

Study of Expression of Claudin-1 In Thyroid Lesions

¹Dr. Mohammed Imran Ali, ²Dr. Jayavardhan D, ³Dr. GRangaswamy,

⁴Dr. S. Sandhya,

¹Assistant Professor, Kakatiya Medical College

^{2,3}Kakatiya Medical College

⁴Professor, HOD, Kakatiya Medical College

Authors Affiliation-: Department Of Pathology, Kakatiya Medical College, Warangal, Telangana, India

*Corresponding Author: ¹Dr. Mohammed Imran Ali

ABSTRACT

Background: Thyroid cancers represent only 1% of all malignant diseases, are among the most common endocrine malignancies [1–3]. The “gold standard” in diagnosis of thyroid nodules is pathologic evaluation using routine hematoxylin and eosin (H&E) staining. However, morphologic overlap between follicular lesions especially the follicular variant of papillary carcinoma (FVPC) is common. Consequently, immunohistochemical and molecular methods were investigated to aid in the diagnosis of these problematic cases [6–9].

Methods: The material of this one year retrospective study included 63 specimens of surgically removed, formalin-fixed and paraffin embedded thyroid lesions that were received at the department of pathology MGM Hospital, Warangal during the period from June 2016 to June 2017. **Results:** The Benign thyroid nodules showed negative claudin-1 expression (staining in <5% of the cells) in 38 out of 41 cases (92.6%). Out of 22 cases of papillary carcinoma of thyroid 21 (95.4%) cases were showed strong and diffuse claudin-1 expression in 80–90% of the tumor cells (score 3) was observed including 9/9 cases of classic Papillary carcinoma of thyroid and 12/13 cases of follicular variant of papillary carcinoma.

Conclusion: Claudin 1 may be a useful immunohistochemical marker in histopathologically overlapping cases especially Papillary carcinoma and benign thyroid nodules with papillary features. Further studies with larger sample sizes are required in order to clarify the diagnostic utility of claudin -1 expression levels in differentiating controversial cases.

Keywords: Multinodular Goitre, Papillary carcinoma of thyroid, Follicular Adenoma, Claudin-I

I. INTRODUCTION

Thyroid cancers represent only 1% of all malignant diseases, are among the most common endocrine malignancies [1–3]. Most thyroid tumors derived from the follicular epithelium [2, 3]. Papillary carcinoma is the most common type of thyroid cancer, comprising approximately 80% of thyroid epithelial malignancies [1–4]. The “gold standard” in diagnosis of thyroid nodules is pathologic evaluation using routine hematoxylin and eosin (H&E) staining. However, morphologic overlap between follicular lesions especially the follicular variant of papillary carcinoma (FVPC) is common. In such cases an objective consistent diagnosis based merely on morphologic assessment is sometimes impossible [5].

Consequently, immunohistochemical and molecular methods were investigated to aid in the diagnosis of these problematic cases [6–9]. Several immunohistochemical markers such as galectin-3, cytokeratin-19 and HBME-1 have been recommended to help in the discrimination between these controversial thyroid nodules [10]. Nonetheless up till now there is no agreed consensus about an immunohistochemical marker that would reliably overcome such diagnostic obstacles.

Claudins, a family of transmembrane proteins, are major components of the tight junction (TJ) [11, 12]. Claudin-1 was the first member of the claudin family to be identified as a tight junction component [13]. So far 24 members of the claudin family have been described, and their role in carcinogenesis and cancer progression has been proposed repeatedly [10, 14–16]. Altered expression of claudins was found in a wide variety of human malignancies including endometrial [17], papillary renal cell [18], colon [19], pancreatic [20], breast [21] and cervical cancers [22]. Claudins as membrane proteins showing differential expression in the normal versus neoplastic tissues [14, 16, 23] may provide new opportunities for targeted cancer therapy [24, 25]

II. MATERIALS AND METHODS

The material of this one year retrospective study included 63 specimens of surgically removed, formalin-fixed and paraffin embedded thyroid lesions that were received at the department of pathology MGM

Hospital, Warangal during the period from June 2016 to June 2017. All available slides were reviewed and the most representative blocks from each case were selected. The selected samples included 11 males and 52 females ranging from 11 to 87 years old.

Cases included 22 Papillary thyroid carcinoma cases, 41 cases of benign thyroid nodules. The 22 PTC cases were further classified into 9 cases of classic PTC and 13 cases of FVPCs. The 41 cases of benign thyroid nodules included 13 cases of multinodular goiter with dominant nodule, 22 cases of follicular adenoma, and 6 cases of Hashimoto's thyroiditis.

Confirmation of the original diagnosis was achieved through separate revision of H&E stained slides by the two authors independently using the well established diagnostic criteria for each lesion. For the diagnosis of PTC we followed the histological criteria proposed by Chan [26] which are divided into major and minor features. The major features include: (1) nuclei are ovoid rather than rounded; (2) nuclei are crowded, often manifesting as a lack of polarization in the cells that line the follicle; (3) nuclei show a clear or pale chromatin pattern; (4) psammoma bodies are found. If one of the four features was lacking, four or more of the following features may occur: (1) presence of abortive papillae; (2) predominantly elongated or irregular shaped follicles; (3) dark staining colloid; (4) presence of rare nuclear pseudo-inclusions; or (5) multinucleated histiocytes in follicle lumen.

Follicular adenomas were defined as completely encapsulated follicular tumors with homogenous morphology, lacking the nuclear features of papillary carcinoma and without capsular or vascular invasion.

III. IMMUNOHISTOCHEMISTRY

All 63 samples were subjected to immunohistochemical staining with claudin-1 antibodies. The paraffin embedded tissue sections were deparaffinized in xylene and rehydrated through absolute alcohol. Antigen retrieval in citrate buffer was used after the sections were treated in a microwave at 8 W for 5–6 min, then at 3 W for 10 min, the sections were then left to cool for 20 min. Peroxidase and protein block were done. After that the slides were incubated overnight with each of the primary antibodies at room temperature using claudin-1 antibody.

Interpretation of immunohistochemical staining of claudin-1

According to Nemeth et al. [27], cases exhibiting membranous claudin-1 staining in >5% of the cells were considered positive. Claudin-1 expression was scored as follows: 0, staining in <5% of the cells; 1, staining in 5–25% of the cells; 2, staining in 26–50% of the cells; 3, staining in >50% of the cells.

IV. RESULTS

An expression of claudin-1 in benign thyroid lesions :

The Benign thyroid nodules showed negative claudin-1 expression (staining in <5% of the cells) in 38 out of 41 cases (92.6%) (table-1), which included 20 out of 22 cases of follicular adenomas (90.1%), 12 out of 13 cases of multinodular goitre (92.3%) and 6 out of 6 cases of Hashimoto's thyroiditis (100%). Positive claudin-1 expression was observed in only 3 cases (7.2%). All of these 3 cases showed claudin-1 positivity in 10–25% of the cells (score 1); two of which were follicular adenoma and one multinodular goitre.

An expression of Claudin-1 in malignant lesions :

Out of 22 cases of papillary carcinoma of thyroid 21 (95.4%) cases were showed strong and diffuse claudin-1 expression in 80–90% of the tumor cells (score 3) was observed including 9/9 cases of classic Papillary carcinoma of thyroid and 12/13 cases of follicular variant of papillary carcinoma (table-1).

Table-1: expression of claudin-1 in benign thyroid lesions and papillary carcinoma

| | apillary carcinoma | Follicular variant of papillary carcinoma | Follicular adenoma | Multinodular goitre | Hashimoto's thyroiditis |
|---|--------------------|---|--------------------|---------------------|-------------------------|
| Claudin-1 expression Negative (% within diagnosis) | 00 (0%) | 01 (7.7%) | 20 (90.1%) | 12 (92.3%) | 06 (100%) |
| Claudin-1 expression Positive (% within diagnosis) | 09 (100%) | 12 (92.3%) | 02 (8.9%) | 01 (7.7%) | 00 (0%) |

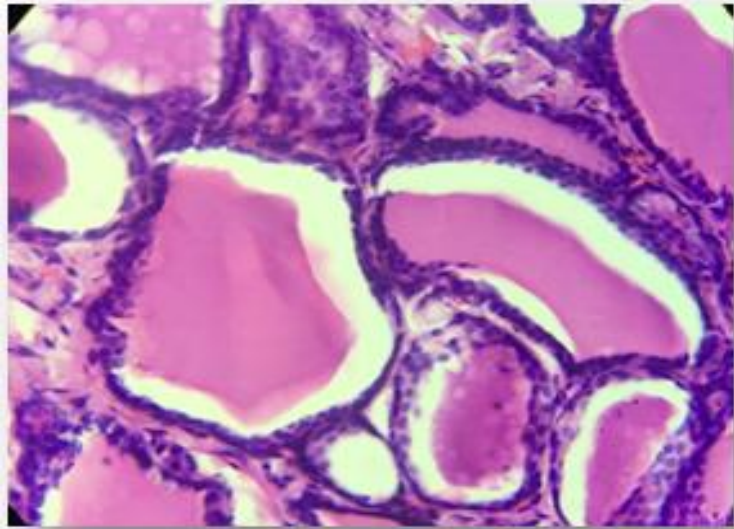


Fig #1 H&E of Multinodular Goitre (40X)

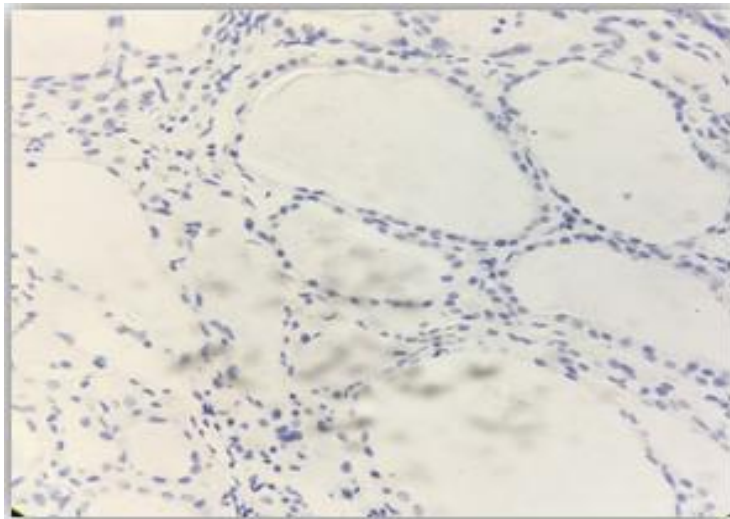


Fig #2: Negative claudin -1 expression multi nodular goitre (40X)

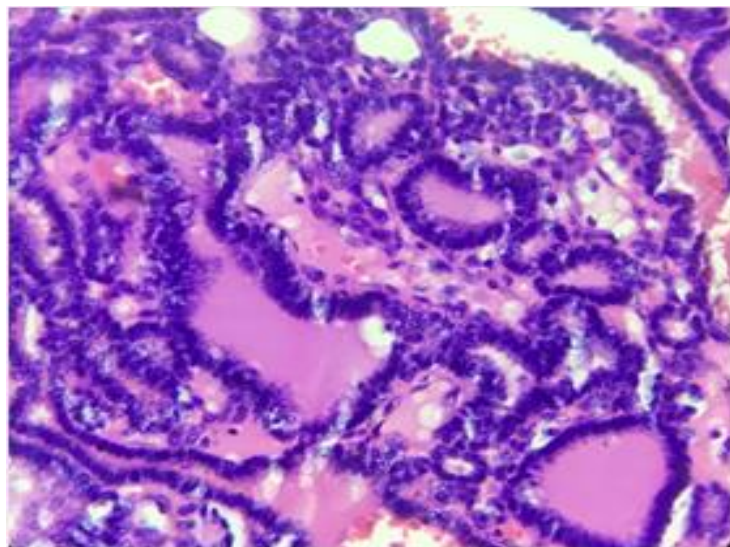


Fig #3: H&E of Papillary Ca of Thyroid (40X)

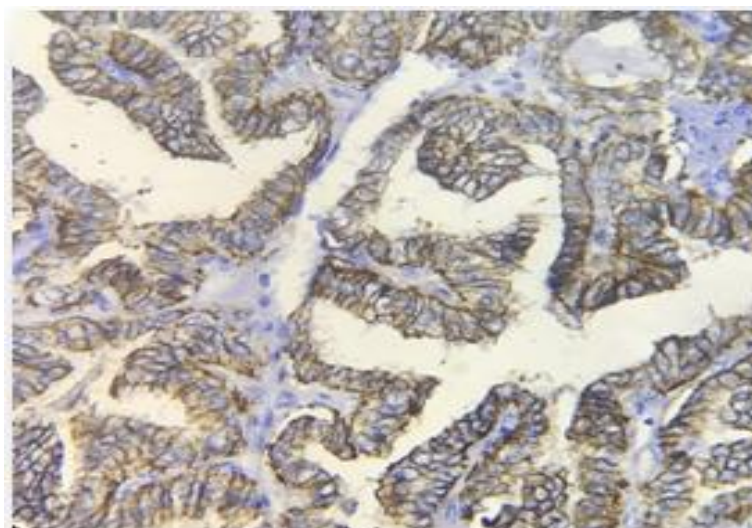


Fig #4: Diffuse and strong claudin -1 expression in papillary carcinoma of thyroid (40X)

There was a high statistically significant difference between papillary carcinoma of thyroid and other benign thyroid nodules as regards claudin-1 expression ($P < 0.001$) (Table 2). There was also a high statistically significant difference between Follicular variant of papillary carcinoma and follicular adenoma (table-4).

Table 2: The sensitivity, specificity, positive predictive value, negative predictive values and accuracy for distinguishing benign and malignant lesions of thyroid

| Antibody | Sensitivity | Specificity | PPV | NPV | Accuracy | P value |
|--------------|-------------|-------------|-------|-------|----------|---------|
| Claudin-1+ve | 95.4% | 92.6% | 87.5% | 97.4% | 93.6% | <0.001* |

PPV: positive predictive value, NPV: negative predictive value.

* Highly significant

Table 3: Comparison between FVPCs and follicular adenoma as regards claudin-1 expression.

| | Follicular Adenoma | FVPC | Total | P value |
|-----------------------------|--------------------|-------|-------|---------------|
| Claudin-1 | | | | |
| Negative | | | | |
| Count | 20 | 01 | 21 | $P < 0.001^*$ |
| (% within diagnosis) | 90.1% | 7.7% | 60% | |
| Positive | | | | |
| Count | 02 | 12 | 14 | |
| (% within diagnosis) | 8.9% | 92.3% | 39.2% | |
| Total | 22 | 13 | 35 | |

* Highly significant.

Table 4: The sensitivity, specificity, positive predictive value, negative predictive values and accuracy for distinguishing follicular adenoma and follicular variant of papillary carcinoma of thyroid.

| Antibody | Sensitivity | Specificity | PPV | NPV | Accuracy | P value |
|--------------|-------------|-------------|-------|-------|----------|---------|
| Claudin-1+ve | 92.3% | 90.9% | 85.7% | 95.2% | 91.4% | <0.001* |

PPV: positive predictive value, NPV: negative predictive value.

* Highly significant

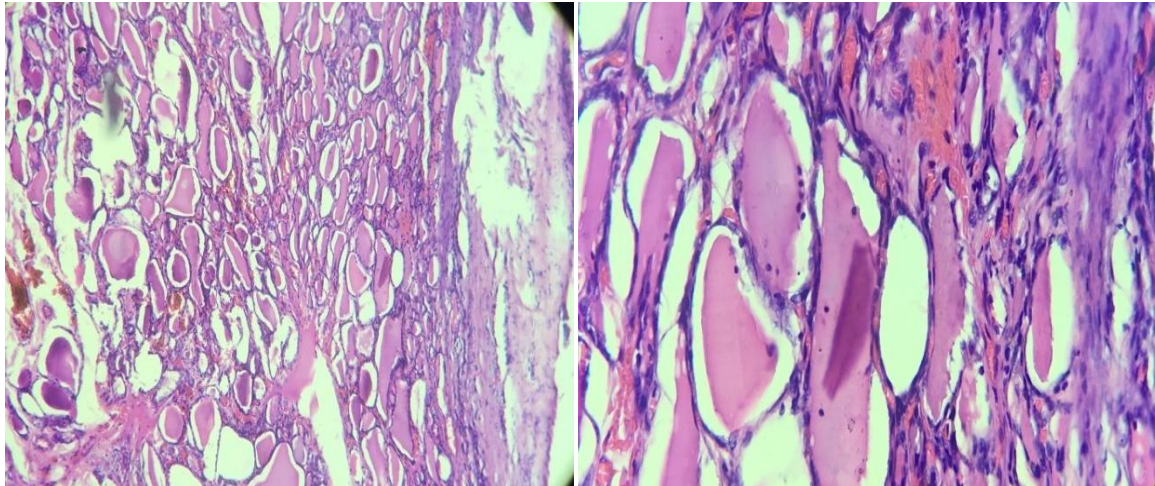
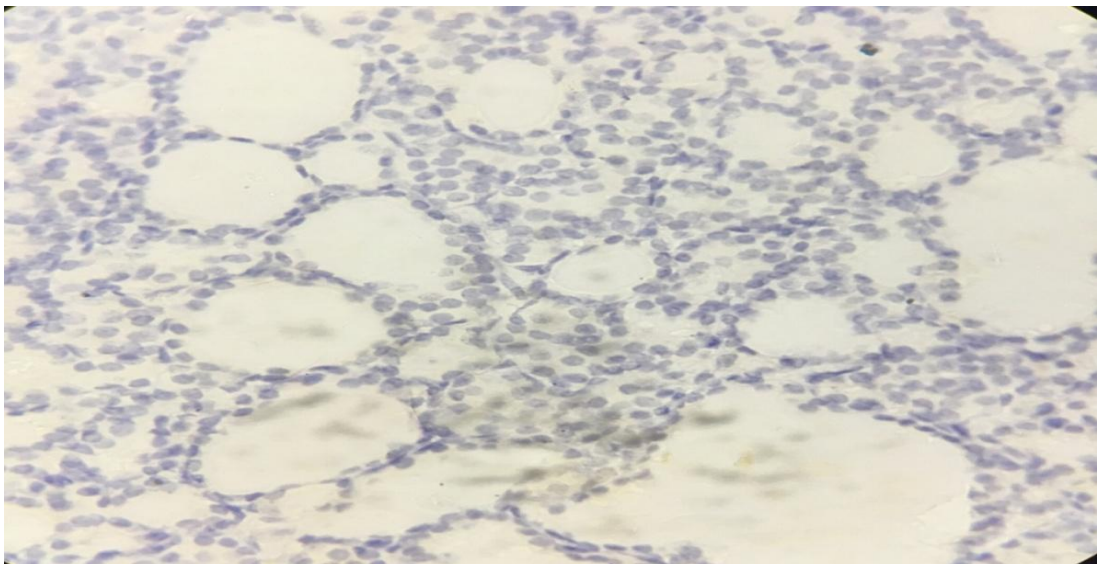


Fig 5&6 # H&E of Follicular Adenoma (10X AND 40X)



Fig#7: Negative claudin-1 expression in follicular adenoma

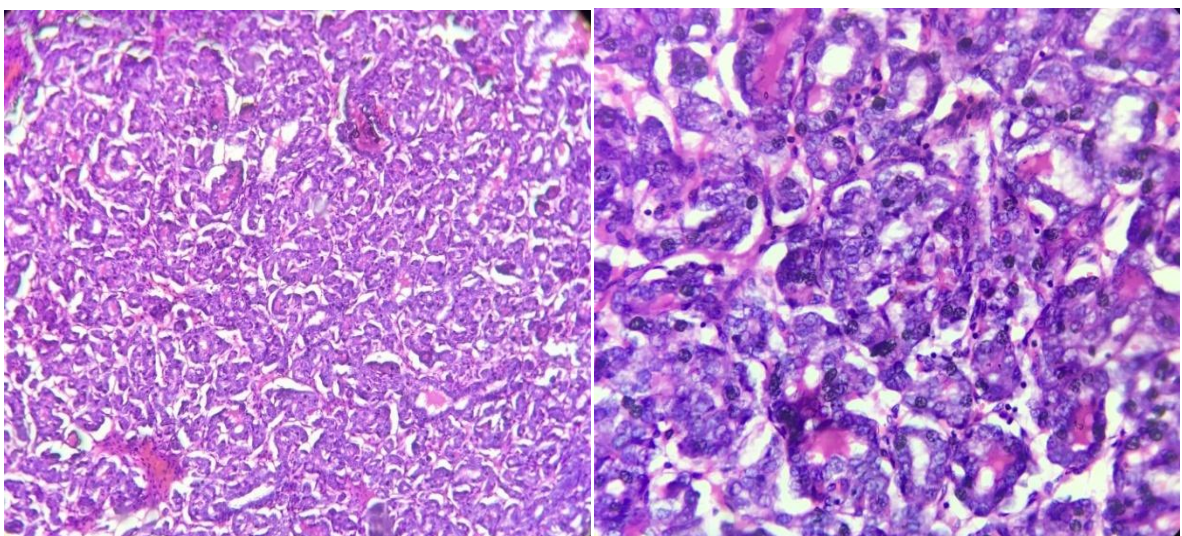


Fig 8&9: H&E of Follicular variant of Papillary Ca (10X &40X)

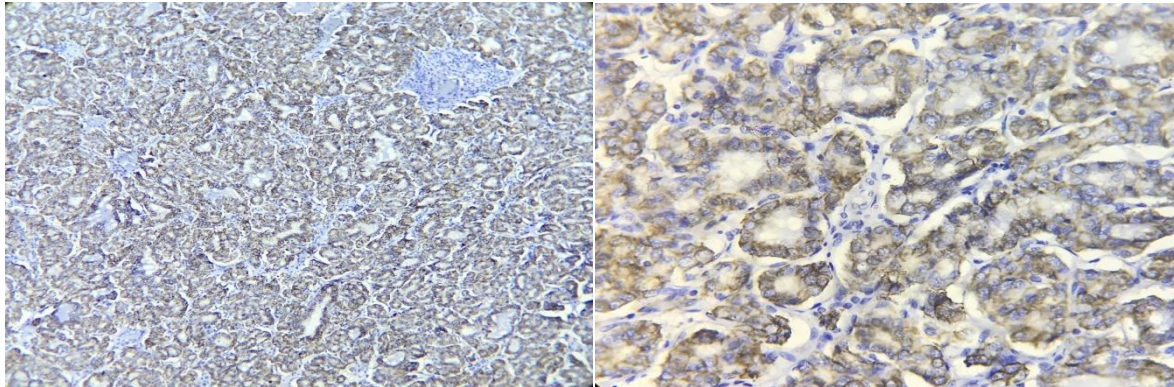


Fig 10 & 11#: Diffuse and strong claudin – 1 positive in follicular variant of papillary carcinoma

V. DISCUSSION

The diagnosis of thyroid lesions is, usually but not always, easily achieved with almost minimal interobserver variability. But in certain conditions it is difficult to diagnose the lesions Eg: follicular adenoma from follicular variant of papillary carcinoma and multinodular goitre with papillary features from papillary carcinoma. Therefore this study investigated the possible role of claudin-1 in resolving such problem.

The role of claudin-1 as a tight junction protein in cancer initiation and progression has been intensively investigated. The upstream signaling pathways influencing claudin-1 expression in thyroid tumors remains elusive. Tzelepi et al. [28] demonstrated that papillary carcinoma being a well differentiated thyroid carcinoma shows high claudin-1 expression, and that dedifferentiation of thyroid tumors involves tight junction impairment via claudin-1 down regulation. In addition, loss of tight junction integrity leads to an increased influx of growth factors, nutrients and other tumor promoting molecules, therefore providing an advantage for tumor development and progression. On the other hand, Hucz et al. [29] and Nemeth et al. [27] associated claudin-1 expression with the invasive and metastatic phenotype of PTC due to preserved claudin-1 strong expression in the lymph node metastasis.

In the current study all 9 cases of classical PTCs (100%) and 11 out of 12 cases of FVPC showed strong and diffuse claudin-1 expression. 92.6% of the benign thyroid nodules showed negative claudin-1 expression. Similar results were elucidated by Hucz et al. [29] and Nemeth et al. [27] who reported high claudin-1 expression in PTC cases compared to negative expression in normal thyroid tissue and in follicular adenomas. This differential expression in the normal versus neoplastic tissues may provide new opportunities for targeted cancer therapy. In addition, our results showed a high statistically significant difference between FVPCs and other benign thyroid nodules as regards claudin-1 expression ($P < 0.001$). Thus claudin-1 immunohistochemistry is proved to be very useful in differentiating FVPCs from other follicular nodules with 92.3% sensitivity and 90.9% specificity, 95.2% NPV and 85.7% diagnostic accuracy.

In conclusion, claudin 1 may be a useful immunohistochemical marker in histopathological overlapping cases especially papillary carcinoma and benign thyroid nodules with papillary features. Further studies with larger sample sizes are required in order to clarify the diagnostic utility of claudin -1 expression levels in differentiating controversial cases.

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***Corresponding Author: ¹Dr. Mohammed Imran Ali**
Department Of Pathology, Kakatiya Medical College, Warangal, Telangana, India