“DIAGNOSTIC VALUE OF CD-10 MARKER IN DIFFERENTIATION OF MALIGNANT THYROID CARCINOMA FROM BENIGN THYROID LESION”

By

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Dissertation submitted to

SBKS MEDICAL INSTITUTE & RESEARCH CENTRE

SUMANDEEP VIDYAPEETH, PIPARIA, VADODARA

In partial fulfillment

Of the requirements for the degree of

M.D.

in

PATHOLOGY

Under the Guidance of

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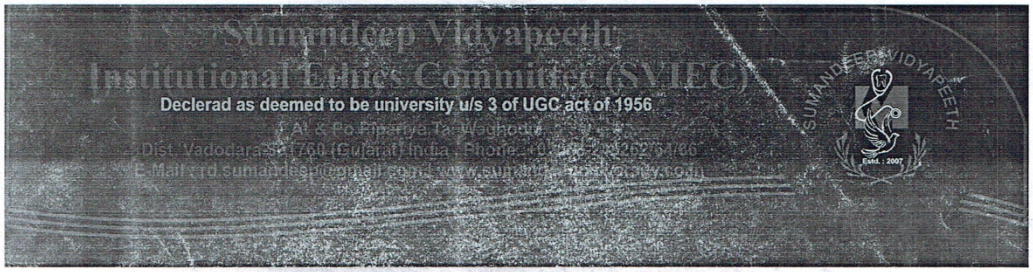
SBKS MEDICAL INSTITUTE & RESEARCH CENTRE,

PIPARIA, VADODARA

YEAR 2015-2018



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Dear Dr. Monil,

The Sumandeep Vidyapeeth Institutional Ethics Committee (SV IEC) is in receipt of your above mentioned study document and as the research study classifies in the minimal risk category; as recommended by HRRP SBKS MI&RC. The SV IEC approves your study to be conducted in the presented form.

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Dr. Niraj Pandit
Member Secretary
SV Institutional Ethics committee

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I hereby declare that this dissertation entitled **"DIAGNOSTIC VALUE OF CD-10 MARKER IN DIFFERENTIATION OF MALIGNANT THYROID CARCINOMA FROM BENIGN THYROID LESION"** is a bonafide and genuine research work carried out by me under the guidance of **DR. R.K. PASALE, Professor & HOD, Department of Pathology, SBKS Medical Institute & Research Centre, Piparia, Vadodara.**

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ACKNOWLEDGEMENT

The writing of this dissertation has been one of the most significant academic challenges I have ever faced. It gave me great pleasure in preparing this dissertation and I take this opportunity to thank everyone who has made this possible.

First and foremost, I bow down to **"The God"** for giving me energy, inspiration, courage to complete this task and for blessing me so abundantly, far beyond what I deserve.

I would like to thank my parents **Dr. Prakash Bhavsar** and **Dr. Chhaya Bhavsar**, to whom I owe all my success and motivation and for giving birth to me at the first place and unconditionally supporting me in every way throughout my life. Also I would like to thank my wife **Mrs. Roshni Bhavsar** for her endless encouragement, love and care.

I am just wordless to express my deep sense of gratitude towards my P. G. Guide, **Dr. R. K. Pasale,** Professor & head of Pathology, S.B.K.S. M.I. & R.C. PIPARIA whose sagacious suggestions, immense interest in subject, keen evaluation and constructive criticism have promoted completeness to this work. His patience tutelage, objective critique and inspiring support at all times have made me able enough to bring this dissertation to its present status. He has always respected my individual thoughts and ideas too. His guidance helped me in all the time of research and writing of this dissertation. I could not have imagined having a better advisor and mentor for my M.D. study. There is much to thank you for, if only the words could suffice. But deep

inside I know I can never repay you for the care you took to nurture me. **Sir, "I am really grateful to you for being there."**

I express my sincere thanks to Hon. **Dr. Mansukh Shah**, President, Sumandeep Vidyapeeth, **Dr. Dixit Shah**, Executive Trustee, Sumandeep Vidyapeeth, **Dr. G. D. Mehta**, Hon. Chancellor and **Dr. J. R. Patel**, Vice chancellor, Sumandeep vidyapeeth for providing all the necessary facilities.

I am thankful to **Dr. G. V. Shah**, Dean, S.B.K.S. M.I. & R.C., Piparia, for providing facility at the institute to do this dissertation work.

I am indebted to all my professors **Late Dr. Y. R. Premalatha**, **Dr. R.K. Tandon**, **Dr. S. S. Goswami**, **Dr. Jasmin Jasani**, **Dr. S. P. Pandya**. They were always ready to guide and solve queries with their critical suggestions and enormous knowledge whenever I was in problem. **Dr. Rippal Bhimani,** Asst. Prof. of Pathology, **Dr. Shashikant Mavadia**, Asso. Prof. of Pathology, **Dr. Kuntal Patel**, Asst. Prof. of Pathology, **Dr. Jigna Patel**, Asst. Prof. of Pathology and **Dr. Ashu Dogra**, Asst. Prof. of Pathology for their guidance, constant encouragement, unselfish cooperation and support throughout my post graduate study.

I express my sincere thanks to the Member Secretary of Institutional Ethics Committee (Human) of Sumandeep Vidyapeeth for permission to carry out and providing facilities for the present study.

I am lucky to have colleagues **Dr. Aviral Chandra**, **Dr. Mansi Balar** and **Dr. Nikita Bansal**. Thank you for helping me throughout my post graduate study and helping out when I was down. I thank to **Dr. Nisarg Champaneria, Dr. Shreya Shah**, **Dr. Prashant Kumar**, **Dr. Bhavya Saxena**, **Dr. Mihir Sharma, Dr. Nirmala Patel** and

Dr Tulsi Zariwala for the stimulating discussions, for the sleepless nights we were working together, and for all the fun we have had in the last three years.

I am also thankful to my seniors **Dr. Mobeen**, **Dr. Kalidash**, **Dr. Mohit**, **Dr. Denis, Dr. Priyanka, Dr. Annie** and my enthusiastic juniors **Dr. Shilpan**, **Dr. Jay**, **Dr. Payal**, **Dr. Vaibhavi** and **Dr. Bhavik**.

I cannot forget to thank the technical staff **(Patel kaka, Mr. Vinod, Mrs. Jyoti, Mr. Navneet, Yogesh and Mr. Heera)** of the pathology department, for their help and support and also **Mr. Kanubhai Parmar** C/O Vishal Graphics for editing and manuscripting of my thesis. Thank you, one and all.

Dr. MONIL BHAVSAR

ABSTRACT

Introduction: Thyroid cancer is considered as the most common endocrine malignancy. PTC (Papillary Thyroid Carcinoma) is the most common form of malignant thyroid tumor. This study was aimed to study the expression and sensitivity of immunohistochemical marker CD 10 for papillary thyroid carcinoma and benign thyroid lesion.

Methods: The present study was carried out in the Department of Pathology, Dhiraj General Hospital & Srimati Bhikhiben Kanjibhai Shah Medical Institute & Research Centre, Sumandeep Vidyapeeth, Piparia. A total of 30 cases of thyroid lesion in which 10 are papillary thyroid carcinoma and 20 are benign follicular adenoma were evaluated after its histological diagnosis with immunohistochemical marker CD 10.

Results: From selected 30 cases studied in two groups of PTC (n = 10) and follicular adenoma (n = 20), CD10 was immunohistochemically expressed in 20% of PTC cases, but in none of the follicular adenoma (0%). There was not significant relationship between expression of CD10 with the type of thyroid lesion, age and sex of the studied population ($P > 0.05$).

Conclusions: The result of present study concluded that, the overall sensitivity of CD 10 marker for detecting PTC is too less to recommend its use in daily practice.

To find out the diagnostic usefulness of CD 10, further studies including larger sample size with adequate number of controls (adenomas) will have to be done.

Keywords: CD10, papillary thyroid carcinoma, follicular adenoma, immunohistochemistry

TABLE OF CONTENT

LIST OF TABLES

LIST OF GRAPHS

LIST OF FIGURES

INTRODUCTION

Thyroid cancer is considered as the most common endocrine malignancy. Its variants includes papillary thyroid carcinoma (PTC) (80%), follicular carcinoma (15%), medullary carcinoma, poorly differentiated carcinoma (<1%) and anaplastic carcinoma (<2%).[1] Thyroid carcinoma constitutes for 1% of all cancers.[2] It is estimated that the incidence rate of thyroid carcinoma world-wide ranges from 0.5-10 cases per 1,00,000.[3]

Most of the thyroid carcinomas (95%) arise from follicular cells while small portion (5%) arise from para-follicular C cells. Classification of thyroid carcinoma is based on growth pattern and histological grading.[4]

According to A.Khan *et al*.,in "Pathology of thyroid gland" Papillary thyroid carcinoma (PTC) is the most common type of malignant thyroid tumor constituting more than 70% of thyroid malignancies.[5]

According to R.A.DeLellis in "Pathology and Genetics of tumour of Endocrine organs" radiation is a genotoxic factor for PTC. The widespread use of X-irradiation in the 1950s for treating conditions such as enlarged thymuses and acne have contributed to the increased incidence of PTC[6]

Patients with PTC usually present with a cold nodule on radioactive iodine scan. A few patients may present with cervical lymphadenopathy. Delphian lymph node involvement is a bad prognostic sign in PTC.[5,6]

A follicular adenoma is a benign encapsulated tumor of the thyroid gland. It is a, homogeneous, firm or rubbery, round or oval tumor. It is surrounded by a thin fibrous

capsule. A follicular adenoma is a common tumor of the thyroid gland. Most patients with a follicular adenoma are clinically and biochemically euthyroid. Approximately1% of follicular adenomas are "toxic adenomas," which are a cause of symptomatic hyperthyroidism.[7]

Follicular pattern in adenomatous nodules and disruption of capsule in adenomas create difficulties in diagnosing thyroid lesions. To overcome the problem, different immunohistochemistry (IHC), cytological, and molecular studies have been developed. IHC markers can aid the better assessment of morphological details of thyroid lesions. Papillary carcinomas are more prone to diagnostic discrepancies among experienced pathologists.[8]

IHC may provide additional support in evaluation of diagnosis of thyroid discrepancies lesions.[9] Significant IHC markers in differentiated thyroid cancer are being used and the most discriminatory markers are Galectin-3, CK-19,CD-10, Aurora-A, p16, AR, HBME-1[10]

CD10 is a 90-110 kda cell matrix metalloproteinase which is a membrane-bound zinc dependent endopeptidase. It is also called as "common acute lymphoblastic leukemia antigen" (CALLA)[11], "neutral metalloendopeptidase" in kidney and "enkephalinase" in brain[12]. Matrix metalloproteinases are metallopeptidases which cleaves extracellular matrix proteins and play an important role in tissue remodeling.[13] It is one of the studied markers which originally was used for diagnosis and classification of malignant lymphomas and leukemia's.[14]

The diagnostic utility of CD10 in different non-hematopoietic lesions including breast, uterus and liver has been reported. In a study "CD10 as a Prognostic Stromal Marker in Breast Carcinoma" by Dr.C.Arun prabhakaran highlights the role of

stromal CD10 expression in predicting tumor response and prognosis and therefore cd10 could be included as a routine marker before giving chemotherapy and concluded that there is wide expression of CD10 in desmoplastic stroma of breast carcinoma and negative immunoreactivity in stromal cells of normal breast[15]. In another study "Role of CD 10 immunochemistry in differentiating hepatocellular carcinoma (HCC) from metastatic carcinoma of liver" by Ahuja *et al* seventeen (68%) of twenty-five cases of HCC were positive for CD10 and Of 25 cases of metastatic carcinoma, four (16%) were positive for CD10 and thus he concluded that CD10 immunostaining is useful in discriminating HCC and metastatic carcinoma of the liver.[16]

The aim of this study was to determine its expression in malignant and benign thyroid lesions.

Use of CD10 in accordance with clinical and histological features of thyroid lesions could be used as both diagnostic and prognostic tool which consequently influence the management and their prognosis for survival of patients with thyroid neoplasm specially PTC[17]

AIMS AND OBJECTIVES

1. To find out the extent to which CD10 is a useful marker in differentiating benign lesions from malignant ones.

2. To study expression of CD10 in benign and malignant thyroid lesions.

3. To analyze the sensitivity of CD10 in benign and malignant thyroid lesions.

REVIEW OF LITERATURE

In the endocrine system, thyroid carcinoma is the most common malignancy. It is now the sixth most common cancer in women and the second most common cancer in women under 40 years of age[18]. The diagnosis of thyroid nodules is made by neck examination and ultrasonography in over 50% of the patient population over the age of 50. However, only 5% of these patients will be diagnosed with cancer. For diagnosing thyroid nodules, fine needle aspiration biopsy is the gold standard, though 10–15% of these biopsies are questionable, which eventually require a diagnostic thyroid lobectomy. Therefore, research in thyroid biomarkers has become an area of active interest. Biomarkers have become useful for detecting thyroid cancer early, and also for detecting recurrent and persistent disease and for predicting the efficiency of surgical removal, radioiodine ablation, and chemotherapy[19].

Liu in his study done in 2014 mentioned that there is no single marker sensitive enough to provide a definitive malignant diagnosis. Therefore, different panels of combined immunomarkers were proposed. The combination of HBME-1, GAL-3, and CK19 was the most common panel evaluated and their diffuse expression has not been reported in benign lesions.

In a study "Application of Immunohistochemistry in Thyroid Pathology" by Haiyan Liu *et al* a distinct membranous staining pattern for trophoblastic cell surface antigen 2 (TROP2), a 35-kDa type 1 transmembranous glycoprotein in PTCs; follicular neoplasms were nonreactive or expressed rare focal, weak cytoplasmic staining. It was concluded that TROP2 is a potential novel immunomarker for the identification of PTC that can be used in a panel to increase accuracy of diagnosis when encountering a difficult lesion derived from follicular cell[20]

In another study by K.T.Mai *et al* "Immunohistochemical study of papillary thyroid carcinoma and possible papillary thyroid carcinoma-related benign thyroid nodules" a combination of immunostaining with HBME, CK, and CD57 (or CD15) is a sensitive and specific test for PTC. To rule out the thyroid nodules having a diagnostic problem with PTC, this panel can be used [21]

The usefulness of CD10 marker in discrimination of different benign and malignant thyroid lesions demonstrated in a report by "CD 10 expression is useful in the diagnosis of follicular carcinoma and follicular variant of papillary thyroid carcinoma" by Tomoda *et al* that CD10 was not detected in normal thyroid tissue, benign lesions and pure papillary carcinomas except for follicular variants. While, CD10 was expressed in 8 of 10 follicular carcinomas (80%) and 7 of 9 follicular variant of papillary thyroid carcinomas (77%) [22]

In a study "Can CD 10 be used as a diagnostic marker in thyroid pathology" by G.Yegen *et al* CD10 was negative in adenomatous nodules, minimally invasive follicular carcinoma, and well-differentiated carcinoma and normal thyroid tissue. It was expressed in nine of 14 (64.2%) conventional papillary carcinomas, four of 24 (16.6%) follicular variant of papillary carcinomas, three of six (50%) papillary microcarcinomas, one of nine (11.1%) widely invasive follicular carcinomas, and three of ten (30%) follicular adenomas[23]

In a recent study "Diagnostic value of CD-10 marker in differentiating of papillary thyroid carcinoma from benign thyroid lesions" done in 2014 by M.Mokhtari *et al* revealed that out of 134 cases of which 67 were PTC and 67 were benign thyroid lesions, CD 10 were immunohistochemically positive in 20 cases (29%) of PTC and none of the benign lesions (0%)[17]

Chu and Arber studied "Paraffin-section detection of CD10 in 505 nonhematopoietic neoplasms. They reported frequent expression in renal cell carcinoma (41 out of 46), transitional cell carcinoma (13 out of 24), prostatic adenocarcinomas (11 out of 18), rhabdomyosarcomas (3 out of 5), pancreatic adenocarcinomas (7 out of 14), schwannoma (5 out of 11), malignant melanoma (12 out of 30) and endometrial stromal sarcomas (5 out of 5)". They got negative expression of CD 10 in 408 non-hematopoietic neoplasms including 55 thyroid tumours[24]

Anatomy of thyroid gland:

The thyroid gland develops from the floor of primitive pharynx during the third week of gestation. The developing gland migrates along the thyroglossal duct to reach its final location in the gland. Normal adult thyroid gland weighs approximately 12-20 g.[25]

The normal adult thyroid gland is composed of two lobes joined by the isthmus, which lies across the trachea anteriorly, below the level of the cricoid cartilage.

Microscopically, the thyroid gland is made up of round or oval follicles. They are lined by a single layer of follicular cells whose shape ranges from flattened to low columnar depending on their degree of activity. Follicular cells with abundant granular acidophilic cytoplasm are referred to as Hürthle cells (a misnomer), oxyphilic cells or oncocytes. They can be detected immunohistochemically with antibodies directed against mitochondrial enzymes.

The blood supply of thyroid is from superior and inferior thyroid artery. Superior, middle and inferior thyroid veins drain into internal jugular vein and brachiocephalic vein.

Lymphatic drainage is multi-directional and drains into pericapsular, internal jugular chain, pretracheal, paratracheal, prelaryngeal, recurrent laryngeal nerve chain, retropharyngeal and retroesophageal nodes.[26]

Classification of thyroid tumors: [26, 27]

I. Adenomas

A. Follicular

B. Papillary

C. Teratoma

II. **Malignant Tumors**

A. Differentiated

1. Papillary carcinoma

2. Follicular carcinoma

B. Medullary carcinoma

C. Undifferentiated

1. Giant cell

2. Carcinosarcoma

Papillary Thyroid Carcinoma:

Incidence:

The National Cancer Institute indicates that "thyroid carcinoma is the most ordinary type of endocrine related carcinoma and in 2016, it estimated 64,330 new cases"[28]

The incidence of thyroid cancer has nearly tripled since the late 20th century, growing from 4.9 cases per 1,00,000 in 1975 to 14.3 cases per 100,000 in 2009 (2.9-fold increase). The whole increase has been due to a rise in the incidence of papillary thyroid cancer, from 3.4 to 12.5 cases per 1,00,000 (3.6-fold increase).[29]

According to American Thyroid Association, papillary thyroid carcinoma is the most common type of thyroid cancer which accounts for 70-80% of all thyroid cancer. It is commonly diagnosed between the age of 30 and 60. Females are affected 3 times more than males.[30]

The American Cancer Society mentioned in its most recent estimates that the new cases of thyroid cancer in the United States for 2017 are about 56,870 (42,470 in women, and 14,400 in men) and about 2,010 deaths from thyroid cancer (1,090 women and 920 men).[31]

The Chernobyl nuclear accident in 1986 led to a noticeable increase in the incidence of PTC in Belarus and other areas of the former Soviet Union close to the accident. Children were most commonly affected.[32]

Histogenesis:

Thyroid neoplasm originates from follicular epithelial cells and parafollicular cells (C cells). Papillary carcinoma originates from follicular epithelial cells and it is a well differentiated tumor.[33]

Etiology: [26,34]

The etiology of PTC is yet not discovered, but a number of associations have been made as given below.

a. Moleclular:

Multiple genetic and epigenetic mutations cause activation of signaling pathways responsible thyroid cancer. *BRAF* and *RAS* genes and chromosomal rearrangements of *RET*/*PTC* and *PAX8*/*PPARG* are common point mutations in PTC. A 10-fold increased risk of thyroid cancer in relatives of patients with thyroid cancer suggests a genetic basis for vulnerability of these tumors. A association between papillary thyroid carcinoma and human leukocyte antigen *(HLA)-DR7* has also been observed. A similar occurrence has been described for this tumor in monozygotic twins.

b. External radiation

External radiation to the neck during childhood increases the risk of papillary carcinoma. The latency period between exposure and diagnosis of thyroid carcinoma is minumum 5 years.

c. Iodine excess

In Vienna and Austria, during a period when iodide intake was low in the population, instead of the expected 80% of all thyroid cancers, PTC constituted only 25% .The addition of iodine to the diet in endemic goiter areas in Europe has increased the proportion of papillary carcinomas relative to follicular thyroid cancer. "A correlation exists between iodine deficiency and *RET*/*PTC* chromosomal rearrangements" was stated by a study of post-Chernobyl thyroid cancer.

Clinical Features:

Eric J Lentsch, MD in 2016 published an article in which he said that the most common feature of thyroid carcinoma is nontender palpable nodule in the midline of neck. Papillary carcinoma may also present as a nodule with enlarged cervical lymph nodes or cervical lymphadenopathy in the absence of a palpable thyroid nodule.[34]

Most carcinomas present with single nodule that moves with the thyroid gland while swallowing. Dysphagia, hoarseness of voice, cough or dyspnea may occur in advanced disease.[35]

Gross:

Most papillary carcinomas are solitary or multifocal.[35] Lesions may be solid, whitish, firm and clearly invasive. Less than 10% are surrounded by complete capsule.[26] Lesional calcification is a common feature.[36] The lesions may contain areas of fibrosis and are often cystic.[35]

Microscopy:

Papillary carcinoma contains abundant papillae which are branching, complex and randomly oriented with a central fibrovascular core and a single or stratified lining of cuboidal cells. The stroma of the papillae may be edematous or hyaline. It may contain lymphocytes, foamy macrophages, hemosiderin, or adipose tissue.[26]

The nuclei of papillary thyroid carcinoma have been described as ground glass (optically clear), empty or Orphan Annie eyed. These nuclei contain hypodense chromatin and they are larger and more oval compared to normal follicular nuclei.[36]

Nuclear grooves are usually arranged along the longest nuclear axis and represent like the pseudoinclusions. Mitoses are very scanty or absent in papillary carcinoma.[26]

Psammoma bodies (calcified structures) are often present within the cores of the papillae. Their presence in a thyroid gland strongly suggests the diagnosis of papillary carcinoma.[26, 35]

Regional lymph node metastases are very common at initial presentation of papillary thyroid cancer. The presence of psammoma bodies in a cervical lymph node is an evidence of a papillary carcinoma in the thyroid.[36]

Variants of Papillary Thyroid Carcinoma: [26,37]

1. Papillary microcarcinoma

This tumor measures 1 cm or less in diameter. It is more common in males than females. Occasionally patients may present with associated cervical lymph node metastases. Non encapsulated tumors show extensive sclerosis and are more aggressive.

2. Follicular carcinoma

Follicular carcinoma is composed of follicles entirely. There is invasive pattern of growth, psammoma bodies, fibrous trabeculation and darker or hypereosinophilic colloid showing scalloping “bubble gum” appearance.

3. Encapsulated variant

It is completely surrounded by a capsule. These lesions are ‘hot’ on thyroid scan and are characterized by pale, vacuolated colloid. Follicular cells are low columnar with basally located normochromatic or hyperchromatic nuclei.

4. Tall cell variant

This variant of carcinoma is characterized by papillae lined by single layer of tall cells (the height being at least 3times the width) and abundant eosinophilic cytoplasm. Nuclear pseudoinclusions are more prominent. There may be an extensive lymphocytic infiltration of the stroma

5. Oncocytic (oxiphilic) variant

On gross examination, these tumors have a distinct brown color and encapsulated or invasive. Nuclear features of this variant are those of papillary carcinoma with ample cytoplasm. Cytoplasm has a granular oxyphilic quality. There is heavy lymphocytic infiltrate in the stroma of the papillae.

6. Columnar cell variant

There are pseudo stratified columnar cells in this variant. Some of which may have supranucleur and subnuclear cytoplasmic vacuoles. Mitotic figures can be found.

7. Diffuse sclerosing variant

These tumors are more commonly seen in patients with age between 15 and 30 years. There is diffuse involvement of one or both lobes of thyroid in this variant. These tumors show extensive squamous metaplasia, prominent lymphocytic infiltration, abundant psamomma bodies and stromal fibrosis. Patients with this variant usually have nodal metastases. Lung metastases are common.

8. Cribriform-morular variant

These tumors are characterized by the presence of a cribriform pattern of growth and morular formations. This rare variant is usually associated with Gardner Syndrome and familial adenomatous polyposis. The tumors usually involve both thyroid lobes with focal papillary architecture and the nuclei focally show clearing and grooves.

9. Solid cell variant

In this variant, sheets of tumor cells show features of typical PTC. Vascular invasion and extrathyroidal extension are usually present. These tumors are more common in children with a history of radiation exposure.

10. Clear cell variant

These tumors have a papillary architecture and cytological features of papillary thyroid carcinoma and comprised of mainly clear cells. Some tumors may have oncocytic and clear cell features.

11. Macrofollicular

It contains macrofollicles and can be easily confused with a hyperplastic or colloid nodules. The cytological features of papillary thyroid carcinoma are seen at higher magnification.

12. Papillary thyroid carcinoma with fasciitis like stroma

There is stromal reaction of the tumor which may obscure the neoplastic epithelial component. It may be misinterpreted as fibromatosis or nodular fasciitis.

Follicular Adenoma:

The term "follicular" is often used to either designate thyroid parenchymal cells which produce the growth pattern of a thyroid lesion that is follicle forming. Follicular lesions are further subclassified into microfollicular, macrofollicular, based on the size of the follicles and totally or partially encapsulated or unencapsulated, based on the type of encapsulation.[38, 39,40] Thyroid nodules are very ordinary. It is predictable that in the general population they are found in 4-7% by palpation and in 50-67% by ultrasonography or at autopsy[41]

Histogenesis:

Follicular adenoma of the thyroid gland is the tumor of follicular cell differentiation that consists of macro or microfollicular pattern with follicles lined by cuboidal epithelial cells. It is a firm or rubbery, homogeneous, round or oval tumor that is encircled by a thin fibrous capsule.[7]

Etiology:

Functioning follicular adenomas occur as a consequence of a monoclonal expansion of thyroid follicular cells with a high frequency of activating mutations in the gene for the TSH receptor and less frequently in the adenylate cyclase-stimulating G alpha protein gene that causes increased thyroid hormone secretion independent of TSH.[7,42] N-RAS and K-RAS mutations may be present in patients with follicular adenoma and play a role in the evolution of follicular adenoma to follicular carcinoma.[7,43] Follicular Aenomas show genetic aberrations similar to Follicular Carcinoma. Follicular Adenomas show chromosomal loss at 3p25 most commonly and PAX8-PPARG rearrangement .[38,44-48]

Clinical Features:

Most patients with a follicular adenoma of the thyroid gland present with a solitary, spherical, encapsulated thyroid nodule. Clinically they are asymptomatic. Patients may present with coughing, dyspnea, hoarseness, or dysphagia due to compression of the trachea, esophagus or recurrent laryngeal nerve. They may complain of neck pain as a result of sudden tumor enlargement from intratumoral hemorrhage or cystic degeneration.[7]

Gross:

Follicular adenomas are average about 3 cm in diameter. Some lesions are also ≥10cm in diameter. Depending on the cellularity and colloid contetnts, they may appear gray-white to red-brown. Areas of hemorrhage, fibrosis, calcification and cystic changes, bone formation are common in follicular adenomas.[35]

Microscopy:

Microscopically, the tumor shows follicles containing colloid which may show variation in size. The hallmark of follicular adenomas is that it is completely enveloped by the thin fibrous capsule. The neoplastic cells show variation in size, shape or nuclear morphology. Occasionally the neoplastic cells acquire bright eosinophilic granular cytoplasm. (oxiphil or Hurthle cell change). Mitosis is rare or absent. [35]

Variants of Follicular adenoma:[26]

1. Macrofollicular adenomas

2. Microfollicular adenomas

3. Normofollicular adenomas

4. Hürthle cell adenoma

5. Hyalinizing trabecular adenoma

6. Atypical adenoma

Immunohistochemistry:[49-55]

"Immuno" means in relation to antibodies used in the procedure and "histo" meaning tissue.

It seeks to exploit the specific results by binding of antibody to antigen. Over the past last two decades, immunohistochemistry has had an immense impact on the practice of diagnostic pathology. The identification of specific and highly selective cellular epitopes in routinely processed paraffin wax-embedded tissues with an antibody and

appreciate labelling system is now a routine procedure in most cellular pathology laboratories in the developed world.

Immunohistochemistry is "a method for localizing specific antigen in tissue or cells based on antigen antibody recognition; exploiting the specificity provided by the binding of antibody with its antigen at light microscopic level"

Basic Principle of IHC:

The basic critical principle of IHC is **"**sharp localization of target components in the cell and tissue, based on satisfactory signal to noise ratio". Amplifying the signal while reducing nonspecific background staining (noise) is the major strategy to achieve a satisfactory and practically useful result.

Immunohistochemistry (IHC) is the method of detection of molecules based on the localization of antigens in tissue sections by the use of labeled antibody as specific reagents through antigen-antibody interactions that are visualized by a marker such as an enzyme coupled with chromogen.

Recent advances and future guidelines:[51]

Immunohistochemistry will play an important role in numerous recent new developments which includes genogenic immunohistochemistry for diagnosis, proteins for targeted therapy, methods to develop better and specific monoclonal antibodies with recombinant technology, "technician-free" automation of the IHC procedures, and "pathologist-free" microscopic image analysis technology for interpretation and evaluation of high-throughput results.

CD 10 :

It is also reffered as Neprilysin, membrane metallo – endopeptidase (MME), neutral endopeptidase (NEP) and common acute lymphoblastic leukemia antigen (CALLA), is an enzyme in humans encoded by MME gene.[56] CD 10 is 90-110 kDa cell membrane metallopeptidase located on 3q21-27 which inactivates bioactive peptides. It is a zinc depedent metalloprotease that cleaves the peptide at the amino group of hydrophobic residues and inactivates bradykinin, glucagon, substance P, oxytonin etc. It also degrades the amyloid beta peptide.[11] CD 10 is expressed in large number of tissues especially in abundance in kidney. It is an important marker in the diagnosis of acute lymphoblastic leukemia. It is present on leukemic cells of pre B phenotype. Hematopoeitic progenitors which express CD 10 are known as common lymphoid progenitors which can further differentiate into B or T lymphocyte or NK cell.[59]

Anti CD 10 antibody is a mouse monoclonal derived having Ig G1 isotype with unknown light chain with cell membrane staining. It is used in clinical pathology for diagnosis in lymphomas and leukemias like acute lymphoblastic leukemia, follicular, Burkitt, Diffuse B cell and Angio - immunoblastic T cell lymphoma.[58] CD 10 expression helps in differentiating clear cell renal carcinoma from oncocytoma and chromophobe renal cell carcinoma.[58] CD 10 expression is one of the important characteristics of the mullerian system derived neoplastic mesenchymal cells.[60]

MATERIAL AND METHODS

The study was conducted at Dhiraj General Hospital & Smt. Bhikhiben Kanjibhai Shah Medical Institute & Research Centre, Sumandeep Vidyapeeth, Piparia. The study was prospective, retrospective and observational in nature. A total of 30 cases were studied during the span of 19 months (From February 2016 to August 2017).

Inclusion criteria:

All cases of benign and malignant thyroid lesions

Age: 18 to 70 years

Sex: Male and female both

Exclusion criteria:

Inflammatory thyroid lesions

Poorly preserved specimens

Poorly differentiated and undifferentiated thyroid carcinoma

A total of 30 cases of papillary thyroid carcinoma and benign thyroid lesion were evaluated for the expression of CD 10 by immunohistochemical technique. All thyroid specimens were processed in the histopathology section of the department of pathology.

The specimen were processed in the tissue processor, paraffin blocks were made, 3-5 micron thick sections were prepared & stained with routine Haematoxylin & Eosin

staining method. The slides were examined microscopically to confirm the diagnosis of PTC and Benign Follicular Adenoma.

After examination of H & E stained sections by light microscopy, preparation of representative sections on specially treated slides were done to demonstrate CD 10 antigen expression immunohistochemically, using Envision flex IHC kit purchased from DAKO.

Dako polylysin coated frosted slides, Coplin jars, Pressure cooker, Hot plate, Envision Flex target retrieval solution (high pH for CD 10), Envision Flex wash buffer, Envision Flex substrate buffer, Envision Flex peroxidase blocking reagent, Primary antibody used for CD 10.

- Monoclonal Mouse Anti-Human CD 10, Clone 56C6 (Ready–to-Use)

Envision Flex labeled polymer/Horse Radish Peroxidase (HRP), Envision Flex Chromogen- diaminobenzidine (DAB) were used for the study.

Staining methods used

The protocol of Haematoxylin & Eosin staining method is shown in Annexure no.3.

Dako kit (Envision flex) for IHC was used CD 10. The protocols of immunohistochemistry method used are shown in Annexure no.4.

The H & E slides & IHC slides were evaluated independently of each other and the observations were recorded.

The results were evaluated carefully by following criteria.

Evaluation

The cytoplasmic and cell membrane stained in case of CD 10 with clear brown color were regarded as positive. The intensity of immunohistochemical staining of positive was graded based on subjective evaluation of color imparted (brown color) by antibody, antigen and chromogen complex and the number of total cells stained:

Negative: (−) no color, 0% of cells.

Weak: (+), light brown color, 1 to 30% of cells.

Moderate: (++), dark brown color, 31 to 70% of total cells.

Strong: (+++), very dark brown color, 71 to 100% of total cells.[61]

Known slides of positive immunostaining were used as controls to compare our staining in all thyroid lesions. All the observations were carried out by two observers in order to eliminate interobserver bias.

Statistics used

Expression of CD 10 expression in all cases was evaluated using mean, standard deviation, Chi Square Test, one sample t test and unpaired t test.

Results obtained were analyzed in tabulated form, bar diagrams and pie charts.

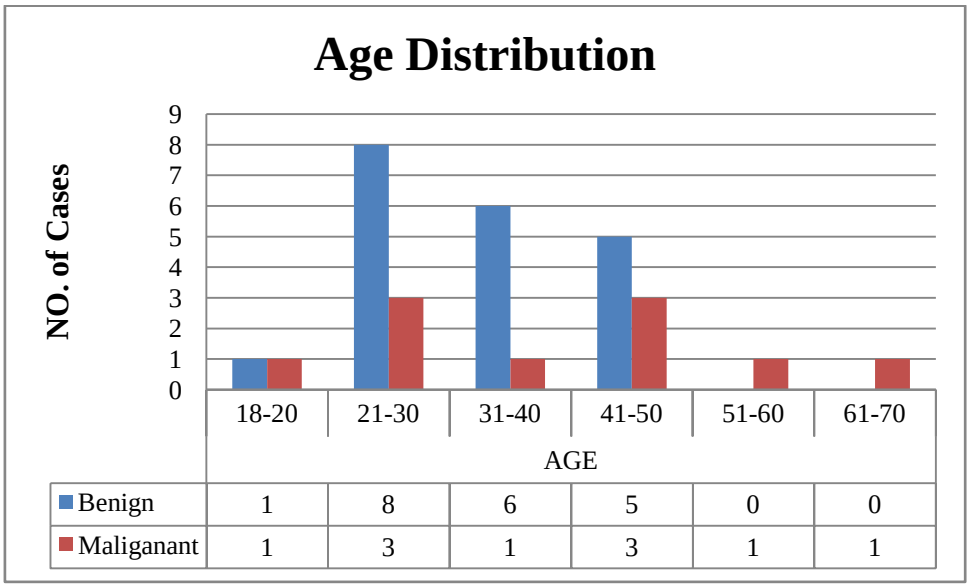
RESULT AND ANALYSIS

The present study was prospective, retrospective and observational in nature. All 30 cases were evaluated histologically. Immunohistochemistry was done by using CD 10 marker.

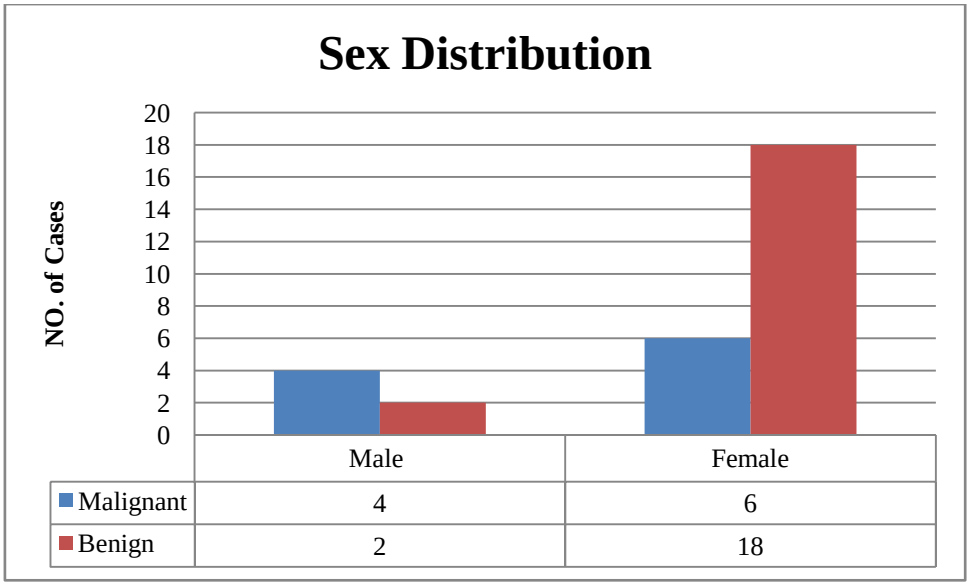
Table 1: Demographic data of all individuals included in study groups.

Age	NO. of Malignant Cases	NO. of Benign Cases
18-20	1	1
21-30	3	8
31-40	1	6
41-50	3	5
51-60	1	0
61-70	1	0

A total number of 30 specimens of thyroid tumors of different age groups as shown in Table 1, were selected out of which 10 cases were malignant and 20 cases were benign according to histopathological diagnosis.



Graph 1: Age distribution of all individuals included in study groups. (Total number of patients=30)



Graph 2: Sex distribution among malignant and benign lesions

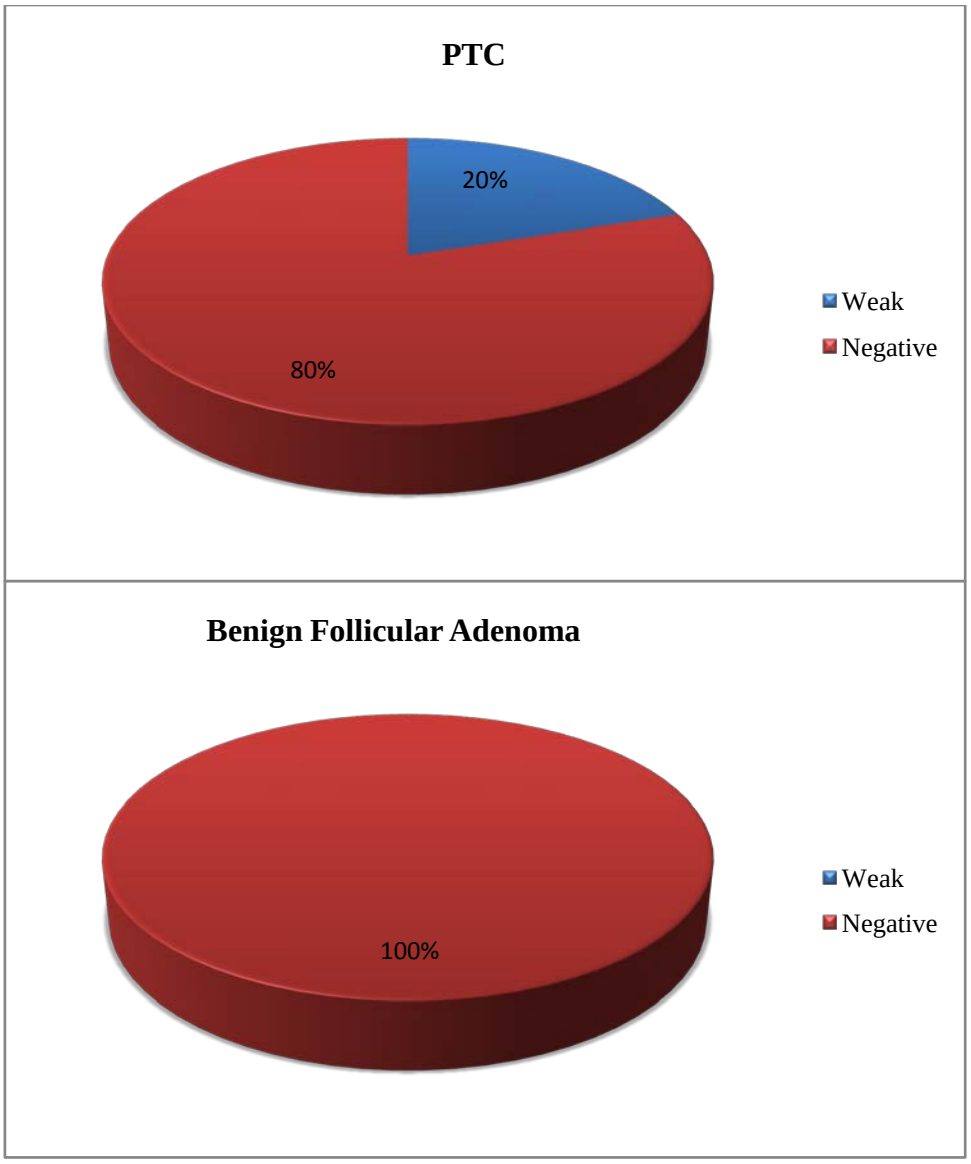
Out of 20 benign tumors 18 were females (90%) and 2 were males (10%). Among malignant tumors, 6 were females (60%) and 4 cases were male (40%).

All the malignant tumors were PTC including conventional papillary carcinoma (5 cases, 50%) and follicular variant (5 cases, 50%). Benign tumors were follicular adenoma.

CD 10 expression was found focally in 2 case of PTC out of 10 cases. CD 10 was not expressed in all the 20 follicular adenomas. Therefore in our study, sensitivity of CD 10 for PTC is only 20%.

Table 2: Comparison of CD10 expression in different study groups. (n=30)

Type	Weak		Negative	
	NO.	%	NO.	%
Malignant	2	20	8	80
Benign	0	0	20	100



Graph 3: Comparison of CD10 expression in different study groups. (n=30)

Table 3: Demographic characteristics including mean age, sex distribution in malignant and benign lesions

Variables	PTC (n=10)	Follicular Adenoma (n=20)	P-value
Sex (F/M)	6/4	18/02	0.05
Age (Mean±SD)	39.8±16.03	32.25±8.44	0.34

Mean age of 30 cases were taken and eventually standard deviation was performed. Obtained P value for sex ratio was 0.05(≤0.05) and for mean age it was 0.34(>0.05). A significant association was not found between age and sex with PTC and follicular adenoma.

Table 4: Mean age and sex distribution of cases with positive and negative CD10 immunoreactivity in patients with PTC

Variables	Positive CD 10 immunoreactivity (n=2)	Negative CD 10 immunoreactivity (n=8)	P Value
Sex (M/F)	1/1	5/3	0.74
Age (Mean±SD)	46±33.94	38.25±12.33	0.32

There was not significant relationship between age and sex with CD10 immunoreactivity ($P > 0.05$)

FIGURES

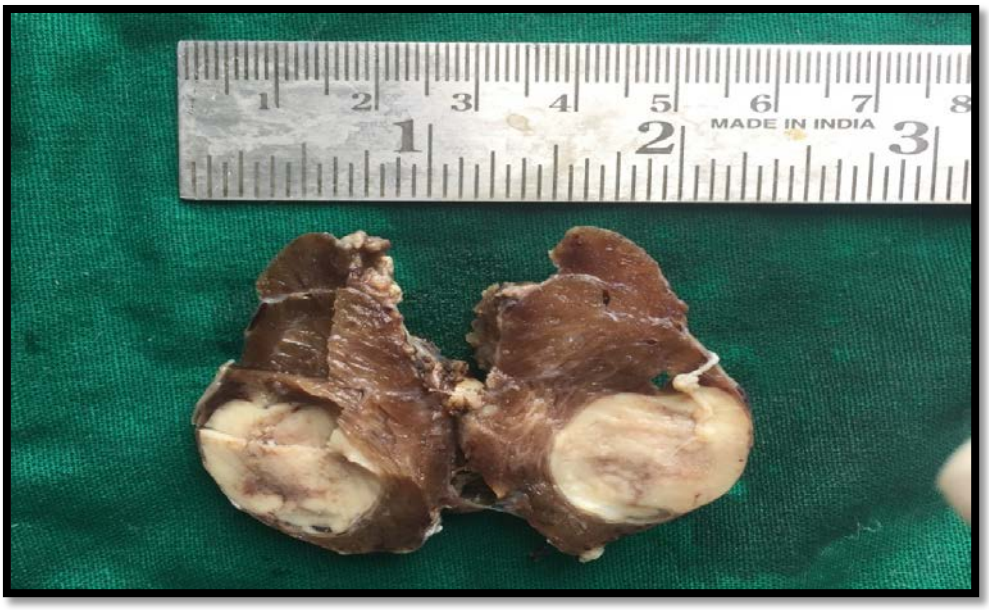


Figure 1: Gross image of Follicular Adenoma

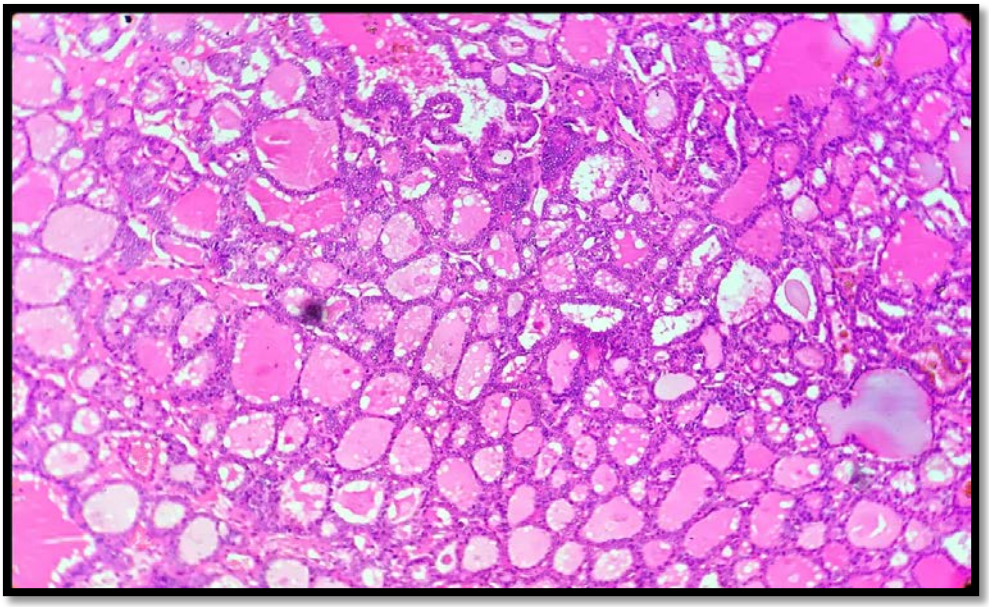


Figure 2: Follicular adenoma showing micro and macro follicles with variablr amount of colloid (10x)

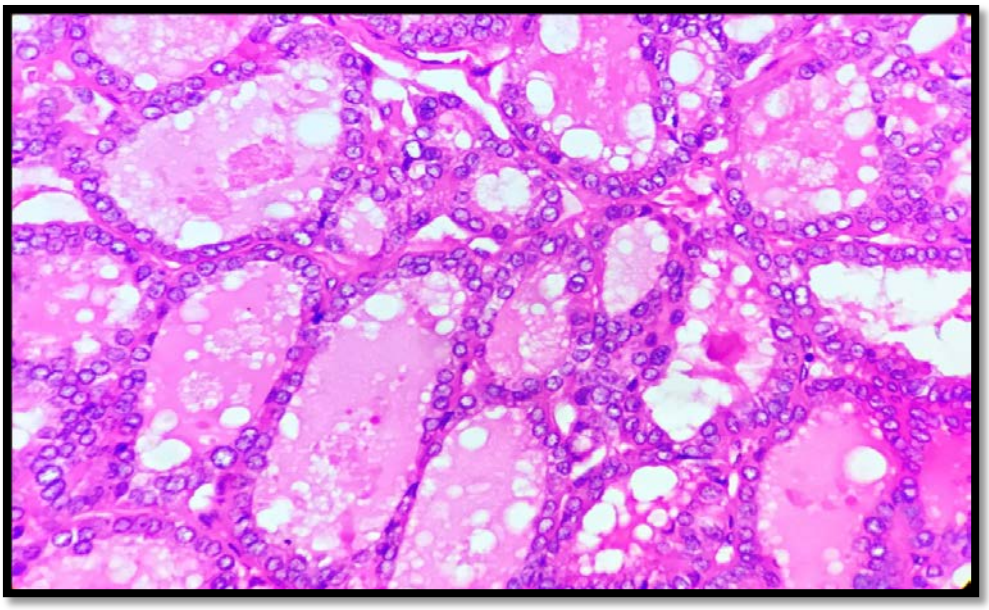


Figure 3: Follicular adenoma showing follicular cells in micro and macrofollicular pattern with variable amount of colloid (40x)

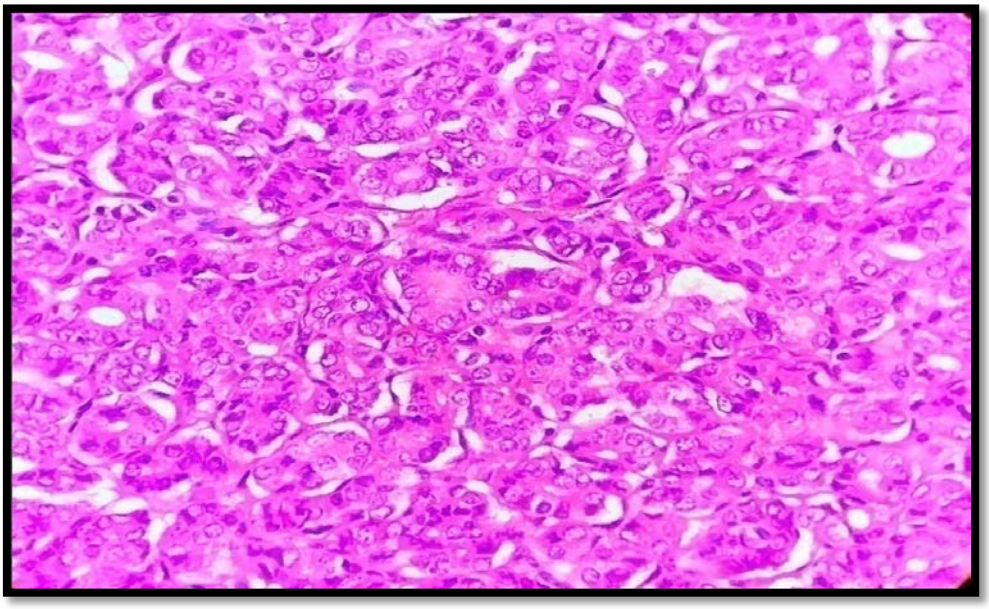


Figure 4: Follicular adenoma showing follicular cells in microfollicular pattern with variable amount of colloid (40x)

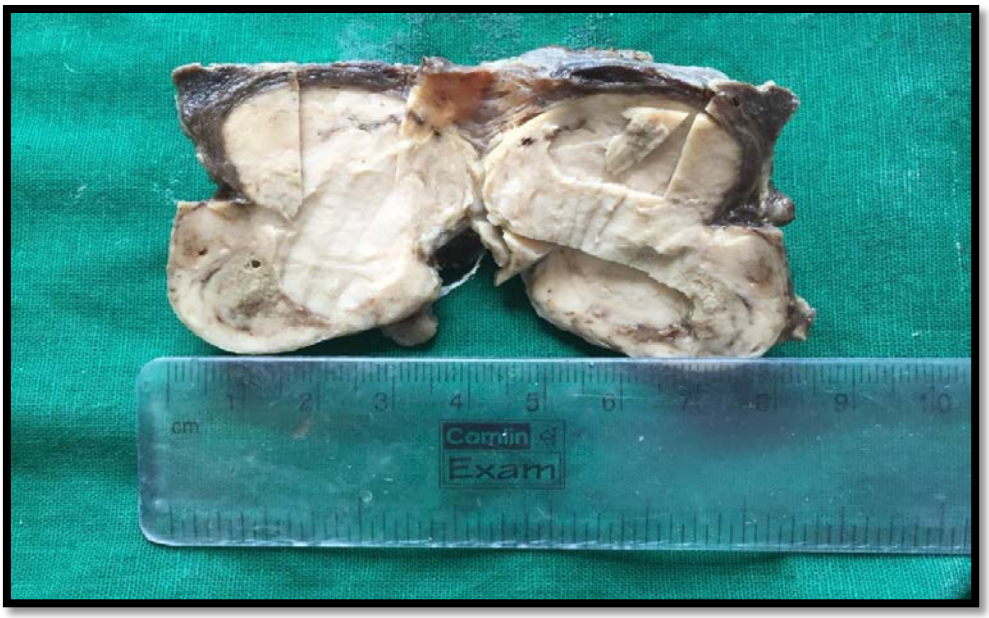


Figure 5: Gross image of Papillary Thyroid Carcinoma (cut-section)

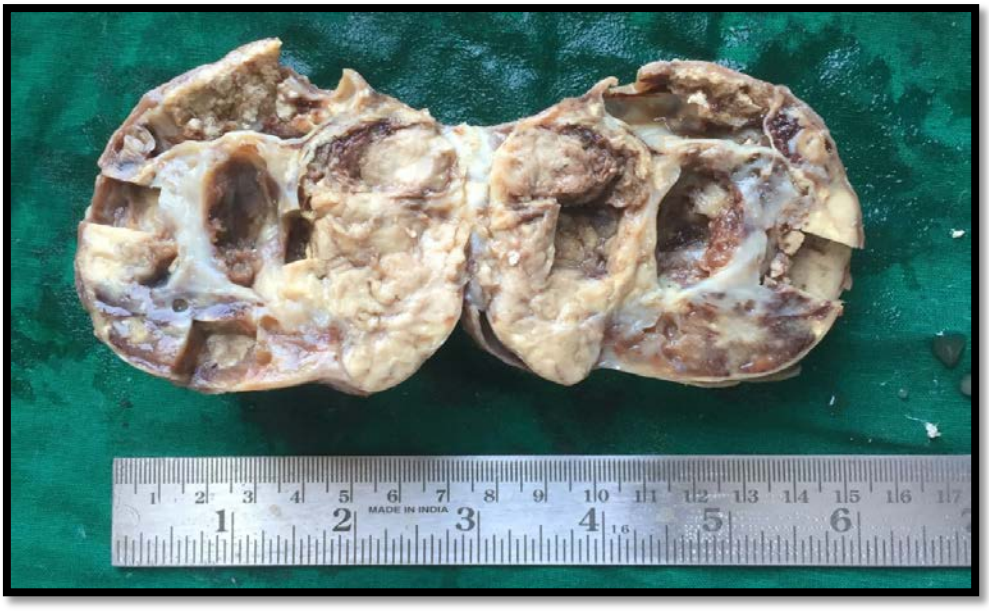


Figure 6: Gross image of Papillary Thyroid Carcinoma (cut-section)

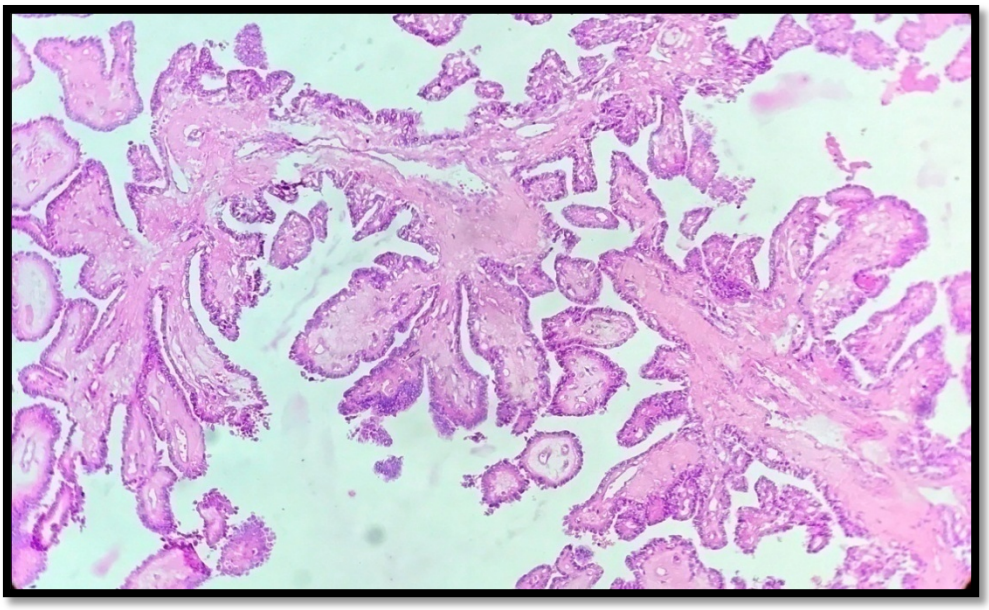


Figure 7: PTC showing papillary pattern of follicles (10x)

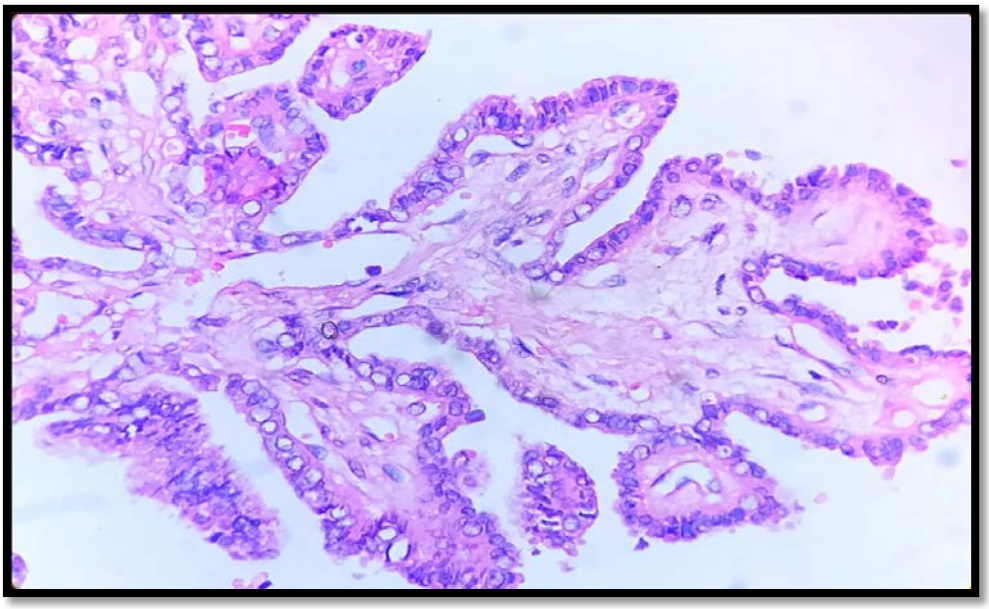


Figure 8: PTC showing follicular cells arranged in branching papillae (40x)

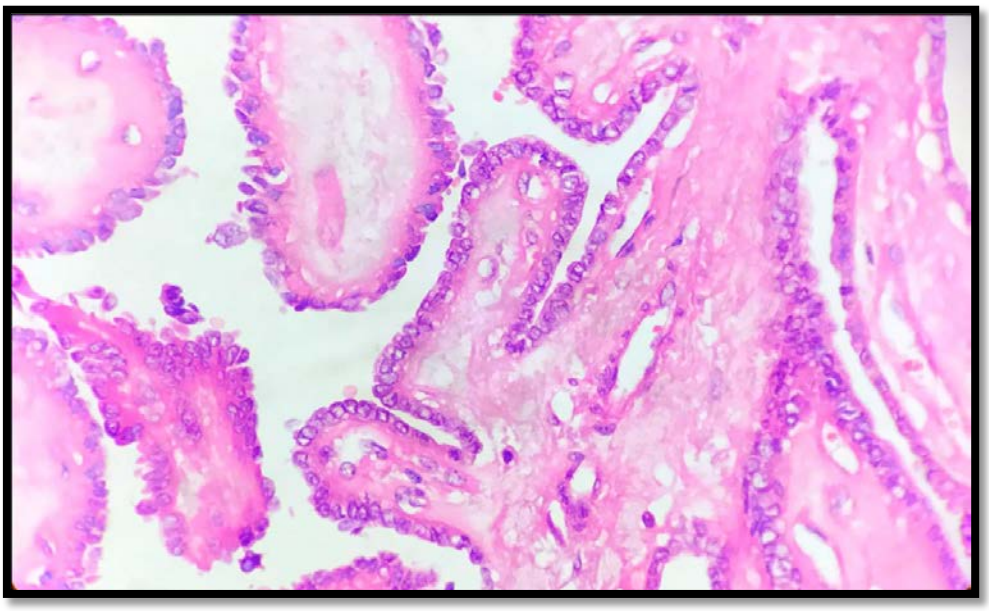


Figure 9: PTC showing follicular cells in branching papillae (40x)

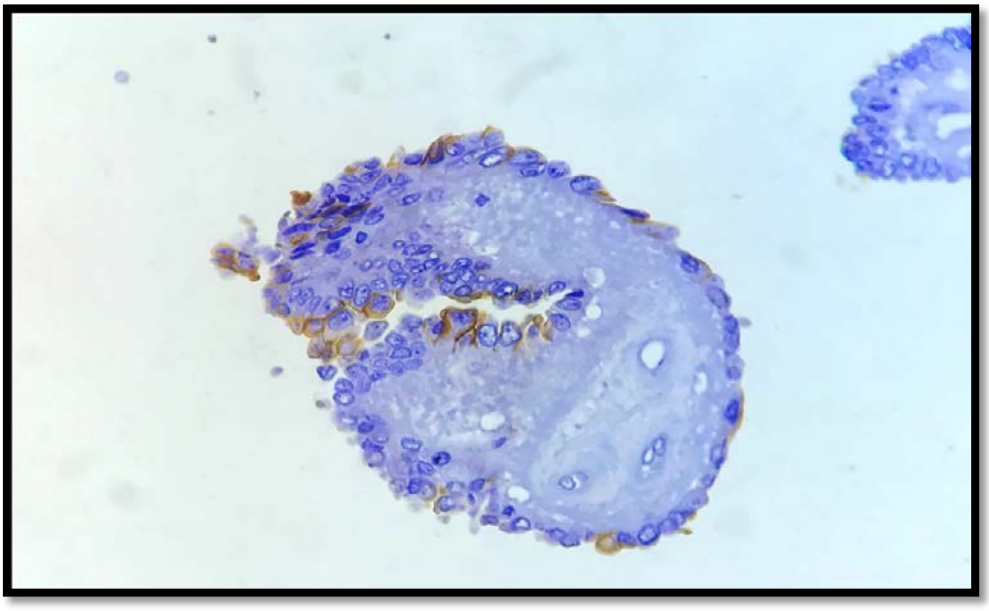


Figure 10: PTC showing focal and weak CD 10 expression (IHC)

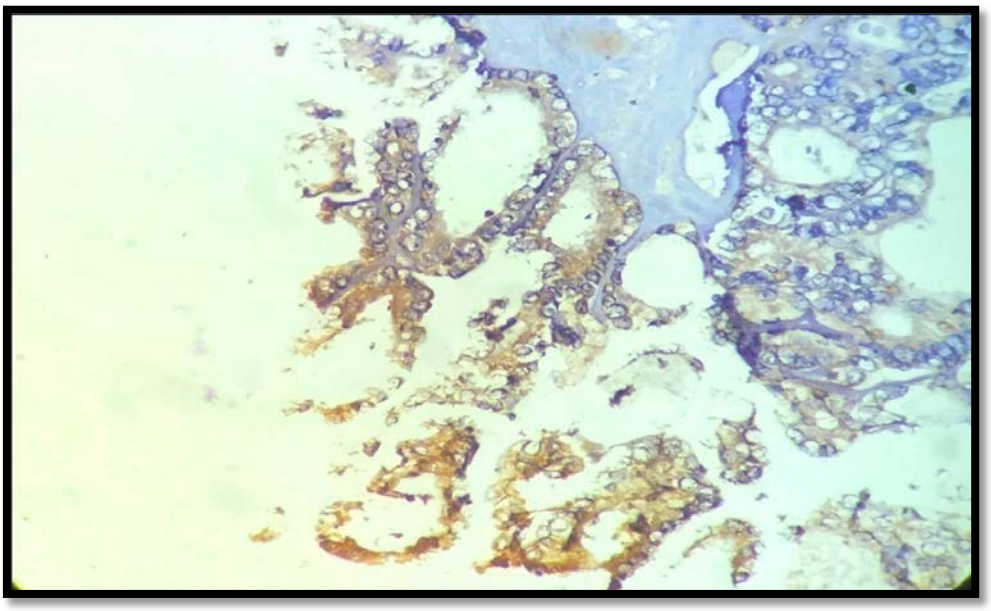


Figure 11: PTC showing focal and weak CD 10 expression (IHC)

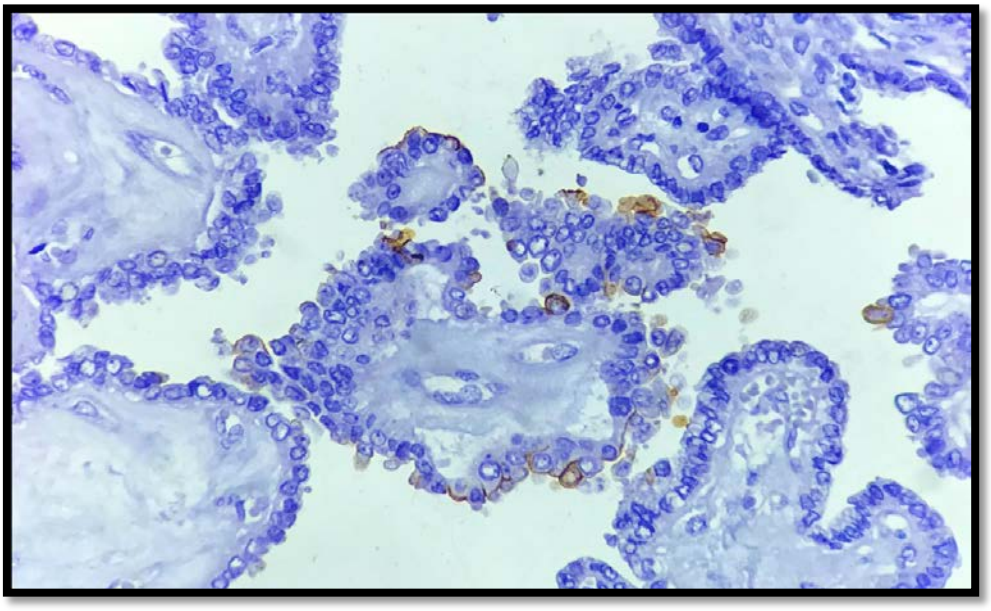


Figure 12: PTC showing focal and weak CD 10 expression (IHC)

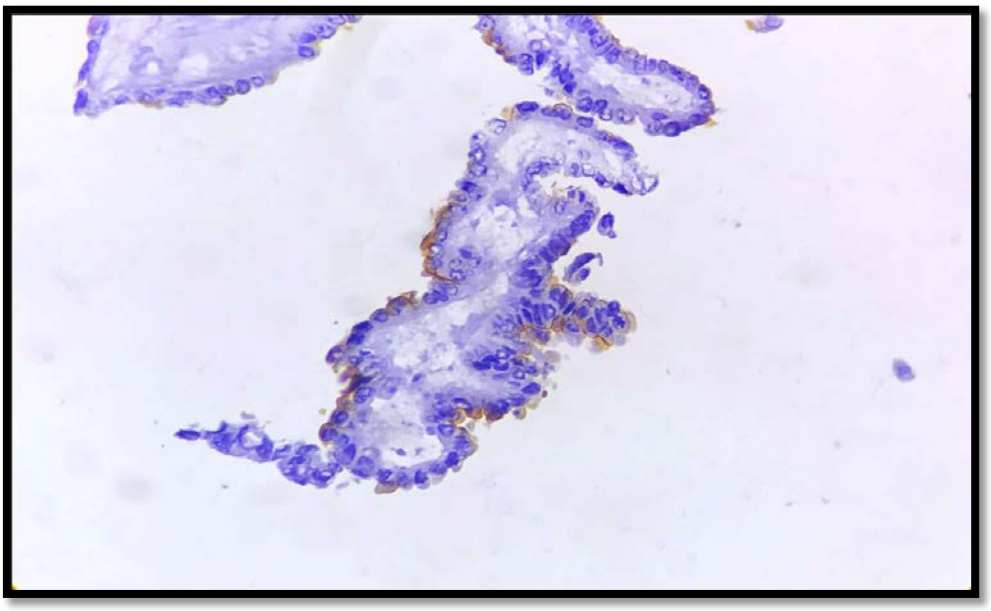


Figure 13: PTC showing focal and weak CD 10 expression (IHC)

DISCUSSION

Pathologic examination using H and E staining is the gold standard in diagnosis of thyroid nodules. Follicular neoplasm and follicular variants of PTC, sometimes show morphologic overlap, which is very common and it is difficult to reach at definite diagnosis in some cases. Considering this fact that incorrect diagnosis (overdiagnosis or underdiagnosis) may cause complications and may even lead to patient's death, so correct diagnosis and subsequently proper treatment are mandatory. Differential diagnosis of these cases may be possible by using IHC.

In the present study, we investigated whether CD 10 could be a useful diagnostic tool in differentiating thyroid carcinoma from benign thyroid lesions. We have found that out of total of 30 cases of both benign and malignant tumors, CD 10 was immunohistochemically positive in 20% of PTC cases, but in none of the benign thyroid lesions (0%).

As reported by Rezk and Khan considering the fact that morphologic diagnosis of some thyroid benign and malignant lesions is challenging and also subjective, so using ancillary procedures such as IHC for some markers including CD10 with conventional pathologic evaluation could be more reliable for diagnosis and classification of different thyroid lesions.[62]

Moreover, mentioned immunohistochemical markers could be used as prognostic tools because during progression of thyroid carcinomas to more poorly differentiated and undifferentiated phenotype, the tumors have not enough and appropriate diagnostic morphologic features of thyroid carcinoma.[62] Thus, in this study we evaluate the diagnostic utility of CD10 marker in this regard. The results of studies in

this field is controversial, some confirmed its utility whereas others did not report any case of CD10 immunoreactivity in thyroid tumors.

Tomoda *et al*. (Japan) in his study titled "CD 10 expression is useful in the diagnosis of follicular carcinoma and follicular variant of papillary thyroid carcinoma" examined the expression of CD10 in 70 thyroid neoplasm. Their findings indicated that CD10 was not positive in benign lesions. CD10 was positive in 80% and 77% of follicular carcinomas and follicular variant of PTC, respectively. That was the first report on the expression of CD10 marker in thyroid neoplasm. They concluded that CD10 expression by IHC considered as a useful marker for classification and diagnosis of benign and malignant thyroid lesions.[22]

Our results were in accordance with the report of **Mokhtari *et al*** (Iran). They have investigated the expression of CD 10 in PTC (n=67) and benign thyroid lesions (n=67). They demonstrated CD 10 positivity in 29.9% of PTC cases and none of the benign thyroid lesions. They concluded that since CD 10 is not seen due to the higher expression of CD10 in PTC than benign thyroid lesions it might be used for differentiating mentioned lesions.[17]

Yegen *et al*. (Turkey) in his study titled "Can CD 10 be used as a diagnostic marker in thyroid pathology?" had investigated the staining pattern of CD10 in 14 benign and 61 malignant thyroid lesions. According to their results CD10 was negative in adenomatous nodules, minimally invasive follicular carcinoma and well-differentiated carcinoma. It was positive in conventional papillary carcinomas (64.2%), follicular variant of papillary carcinomas (16.6%), papillary microcarcinomas (50%), widely invasive follicular carcinomas (11.1%) and follicular adenomas (30%). They concluded that, though CD10 had strong positivity in conventional papillary

carcinoma but it could not be used as a useful marker for differentiating benign and malignant thyroid lesion.[23]

Chu and Arber have studied the expression of CD10 in 505 non-hematopoietic neoplasms including 55 thyroid tumors {follicular adenoma (*n* = 24), papillary carcinoma (*n* = 10), medullary carcinoma (*n* = 16) and Follicular carcinoma (*n* = 5)} by IHC. According to their results CD10 expression was negative in all studied thyroid tumors.[24]

Yasuda *et al* **(**Japan) have investigated the availability of CD10, as a immunohistochemical marker in different non-hematopoietic neoplasms. They showed that CD10 was not present in thyroid tumors and has not any diagnostic value for this group of non-hematopoietic neoplasms.

The limitation of present study was that the benign lesions were Follicular Adenoma and the malignant lesions were PTC. Subgroups of PTC were not evaluated separately. In addition, we did not evaluate other malignant lesions (follicular or other malignant tumors) of thyroid gland.

The results of present study indicate that CD 10 was expressed in only 20% of PTC case, which is very low positivity and therefore it cannot be used as a diagnostic tool to differentiate PTC from benign thyroid lesions. However, its consistent non-expression in all the benign lesions might be useful in some difficult cases in differentiating between benign tumors and PTC. To find out its diagnostic usefulness in day to day practice, studies using large samples of PTC with adequate number of controls (adenomas) will have to be done. Therefore the real usefulness of CD 10 in thyroid tumors is not yet established.

CONCLUSIONS

In the present study, out of total of 30 tumors (20 follicular adenoma and 10 PTC), none of the benign tumors expressed CD 10. Only two of the PTC tumors expressed CD 10 (20%). The result of present study concluded that, the overall sensitivity for detecting PTC is too less to recommend its use in daily practice.

To find out the diagnostic usefulness of CD 10, further studies including larger sample size with adequate number of controls (adenomas) will have to be done.

SUMMARY

1. Some investigators have proposed using CD 10 as a biomarker to differentiate between PTC and benign thyroid lesions including follicular adenoma.

2. With the objective to find out the usefulness of CD 10 marker in thyroid tumors, the present study was done. It included 30 thyroid tumors.

3. In a total of 30 tumors, thyroid adenoma constituted 20 cases and PTC constituted 10 cases.

4. None of the benign tumors expressed CD 10.

5. Two of the malignant tumors expressed CD 10 (20%).

6. The overall sensitivity for detecting PTC is too less to recommend its use in daily practice.

7. In some selected cases, one can use CD 10 to differentiate between benign and malignant one.

8. More studies with larger sample size and adequate controls are necessary to find out the usefulness or otherwise of CD 10 in thyroid tumors.

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ANNEXURES

ANNEXURE 1 - OVERNIGHT SCHEDULE FOR TISSUE PROCESSING

ANNEXURE 2 - PREPARATION OF HAEMATOXYLIN AND EOSIN STAIN

ANNEXURE 3 - METHOD OF H & E STAINING

ANNEXURE 4 - METHOD OF IHC STAINING

ANNEXURE 5 - ABBREVIATION

ANNEXURE 6 - CASE RECORD FORM

ANNEXURE – 1

OVERNIGHT SCHEDULE FOR TISSUE PROCESSING

(John D Bancroft, 6th edition)[62]

- 10% Formalin - 0 hr
- 70% Alcohol - ½ hr
- 80% Alcohol - ½ hr
- 90% Alcohol - ½ hr
- Absolute Alcohol - 1 hr
- Absolute Alcohol - 1 hr
- Absolute Alcohol - 1 hr
- Absolute Alcohol - ½ hr
- Xylene - 1 hr
- Xylene - 2 hrs
- Wax - 2 ½ hrs
- Wax - 4 hrs
- Paraffin embedding & block making
- Lockhart's moulds were placed on a tray or metal piece.

- Fresh molten wax was poured into the mould.

- Tissue was lifted with the forceps and pressed to the bottom, with the cutting surface facing downwards.

- The label bearing the number of the specimen was fixed to the corner of the solidifying wax.

- When the block became hard, moulds were removed.

- Trimming - The four sides of the above prepared block were cut down to square by setting the thickness adjusted at 15 p.m.

- Sectioning – The blocks of prospective cases were inserted with object carrier to the microtome and clamped firmly in place. The object carrier was then adjusted, so that the surface of the block was parallel to the knife edge and locked in that position. Block paraffin was then cut with the microtome knife as usual in rather thick sections till the tissue was reached. At this point the block was readjusted so as to cut the section of 5-6 pm thickness. The section cut in ribbons were received in a tray containing tap water and they were stretched immediately with a pair of needles to prevent wrinkling and further spreading was done by pouring hot water around the section.

- The section was then made to float over clean slides on which a minimum quality of mover's albumin glycol fixative was spread. Finally, the sections were fixed to the slides by heating on the hot plate at 60°C before staining.

Staining – Haematoxylin & Eosin staining (John D Bancroft, 6th edition).

ANNEXURE 2

PREPARATION OF HAEMATOXYLIN AND EOSIN STAIN:

Harris Haematoxylin:

- Haematoxylin crystals 2.5 gms
- Absolute alcohol 50 ml
- Ammonium or potassium alum 50 gms
- Distilled water 500 ml
- Mercuric oxide 1.5 gms
- Glacial acetic acid 20 ml

Preparation

- Dissolve haematoxylin in alcohol.
- Dissolve alum salts in water by heat.
- Remove from heat and mix 1 & 2 solution.
- Bring to boil rapidly.
- Remove from heat, cool and add mercuric oxide.
- Reheat until dark purple color is obtained.
- Remove and plunge the vessel into cold water until its cools.
- The stain is ready to use as soon as it becomes cold.
- Addition of glacial acetic acid gives more precise and selective staining for nuclei.
- Filter every time before using.

Eosin:

- Eosin Y, water soluble 1 gm
- Distilled water 20 ml
- Dissolve and add alcohol 95% 80 ml

ANNEXURE 3

METHOD OF H & E STAINING

Sections were dewaxed in 2 jars of xylene, each for 2 min.

Xylene was removed by keeping slides in 2 jars of absolute alcohol, each for 2 mins.

Treatment with descending grades of alcohol.

In 90% alcohol for 1 min.

In 70% alcohol for 1 min

Rinsed in water

Sections were stained in Harris Haematoxylin for 2-5 min.

It was followed by washing in running water till sections turned blue.

Sections were decolourised with 1% acid alcohol solution.

Again washed in running water for 5-15 min

Counterstained with water soluble eosin for 2 min

Washed rapidly in water

Dehydrated in several changes of 70%, 80%, 90% and absolute alcohol.

Cleared in xylene & mounted in DPX

Results: Nucleus stained blue

Cytoplasm stained pink.

The slides were then examined.

ANNEXURE 4

METHOD OF IHC STAINING

- Working solution (1:50) is prepared of Envision flex target retrieval solution (1:50) with (distilled water) - D/W (200 ml D/W and 4 ml retrieval solution), High pH was used for SMA, Desmin, CD 10 and Low pH was used for Ki-67.

- *retrieval solution will retrieve the antigen on tissue section.

- Antigen retrieval solution is preheated in microwave at 100°C for 1 min

- Slides were dipped in antigen retrieval solution in a coplin jar (care is taken so that tissue section on slide is completely dipped).

- Coplin jar was kept in a pressure cooker on a hot plate till 1 whistle comes.

- Slides was to cooled for 20 mins

- Slides were rinsed with Envision Flex wash buffer 20X for 5 mins (1:20 conc). Fifty ml wash buffer in 1 liter of D/W [once constituted can be used for 6 months]

- Tissue sections were incubated with 200 µl Envision Flex peroxidase blocking reagent for 5-10 mins (in moist chamber).

- Slides were washed with buffer for 3 mins (use new wash buffer everytime)

- Tissue section were incubated with ready –to-use Primary antibody – for 45 mins (in moist chamber)

- Slides were washed with buffer for 3 mins

- Tissue section are incubated with Envision labelled polymer (HRP) for 30 mins (in moist chamber)
- Slides were washed with buffer for 3 mins

- Tissue section were incubated with Chromogen-DAB for 8-10 mins (in moist chamber) [1ml of DAB substrate buffer + 25 µl DAB concentrate – once reconstituted can be used for 5 days – and store at 2-8°C]

- Slides were washed with buffer for 3 mins

- Slides were counterstained with haematoxylin – for 5 mins

- Slides were washed in water / Dipped in alcohol once and dried

- Slides were mounted.

- Slides were examined along with their controls under microscope and then analysed for percentage of positive cells and staining intensity for SMA, Desmin, CD 10 and Ki 67.

ANNEXURE 5

ABBREVIATION

CD	:	Clusters of differentiation.
CALLA	:	Common acute lymphoblastic acute leukemia antigen.
MME	:	Membrane metallo endopeptidase.
PTC	:	Papillary thyroid carcinoma
IHC	:	Immunohistochemistry
CK	:	Cytokeratine
HBME	:	Hector Battifora and MEsothelioma

ANNEXURE 6

CASE RECORD FORM

Proforma used for collecting patient's information

PATIENT DETAILS:

1. Sr. No:
2. Date:
3. IPD no. :
4. Name of the patient:
5. Age (years):
6. Ward:
7. H.P. No:
8. Date:

PATIENT MEDICAL HISTORY:

1. Relevant medical history
   General examination
   Systemic examination

2. Provisional Diagnosis:

3. Final Diagnosis:

4. Secondary or associated diseases:

5. Investigations details:
   A) Routine laboratory investigation - CBC, ESR
   B) X-RAY-
   C) CT SCAN-
   D) CYTOLOGY (FNA)-
   E) HISTOPATHOLOGY-
      GROSS:
      MICROSCOPY:
      IMMUNOHISTOCHEMISTRY: