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# The Combined Utility of hbme-1 Galectin-3 and Brafv600e Mutations in the Diagnosis of Papillary Thyroid Carcinoma

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## Abstract

Newer diagnostic modalities have revolutionized the pathologist ' s approach to diagnosis of thyroid malignancies. Molecular characterization of these malignancies has helped circumvent common morphologic diagnostic difficulties by integrating their genotypic, phenotypic and immunohistochemical features. BRAFV600E mutation has been characterized as highly specific for thyroid carcinoma especially papillary thyroid carcinoma. HBME-1 and Galectin-3 are also such markers which are highly specific for papillary carcinoma of thyroid (PTC). We propose to study HBME-1 and Galectin-3 expression and BRAFV600E mutation in thyroid neoplasms and do a comparative analysis to determine whether there is a correlation between BRAFV600E expression and expression of HBME-1 and Galectin-3. As molecular analysis is time consuming and not very cost effective to the patient the utility of these markers as a surrogate marker for BRAFV600E mutation will also be considered in this study.

**Keywords:** Thyroid; Malignancy; HBME-1; Galectin-3; BRAFV600E

## Introduction

Diseases of thyroid are more common worldwide. In India too diseases of thyroid are common and are on the rise. From various studies done in the past, it has been calculated that about 42 million Indians suffer from thyroid disorders. The Indian Council of Medical Research established the National Cancer Registry Program, and the NCRP has collected the data of more than 3, 00,000 cancer patients between the periods 1984 and 1993. The nationwide relative frequency of thyroid cancer among all the cancer cases was 0.1%-0.2%. The age-adjusted incidence rates of carcinoma of the thyroid per 100,000 populations are about 1 for males and 1.8 for females according to the Mumbai Cancer Registry. This covered a population of 9.81 million subjects [1-10].

## Review of Literature

Neoplasms arising from the lining follicular epithelial cells are the most common. Nearly 95% of thyroid neoplasms originate from follicular cells. From a hospital based cancer registry in India covering 1185 new cases of thyroid neoplasms, it was shown that the commonest epithelial neoplasm arising from follicular cells are papillary carcinoma followed by follicular neoplasms [11]. Medullary carcinomas which are derived from Parafollicular C cells represent about 3% of thyroid malignancies [12].

Papillary thyroid carcinomas are characterized by distinct nuclear features and usually have a favourable prognosis. Follicular thyroid carcinomas are less frequent and have a worser prognosis because of hematogenous dissemination [13]

The prognosis of thyroid neoplasms depends on the age at presentation, gender, staging, multicentric origin and histologic type. Each histological type shows marked differences based on genotype and therefore a varied clinical course. Most of these can be effectively treated by surgical resection with or without adjuvant chemotherapy.

Thyroid diseases are different from other diseases in terms of their ease of diagnosis, accessibility of medical/surgical treatment, and the relative visibility that even a small swelling of the thyroid offers to the treating physician. So, early diagnosis and treatment is the cornerstone of management of these lesions. Thyroid malignancies can be accurately diagnosed using strict histopathological criteria. But frequently the pathologist is encountered with lesions with subtle changes or in transformation, posing a challenge to the pathologist. In such scenarios the pathologist depends on ancillary techniques like immunohistochemistry and molecular studies for an accurate diagnosis. A recent study done in India also stressed the importance of usage of ancillary studies such

as immunohistochemistry and genetic profiling of thyroid neoplasms in the near future as a routine basis [10].

## Papillary Carcinoma

Papillary thyroid carcinoma (PTC) is the most common malignant neoplasm of the thyroid. 85% of thyroid neoplasms are papillary carcinomas [14]. They are very indolent tumours with a good prognosis. They are common in the middle age. They have a common aetiology of exposure to radiation. They vary in sizes from 2-3 cm to large or subcentimeter nodules. The cut surface is firm and white with focal areas of calcification and cystic change. Microscopically, the papillary carcinoma is composed of true papillae with thin fibro vascular cores. These papillae are lined by stratified columnar or cuboidal cells with nuclear overlapping, crowding, nuclear grooves, inclusions and clearing **(Figures 1 and 2)**. These nuclear features are diagnostic of papillary carcinomas **(Figure 2)**. Psammoma bodies, cystic change and haemorrhage can also be seen in these neoplasms **(Figure 3)** [15]. These neoplasms spread through lymphatics and nearly a significant half of these cases present with lymph node metastases at the initial presentation.

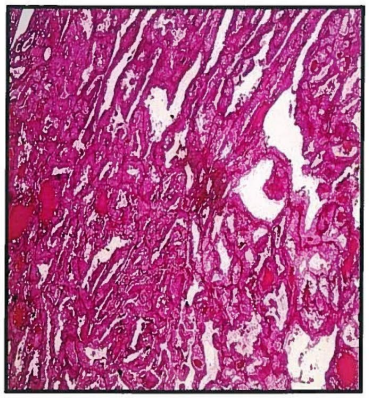


**Figure 1:** Papillary carcinoma-Neoplastic cells arranged in papillary pattern, H&E (x100)

### Papillary microcarcinoma

Papillary microcarcinomas are incidental findings when the thyroidectomy is done for other indications. These lesions are defined by the size which is 1cm or less in diameter. Grossly, these lesions are mostly unencapsulated and occasionally present with metastasis to regional lymph nodes [16].

### Follicular variant of papillary carcinoma

The presence of follicular structures with nuclear features characteristic of papillary thyroid carcinoma and the presence of capsular invasion is in favour of a follicular variant of papillary thyroid carcinoma. These lesions are prone for vascular invasion and metastasis to distant sites [15,17].

### III. Tall cell variant

These tumours are very rare and consist of tall cells. 50% of the tumour should be showing tall cells to be diagnosed as a tall cell variant [15].

### IV. Oncocytic variant

These neoplasms grossly have a distinct brown colour. The cells are polygonal and have an abundant granular eosinophilic cytoplasm [18].

### V. Warthin tumour like variant

The characteristic features of these lesions are the presence of oncocytic cells in the papillae with a prominent infiltrate with lymphocytes and plasma cells in the papillary stalks [16].

### VI. Cribriform variant

This variant of papillary carcinoma is rare and is common among young women. They usually present as multifocal lesions. They have a prominent cribriform architecture [14].

### VII. Diffuse sclerosis variant

They represent 3% of all papillary carcinomas of the thyroid. It most often affects children and young adults and also may present as a bilateral goiter [14].

### VIII. Clear cell variant

These neoplasms are predominantly composed of clear cells arranged in papillary pattern [15].

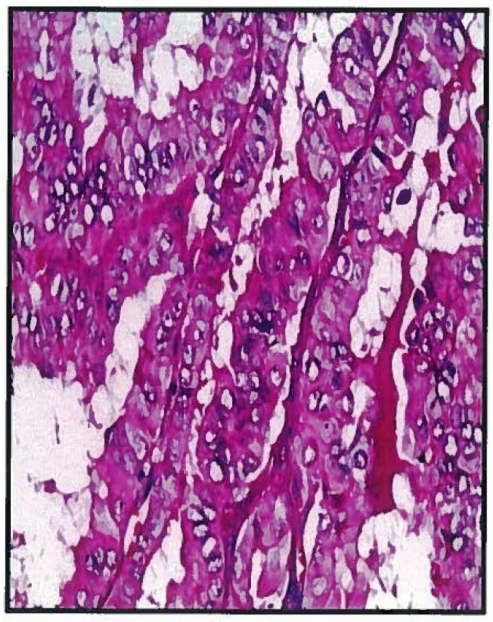


**Figure 2:** Nuclear features of conventional papillary thyroid carcinoma, H&E (x400)

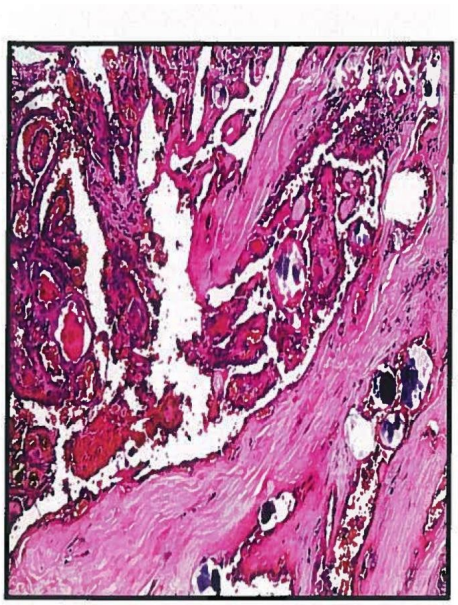
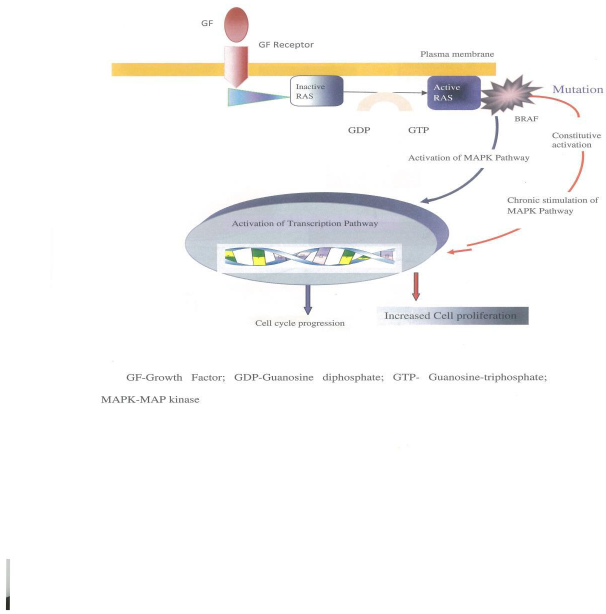


**Figure 3:** Papillary carcinoma with calcification and psammoma bodies, H&E (x 100)




GF-Growth Factor; GDP-Guanosine diphosphate; GTP- Guanosine-triphosphate; MAPK-MAP kinase

**Figure 4:** Mechanism of BRAF mutation oncogenesis.GF-Growth Factor; GDP-Guanosine diphosphate; GTP-Guanosine-triphosphate; MAPK-MAP kinase.

## Mutations in Thyroid Neoplasms

Morphological assessment alone is often adequate for diagnosing thyroid malignancies. Ancillary studies like immunohistochemistry are useful when there are unusual patterns to confirm diagnosis and prognosis.

FNACs play a major role in diagnosis and also in treatment outcome of the patient. More and more FNACs are being done on early lesions as the imaging technology has improved drastically. Added to this targeted therapies also compound to the challenges faced. Therefore knowledge of mutations becomes important. In the last two decades there has been a significant improvement in the molecular alterations of thyroid neoplasms.

The thyroid follicular epithelial cell is acted up on by two hormones, the thyroid stimulating hormone (TSH) and growth factors (GF). These hormones can act on the epithelial cells through any of the two cell signalling pathways discussed below. The thyroid stimulating hormone acts on a seven transmembrane domain G protein coupled receptor. This in turn activates the GS α adenyl cyclase cyclic AMP pathway, which allows the synthesis of thyroid hormones. Growth factors act by binding to the receptor and inducing receptor tyrosine kinase dimerization. This is followed by phosphorylation of tyrosine residues which activate the MAP kinase pathway. Mutations in well differentiated thyroid carcinomas commonly involve the MAP kinase pathway [12].

BRAF mutations are very common among thyroid malignancies. The BRAF mutation can either be a point mutation or a chromosomal rearrangement and is seen in 7% of human neoplasms. They are very commonly seen in malignant melanoma, thyroid neoplasms, serous tumours of ovary, adenocarcinoma of colon, hepatocellular carcinomas, biliary carcinomas and hairy cell leukaemia's.

The BRAFV600E mutation is a transverse mutation resulting in a thymine to adenine substitution which localizes to the kinase domain on exons 11 and 15 of the gene. The BRAF V600E gene is situated on chromosome 7 and is very potent in activating the MAP pathway. The mutation targets the RAS-RAF-MEK-ERK cascade, which is the main pathway of regulation for growth of cells, proliferation and differentiation. The mutation activates the RAS family of GTPases, which in turn activate the RAF kinase family complex. This leads to hyper activation of the BRAF kinase with consequent enhanced downstream signalling. These further activate other relevant parts of the MEK/ERK cascade signalling to over 150 downstream targets. Following this, there is increased transcription of genes which are responsible for cell survival and turn over **(Figures 4 and 5)** [19].

Apart from acting on the MAP kinase pathway, the activated BRAF oncogene can act along with nuclear factor (NF)-□b, Tissue inhibitor of metalloproteinases (TIMP-1) and its cell surface receptor CD63. All these changes trigger molecular events responsible for tumorigenesis.

The activated BRAF V600E oncogene influences (NF)-□b activation through I□B-a phosphorylation. Activated (NF)-□b, triggers further oncogenic pathways by two mechanisms. It can translocate into the nucleus inducing the transcription of several known genes responsible for cell survival, proliferation and immune regulation. Or, after activation (NF)-□b results in

increased TIMP-1 expression. TIMP-1 then binds to its receptor CD63 on cell surface membrane and activates Akt signalling pathway, which is eventually responsible for its antiapoptotic activity [20].

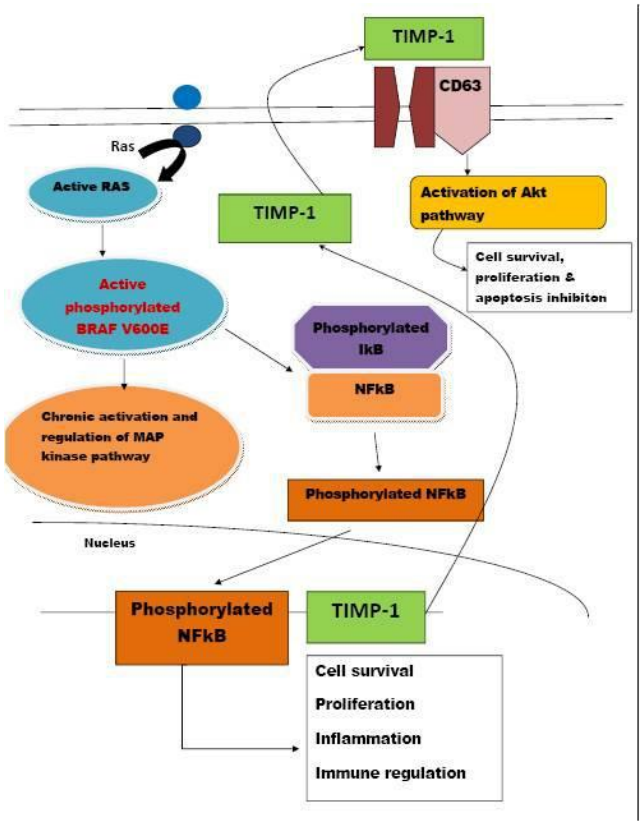



**Figure 5:** BRAFV600E, NFkB and TIMP-1 interaction.

The BRAF positive thyroid neoplasms follow a typical phenotype genotype correlation. They are more common in papillary carcinoma of thyroid and its variants. The BRAF mutation is exclusive for PTC and its variants. So screening for BRAF mutation can be used to differentiate PTC from other thyroid neoplasms.

BRAFV600E mutated papillary micro carcinomas have distinct morphologic characteristics. The neoplastic cells are plump and eosinophilic with classical nuclear features of papillary thyroid carcinoma. They have are unencapsulated lesions with an infiltrative growth pattern and surrounding desmoplasia. They are more prone for extrathyroidal extension [3].

The presence of BRAF mutation correlates directly with the aggressiveness of PTCs. These neoplasms which are positive for BRAF mutations are more prone for metastasis to lymph nodes, extension to extrathyroidal tissue, persistence of the tumour and recurrence. Eventually, most of these neoplasms have an advanced pathological (TNM) staging. Recurrent BRAF positive thyroid neoplasms are also more prone for refractoriness to radio therapy. Thus data available till today suggest that the presence of BRAF mutation in a papillary thyroid carcinoma indicates a poor prognosis [21].

Therefore screening for BRAF mutations has an indispensable value as a diagnostic and prognostication marker. The diagnostic applicability of BRAF mutations can be extra polated to its detection on fine needle aspiration cytological specimens as well as surgical specimens. So information obtained by screening for BRAF mutation can be used along with other clinicopathological factors in planning management of the patient and modalities of surveillance [19].

As molecular diagnosis is time consuming and expensive, there is always a search for other modalities for diagnosis. Immunohistochemical markers are such markers which can be used for diagnosis and prognosis. Two such markers for thyroid expression are HBME-1 and Galectin-3.

## HBME-1

The anti-HBME-1 antibody was first described by Battifora et al. in 1992. It is a mono clonal antibody and it recognizes an unknown antigen present in normal tracheal epithelium, microvilli of mesothelioma cells and adenocarcinoma of breast, lung and pancreas [5].

In general, more than 10% staining is considered positive. A positive reaction is indicated only by cells with distinct strong membranous staining [4]. The staining pattern of HBME-1 is evaluated as follows:

- Membranous staining; this occurs on the luminal side of the cytoplasmic membrane in a fringed or lined pattern.
- Cytoplasmic staining; Here the staining is seen in the cytoplasm of the follicular cell.
- Mixed staining; when both of the above staining patterns are present in a cell [22]. HBME-1 shows greater immunostaining with malignant lesions than benign lesions [22]. Many studies done in the past proves that HBME-1 shows preferential reactivity with malignant thyroid nodules and is a very useful immunomarker to assess malignancy [5].

The staining pattern of HBME-1 is predominantly membranous with variable cytoplasmic positivity. In some cases of papillary thyroid carcinomas, the staining is predominantly membranous or luminal. Other lesions show diffuse moderate to strong reactivity in the cytoplasm [23]. Membranous or luminal pattern of staining is predominantly observed in malignant lesions. Cytoplasmic pattern of staining is mainly seen in benign lesions [6].

Extensive studies on HBME-1 immunohistochemical staining have been done in thyroid neoplasms. These studies have shown that HBME-1 is the most specific marker for differentiating papillary thyroid carcinoma from other thyroid neoplasms [4,7]. The sensitivity and specificity of HBME-1 in detecting cases of papillary thyroid carcinoma are 80% and 96% respectively. Also HBME-1 has a very high positive predictive value of 96.7% in assessment of cases of papillary thyroid carcinoma [5].

This immunomarker is positive in classical and follicular variant of papillary thyroid carcinomas and also in follicular

carcinomas [6]. Therefore HBME-1 can also be used to distinguish follicular variant of papillary thyroid carcinoma from other benign follicular neoplasms. The diagnostic accuracy of HBME-1 with respect to the above fact is 86% [24].

In a study done by Papotti et al. HBME-1 was positive in 70% of cases of well differentiated tumours of undetermined malignant potential. (Lesions with unequivocal nuclear features of papillary thyroid carcinoma). The lesions showed strong and diffuse positivity for HBME-1 and some of the lesions showed >75% of positive immunostaining. These lesions positive for HBME-1 are classified as well differentiated tumours of undetermined malignant potential [7]. Hence, a detailed analysis should be sought in suspicious adenomas or dominant hyperplastic nodules showing HBME-1 reactivity. These lesions could be possible putative precursors to papillary thyroid carcinoma [7]. HBME-1 positivity in thyroid lesions indicates malignancy. But it does not necessarily indicate papillary differentiation of the lesion [23].

## GALECTIN-3

Galectin-3 is a member of the lectin family and is a beta-galactosidase-binding polypeptide. It is a 31-kDa protein which is constitutively expressed by several epithelial cells and immune cells [25]. It plays a role in differentiation of cells, cell growth, apoptosis, cell adhesion and interaction with cell matrix **(Figure 6)**. It also plays a significant role in mRNA splicing, neoplastic transformation, splicing of mRNA and in metastasis.

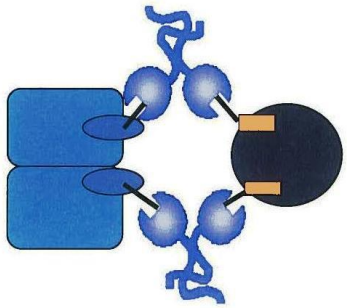


**Figure 6:** Structure of Galectin-3.

The expression of Galectin-3 is predominantly cytoplasmic. But variations such as membranous, extracellular and nuclear pattern can also be seen depending on the cell type. Cytoplasmic expression of Galectin-3 is the sign of a possible malignant transformation while nuclear localization is connected only with cell proliferation [22,26].

Galectin-3 expression in non-malignant lesions is absent or weak. The sensitivity of Galectin-3 in distinguishing benign and malignant thyroid nodules is 85.2%. In a study done by Prasad et al. Galectin-3 was found to be the most sensitive and accurate marker for distinguishing benignand malignant follicular lesions of thyroid [4]. Galectin-3 is immunohistochemically expressed at the highest levels in classical PTC with a papillary architecture, and shows strong and diffuse positivity [5].

Further Galectin-3 can also be a useful marker for assessment of lesions with questionable features of capsular and vascular invasion and nuclear features of papillary thyroid carcinoma. These lesions are termed as neoplasms of undetermined malignant potential. Galectin-3 immunoexpression has been studied in such lesions in which they show a positivity of 86%. Staining patterns of galectin-3 can help in classifying these lesions as WDT-UMP [4].

These lesions can be identified as morphological precursors to follicular variant of papillary thyroid carcinoma [7].

The combined sensitivity and specificity of HBME-1 and Galectin-3 in distinguishing benign from malignant thyroid nodules are 86% and 68.4% respectively. They have a combined positive predictive value of 0.600 and a combined negative predictive value of 0.899 [12].

Therefore we propose to study BRAFV600E mutations, HBME-1 and Galectin-3 expression in thyroid neoplasms and its correlation with both genotype and phenotype.

# Materials and Methods

33 cases of PTC were identified from our archives. The H&E slides were analysed for various morphologic features of PTC. Relevant paraffin blocks with high tumour density were selected for the study. For IHC Dextran polymer detection system was used. Antigen retrieval was done with pressure cooker using citrate buffer (pH 6.0). Peroxidase and power blocks were done.

After this primary antibody HBME-1 and Galectin-3 were applied. Then secondary antibody i.e. polymer horseradish peroxidase was applied. Colour development with working color development solution was done. The slides were then assessed for staining intensity. From the same tissue blocks used for HBME1 and Galectin-3 DNA was extracted and BRAF V600E mutational analysis was done using the following procedure.

## Tissue preparation from Formalin Fixed Paraffin Embedded (FFPE) blocks

Using a pen, the area of the tissue containing the maximum tumour was marked on hematoxylin-eosin stained slide. 10-15 µm sections were cut from formalin fixed paraffin embedded tissues and placed over the plain glass slide. The sections were deparaffinised by immersion in xylene, followed by hydrated in graded alcohol The H&E stained slide with the marked tumour area was kept over the unstained section slide. Using the circled area of interest on the unstained tissue section slide as a guide, a clean scalpel blade was used to scrape the tissue in the area containing tumour tissue. The tissue was placed in a 2 ml of Eppendorf tube.

## Isolation of DNA from FFPE

The tissue was incubated at 50°C until it gets dried up completely. To the dried tissue 500 µL lysis buffer and 35 µL Proteinase K (20 mg/mL) were added, mixed well and the solution was incubated at 60°C for 2-3 hours until the tissue gets dissolves. The temperature was raised to 95°C and the tubes were incubated for 8 min to inactivate Proteinase K. Contaminating RNA were removed by addition of 15 µL RNase (10 mg/mL) and incubating at 37°C for 15 minutes. One ml of phenol chloroform mix (1:1 ratio) was added to each tube vortex and centrifuged at 13,000 RPM for 5 minutes. The supernatant was transferred to fresh tube to which equal volume of chloroform was added, vortex and centrifuged at 13,000 RPM for 5 minutes. The supernatant was transferred to fresh tube DNA was precipitated by adding 1 mL of 100% ethanol and 50 µL of 30M sodium acetate. Further the solution was centrifuged at 13,000 RPM for 5 minutes to pellet down DNA. To the pellet one ml of 75% ethanol was added and centrifuged at 13,000 RPM for 5 minutes. The pellet was dried and suspended with 35 µL of TE buffer.

## PCR for amplification of exon 15 of BRAF gene

PCR was carried for amplification of 224 bp fragment of the exon 15 of BRAF gene using DNA isolated from FFPE tissues as template and BRAF 15F and 15R primers. PCR reactions were performed in 20 µl of 1.5 mM MgCl2 with 200 µM deoxynucleoside triphosphates, 50-100 ng of DNA, 0.5 µM of each primer and 2.5 U Taq polymerase. PCR was carried out for forty cycles with denaturing at 95°C, annealing at 59°C and extension at 72°C. PCR amplification was confirmed on 2% Agarose gel with expected PCR product size around 224 bp **(Figure 7).**

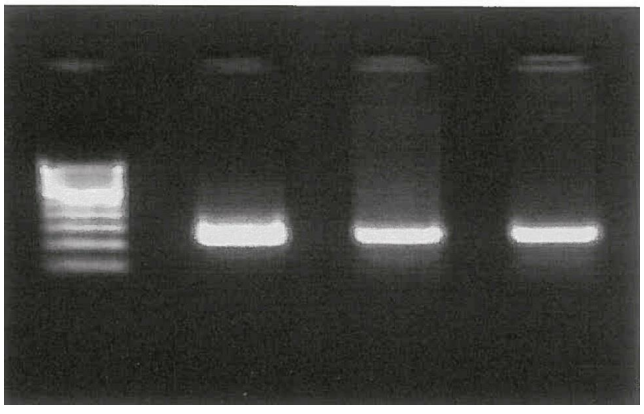


**Figure 7:** Agarose gel electrophoresis: PCR amplification. Image of gel electrophoresis done on polymerase chain reaction amplified products, by using 15F and 15R primers. The polymerase chain reaction product size is 224 bp.

## Clean up of PCR product

PCR products were cleaned up using HIPURA-HIMEDIA PCR clean up kit (Himedia, India). The procedure was followed as per manufacturer instructions.

1.Take PCR product in 1.5 mL tube.

2.Add 4-5 Volume of SPB binding solution (PCR binding solution).

3.Mix thoroughly by gentle pipetting, spin the tube.

4.Load lysate in Miniprep spin column. Centrifuge at 10,000 X g for a minute at RT. Discard flow through.

5.Wash with 700 µL of diluted wash solution (HPE) and centrifuge at 10,000 X g for a minute at RT. Discard flow through.

6.Add 500 µL of diluted wash solution (HPE) and centrifuge at 10,000 X g for a minute at RT. Discard flow through.

7.Centrifuge empty tube for 2 min at 13000 X g to dry column matrix

8.Transfer column to new 2 mL tube, add 30–50 µL of elution buffer

9.Centrifuge 1 min at 13000 X g to elute DNA

10.Quantify the DNA using NANODROP.

## Restriction Fragment Length Polymorphism (RFLP)

For genotyping, RFLP was carried out using TspRI enzyme (Thermo Fisher, USA). This enzyme cuts the wild type PCR product to gives two band of size 125 bp and 87 bp respectively.

Approximately 0.3 µg of PCR product was added to 30 µl reaction mix containing 2 µl of 10x Fast digest green buffer and 1 µl of Fast digest enzyme. The reaction mix with PCR product was incubated at 65C for 1 1/2 hours and the product was resolved using 12% Polyacrylamide gel Electrophoresis (PAGE). PAGE was performed using Amersham electrophoresis system at 100 V for about 5 hours and the gel was stained with ethdium bromide. The stained gel was viewed under UV and documented using gel documentation system. The wild type homozygous alleles showed two bands around 125 bp and 87 bp whereas mutant alleles showed one band around 212 bp. Heterozygous was distinguished from wild type and mutant homozygous by the presence of three bands (212 bp, 125 bp and 87 bp) **(Figure 8)**.

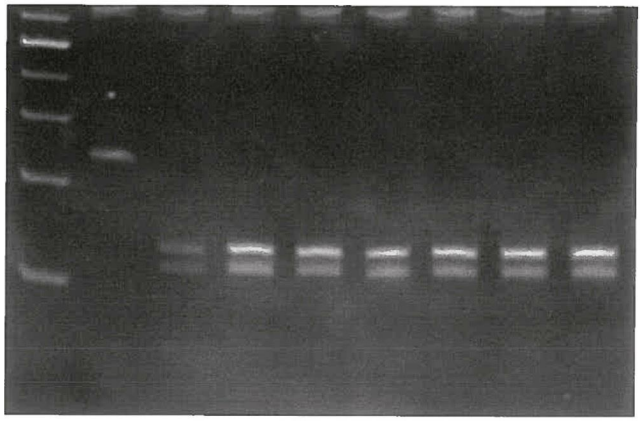


**Figure 8:** Polyacrylamide gel electrophoresis: Restriction enzyme digestion. Control: Well no-1 224 base pairs. Wild type: Well no-2,3,4,5,6,7,8 (120 bp+104 bp)

## Results

In our study we found a 85% expression of HBME-1(in papillary thyroid carcinomas (28 out of 33 cases) This correlates with the other studies where 90% of papillary thyroid carcinoma were immunoreactive for HBME-1 **(Figures 9 and 10)** [7]. In our study Galectin-3 showed expression in 75.75% (25 out of 33 cases) of papillary thyroid carcinoma **(Table 1).**

**Table 1:** HBME-1 and Galectin-3 expression in papillary thyroid carcinoma.

| | Positive | Negative |
|---|---|---|
| HBME-1 | 28/33 cases (85%) | 5/33 cases |
| Galectin-3 | 25/33 cases (75.75%) | 8/33 cases |

BRAFV600E is the most common genetic alteration in thyroid neoplasms especially papillary thyroid carcinoma. Our study revealed that papillary carcinomas of thyroid are associated with increasing incidence, female predominance, younger age at presentation and as an incidental finding among thyroidectomy specimens. Cost factors play an important role in a country like India. So a surrogate immunohistochemical marker for mutational screening will be of immense value.

BRAFV600E mutation is more specific for papillary thyroid carcinoma. In our study we had 33 cases of papillary thyroid carcinoma of which DNA was extracted for 31 cases. BRAFV600E was positive in 9 cases giving an incidence of 27% **(Table 2)**. HBME-1 and Galectin-3 on all these cases (9/9 cases) showed high positivity. This gives an incidence of 100% expression of HBME-1 and Galectin-3 in BRAFV600E mutations **(Table 3)**. The overall expression of HBME-1 in papillary thyroid carcinoma was 28 out of 33 cases and Galectin-3 was 25 out of 33 cases **(Table 1)**.

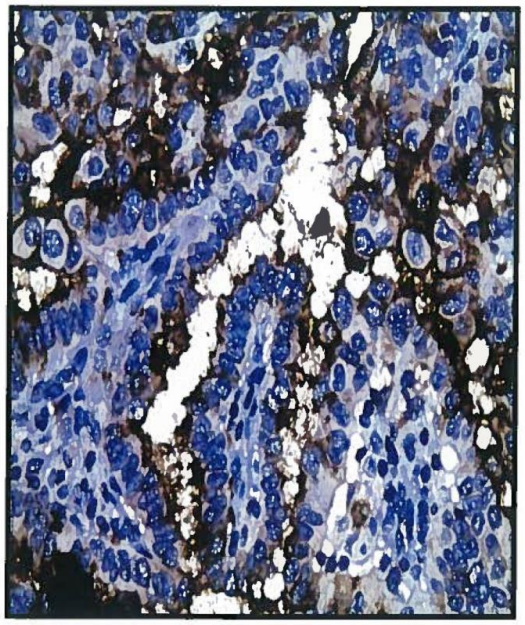


**Figure 9:** Papillary thyroid carcinoma: The cells show strong immunostaining for HBME-1. IHC (x400).

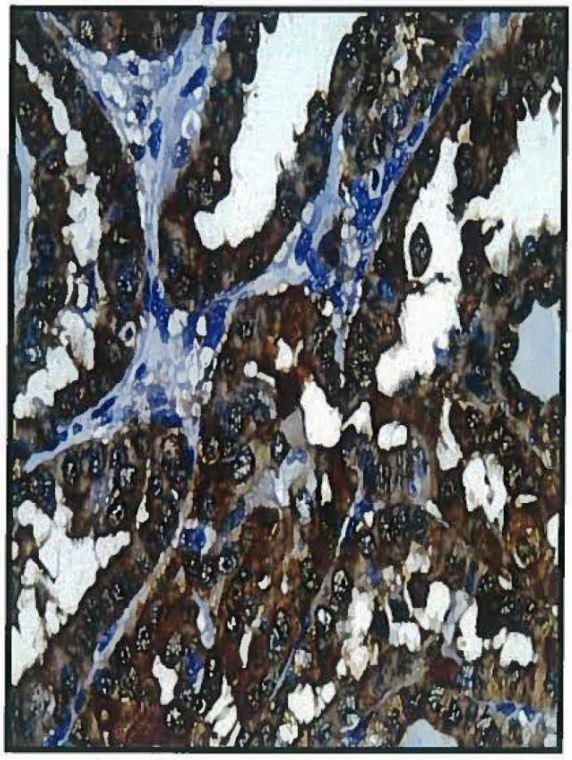


**Figure 10:** Papillary thyroid carcinoma: The cells show strong immunostaining for Galectin-3. IHC (x400).

**Table 2:** Number of BRAF V600E positive thyroid neoplasms.

| Neoplasm | Number of cases |
|---|---|
| Papillary thyroid carcinoma | 9/33 cases(27%) |

**Table 3:** HBME-1 and Galectin-3 immunohistochemical staining among BRAF positive papillary thyroid neoplasms.

| BRAF positive papillary neoplasms N=9 | Positive | Negative |
|---|---|---|
| HBME-1 | 9 (100%) | - |
| Galectin-3 | 9 (100%) | - |

From this study we infer that HBME-1 and Galectin-3 were highly specific for papillary thyroid carcinoma. These markers had no specific correlation with stage, age and sex of the patient. It was observed that HBME-1 and Galectin-3 were 100% positive in all BRAF positive papillary thyroid carcinomas. But a significant number of cases (19 out of 28 cases) were positive for HBME-1 and Galectin-3 where BRAFV600E mutations were negative. So, we can conclude that, HBME-1 and Galectin-3 were expressed in most of the cases of papillary thyroid carcinoma, irrespective of mutational status. So, a larger study is essential among papillary carcinomas of thyroid exclusively with mutational studies and immunomarkers to prove the utility of HBME-1 and Galectin-3 to be used as surrogate marker for the mutation. Therefore from this study we infer that HBME-1 and Galectin-3 are more specific markers for papillary carcinoma of thyroid. When the pathologist is challenged with indeterminate lesions of thyroid neoplasms, these markers can be used to identify cases of papillary carcinoma of thyroid among these lesions. As the study population is small a larger study will prove the usefulness of these.

## Conclusion

The combined use of HBME-1, Galectin-3 along with BRAFV600E mutation study can improve the diagnostic accuracy of potentially aggressive,equivocal cases of PTC. A similar study with larger sample size can confirm their utility as surrogate markers for BRAFV600E mutations.

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