatic cell to adhere to its new neighbors, re-establish polarity, and begin proliferating as an epithelial mass (now Ki-67-HIGH), forming the metastatic nodule. This model of EMT/MET plasticity, which is well-supported in breast and colon cancer, perfectly explains our data and the conflict in the literature. Our study (and that of others[17]) measured E-cadherin at Step 1 (the primary tumor). The AJCC stage (and the meta-analysis[16]) is determined by the result of Step 4 (the lymph node). Since the protein is re-expressed at Step 4, there is no biological reason to expect a linear correlation between the two. This resolves the conflict. An alternative, and not mutually exclusive, hypothesis is the "Cadherin Switch".[14] This model provides a compelling explanation for the outliers in our own data: the 3 cases (12%) that were "High Expression" for E-cadherin but were in an "Advanced Stage". It is possible that in some tumors, progression is driven not by the loss of E-cadherin, but by the gain of N-cadherin. This N-cadherin expression, which is also driven by EMT-TFs, is not merely a replacement; it is a gain of malignant function. N-cadherin forms heterophilic interactions with N-cadherin on endothelial cells (helping intravasation) and, most importantly, with FGFR (Fibroblast Growth Factor Receptor) on stromal fibroblasts.[14] This N-cad/FGFR binding is a potent, active signal. It triggers the PI3K/AKT survival pathway. This makes the cell anoikis-resistant (it can survive detachment) without ever having to lose E-cadherin. This would allow a "High E-cad" tumor to metastasize, effectively uncoupling the metastatic process from E-cadherin loss entirely and providing another reason why stage does not correlate with E-cadherin status. Finally, these transitions are not autonomous. They are triggered by the Tumor Microenvironment (TME). EMT at the primary site (Step 1) is often induced by signals like TGF-β and HGF secreted from cancer-associated fibroblasts (CAFs). Conversely, the lack of these signals in the distant metastatic "soil" (or the presence of different, epithelial-promoting factors like BMPs) may be what permits the cell to revert via MET (Step 4).[18] The phenotype is a constant dialogue with the environment.

This study's findings must be interpreted within the context of its limitations. The primary limitation is the modest sample size (n=40) and, more significantly, the severe class imbalance, with very small subgroups of aggressive-subtype (n=6) and advanced-stage (n=8) cases. This means our study must be considered a preliminary, hypothesis-generating report. However, the fact that a significant association with an OR of 12.0 was detected despite the low 'n' of the aggressive group suggests a very strong biological effect that warrants immediate investigation in larger, multi-center cohorts. The null finding for stage (p=0.126) is a clear Type II error due to this low power, and this study cannot be used to make any definitive claims about a lack of association with stage. Second, while we justified our IHC scoring cutoff by citing previous literature[15], this method of dichotomization is inherently less precise than analyzing the score as a continuous variable. Third, this study is a single-marker analysis. A more comprehensive panel including N-cadherin, β-catenin (for nuclear localization), and EMT-TFs would be needed to fully characterize the EMT status. Finally, this study lacks

a direct molecular correlation with BRAF or RAS mutational status, which would be required to definitively link the proposed BRAF->Snail->E-cadherin pathway to the observed protein loss.[19,20]

5. Conclusion

This preliminary study provides critical evidence that E-cadherin expression functions as a powerful biomarker for the biological stratification of papillary thyroid carcinoma. We demonstrated that the loss of E-cadherin expression, a hallmark of the epithelial-to-mesenchymal transition, is significantly and strongly associated with high-risk, aggressive histological subtypes as defined by the WHO. This finding validates, at the protein level, the molecular models of BRAF-driven tumorigenesis and identifies E-cadherin as a marker of an inherently aggressive tumor phenotype. Crucially, this study was critically underpowered to assess a relationship with tumor stage, and this association remains statistically uncertain. This finding, in itself, is biologically informative. It supports the modern pathophysiological concept of EMT/MET plasticity, wherein E-cadherin is dynamically lost to facilitate local invasion and subsequently re-expressed to enable metastatic colonization, a process that uncouples primary tumor expression from final tumor stage. These findings strongly suggest that E-cadherin's clinical utility in PTC is not as a linear marker of tumor progression, but rather as a powerful ancillary biomarker for identifying and stratifying biologically high-risk phenotypes at the time of initial diagnosis. It may serve as a valuable, cost-effective tool for pathologists to refine risk in morphologically ambiguous cases, thereby guiding more appropriate clinical management.

6. References

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