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CANCER ASSOCIATED FIBROBLASTS IN PAPILLARY THYROID CARCINOMA-AN IMMUNOHISTOCHEMICAL STUDY

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ABSTRACT
Papillary thyroid carcinoma is the most common histological type of thyroid cancer. Fibroblasts and myofibroblasts are the main connective tissue cells that collaborate with tumour cells, which produces all extracellular connective matrix elements and synthesizing and secreting growth factors. The change occurring in fibroblast phenotype and activity, their transformation from normal fibroblasts to tumour fibroblasts (referred to as carcinoma associated fibroblasts), appears to occur in the initial stages of carcinogenesis. In papillary thyroid carcinoma, cancer-associated fibroblasts are a risk factor for lymph node metastasis. The present study aimed to investigate the presence of cancer associated fibroblasts in papillary thyroid carcinoma patients using immunohistochemical markers CD 34 and Alpha smooth muscle actin. This will be beneficial for patients with cancer associated fibroblasts as they can receive targeted therapy. 80 thyroidectomy specimens received in the department of pathology which were diagnosed as PTC during the study period were included in this study. Immunohistochemical staining for CD 34 and Alpha Smooth Muscle Actin were done and their expression were studied.66 percent of the cases showed CD 34 positivity and 56 percent showed Alpha Smooth Muscle Actin positivity. This study concluded that both CD 34 and Alpha Smooth Muscle Actin can be used together as markers of cancer associated fibroblasts which can serve as target for drug therapy in Papillary Thyroid Carcinoma.

KEY WORDS: *Papillary thyroid carcinoma, cancer associated fibroblasts, CD 34, Alpha Smooth Muscle Actin.*

INTRODUCTION
Thyroid cancer represents, world widely about 1% of all epithelial cancers and it is regarded as the most common endocrine neoplasm.[1] Of all the histopathological forms, papillary thyroid carcinoma is the most common histological type of thyroid cancer, accounting for 80-90% of all thyroid malignancies.[2]

Papillary thyroid carcinoma, similar to any other solid tumour is composed of two distinct, still interrelated components: carcinoma cells and tumoral stroma. The tumoral stroma is reshaped by the tumoral cells in order to ensure the proliferation, growth, invasion of neighboring tissue structures and distant metastasis.[1] There is an increasing recognition of the significance of the tumor microenvironment as a non-transformed element that is located adjacent to the cancer cell. Fibroblasts and myofibroblasts are main connective tissue cells that cooperate with tumor cells, which produces all elements of extracellular connective matrix, secreting and synthesising cytokines and growth factors and promoting neovascularization. The change in this phenotype and activity of fibroblasts and their transformation from normal fibroblasts into tumor fibroblasts (carcinoma associated fibroblasts) apparently occurs in initial stages of carcinogenesis.[1] The tumour microenvironment biology which is affected by CAF and myofibroblasts fosters the progression of cancer via different adhesional patterns and transient epithelial to mesenchymal transition induction apart from angiogenesis.[3] CAF are abundant at the margin of the tumor, where they release proinvasive factors for tumor cells.[4]

CD 34 (also called hematopoietic progenitor cell antigen) is an intracellular adhesion protein and cell surface glycoprotein.[5] It mediates the attachment of hematopoietic stem cells to bone marrow extracellular matrix or directly to stromal cells. CD 34 + cells in thyroid stroma are dendritic interstitial cells.[6]

ASMA (Alpha Smooth Muscle Actin) is used to identify smooth muscle cells, myofibroblasts, decidual stromal cells etc.

Myofibroblastic differentiation in papillary thyroid

carcinoma can be exemplified by CD 34 and Alpha smooth muscle actin expression.[7] Desmoplastic stromal reaction is also responsible for the invasive behavior of papillary thyroid carcinoma associated with metastasis to lymph node.[8] Application of immunohistochemical technique can be used as an additional tool to H&E staining method.

At present few studies exist on cancer associated fibroblasts in papillary thyroid carcinoma in India. This study is aimed to investigate the presence of carcinoma associated fibroblasts in papillary thyroid carcinoma patients through immunohistochemical markers CD 34 and Alpha smooth muscle actin.

MATERIAL AND METHODS

The study was conducted on 80 thyroidectomy specimens histopathologically diagnosed as papillary thyroid carcinoma in pathology department, Govt. Medical College, Thiruvananthapuram from January 2019 to may 2020. Surgically resected thyroidectomy specimens were fixed in 10% neutral buffered formalin. Following the cutting, bitting and tissue processing of selected specimens, paraffin embedded blocks were made. Sections were cut from the blocks about 4-5 micrometres thick and H&E staining was done. Diagnosis of the slides were made under optical microscope and the proforma was filled.

Unstained slides were made from the tumor containing tissue blocks. APES coated slides were used to take thinner sections and were kept in the incubator overnight for the adequate fixation. Immunohistochemical staining for CD 34 and ASMA was done in our immunohistochemistry laboratory adhering to the standard IHC protocol, followed by mounting and labelling of the stained slides. Interpretation of CD 34 and ASMA were done and the results were entered as negative or positive.

IMMUNOHISTOCHEMICAL EVALUATION

CD 34 and ASMA staining was evaluated using regular light microscope under 40x magnification. The percentage of stromal cells expressing each antigen was graded as:

0 – up to 5%
+ - >5% and up to 25%
++ - >25% and up to 50%
+++ - >50% of stromal cells

DATA ANALYSIS

MS Excel sheet was used to enter the collected data with necessary details and was analysed using the statistical software SPSS trial version 23.0. The qualitative variables were expressed in percentage.

RESULTS

The present study included histopathologically diagnosed cases of papillary thyroid carcinoma from thyroidectomy specimens received at the Pathology department ranging in age between 17 to 71 years. People belonging to less than 45 years age group showed a higher incidence of papillary carcinoma, with 45 cases in the less than 45 years age group and 35 cases in the more than or equal to 45 years age group.(Refer table 1,figure 1)

Table 1: Percentage distribution of the sample according to age.

Age	Count	Percent
<45 years	45	56
>=45 years	35	44

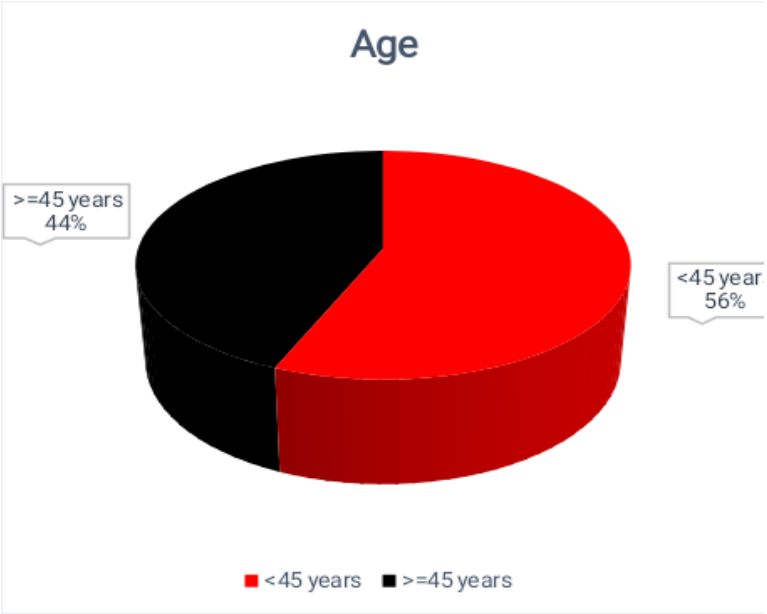


Fig 1: Percentage distribution of the sample according to age.

There were 69 female patients and 11 male patients among the 80 cases, with a female predominance of 86 %. (Refer table 2, figure 2)

Table 2: Percentage distribution of the sample according to gender.

Gender	Count	Percent
Female	69	86
Male	11	14

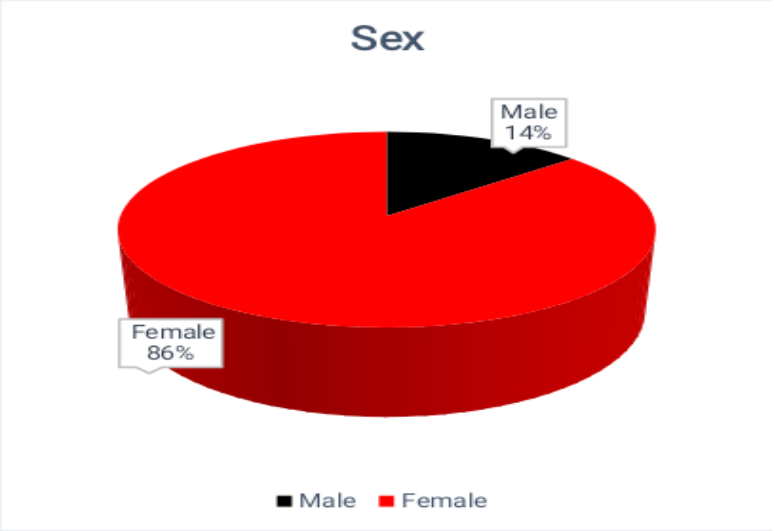


Fig. 2: Percentage distribution of the sample according to gender.

Metastasis to lymph node was found in 26 of the 80 cases of PTC, accounting for 32 % of the total (Refer table 3, figure 3), which was shown to be more common in people over the age of 45 ,with 34% developing metastasis. In the age group under 45 years,31% of the cases developed metastasis. (Refer table 4,figure 4).Lymph node metastasis was more prevalent in the male population, with 83 % developing metastasis ,compared to only 30% in the female population.(refer table 5, figure 5)

Table 3: Percentage distribution of the sample according to lymph node metastasis.

Lymph node metastasis	Count	Percent
Present	26	32
Absent	54	68

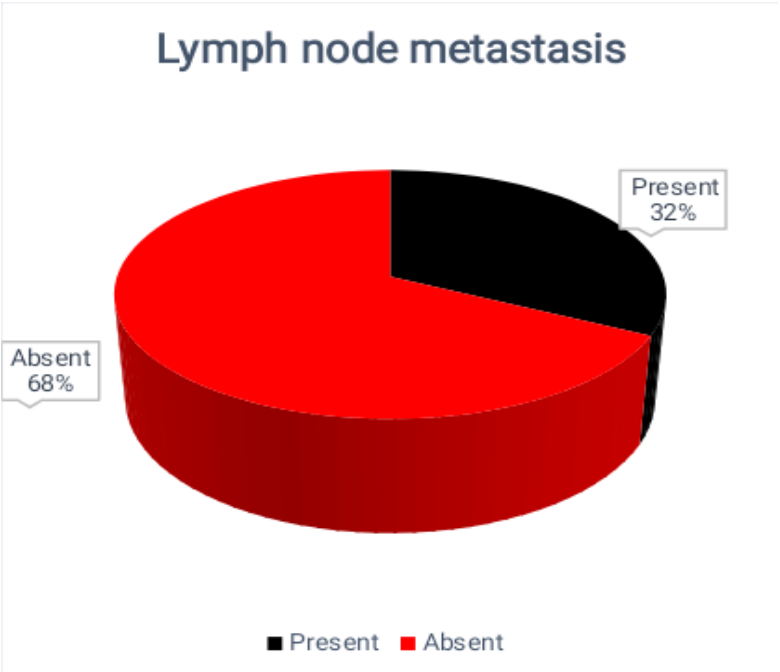


Fig. 3: Percentage distribution of the sample according to lymph node metastasis.

Table 4: percentage distribution of lymph node metastasis among age groups.

Lymph node metastasis	Age<45 years		Age >= 45 years	
	Count	Percent	Count	Percent
Present	14	31	12	34
Absent	31	69	23	66

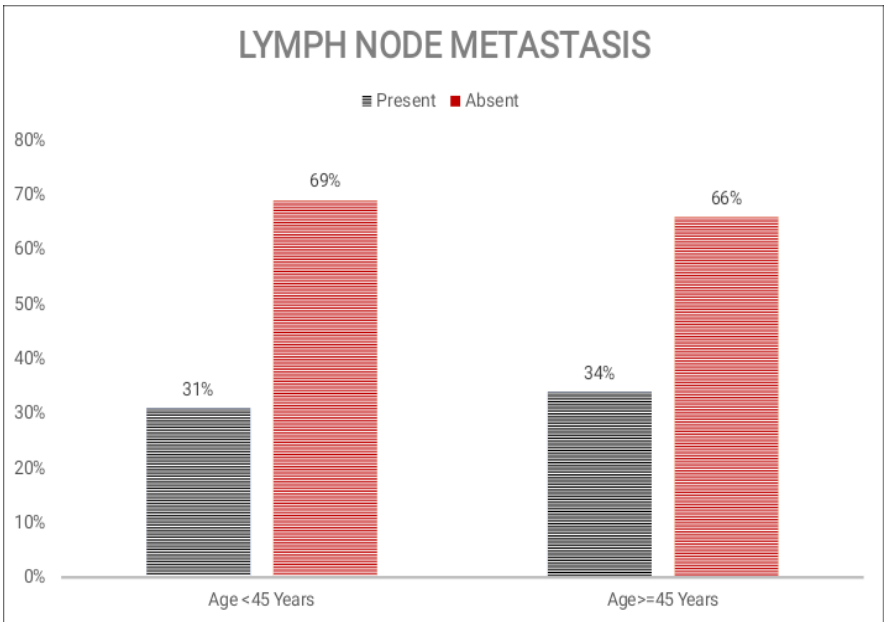


Fig. 4: Percentage distribution of lymph node metastasis among age groups.

Table 5: percentage distribution of lymph node metastasis among gender.

Lymph node metastasis	Male		Female	
	Count	Percent	Count	Percent
Present	5	83	21	30
Absent	6	17	48	70

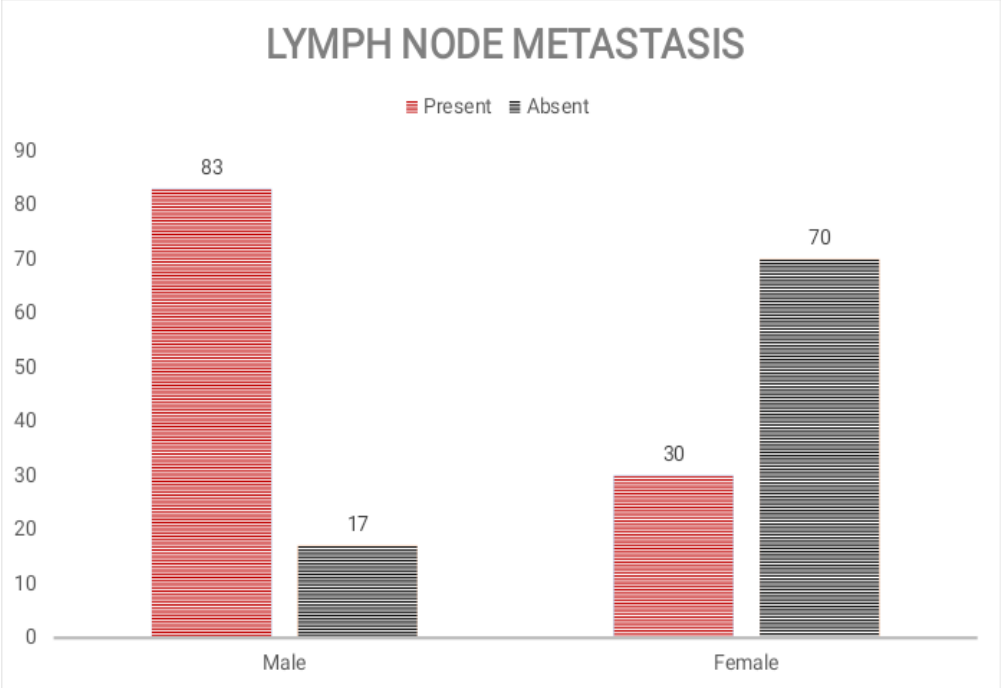


Fig. 5: percentage distribution of lymph node metastasis among gender.

CD 34 expression in papillary thyroid carcinoma

Out of 80 PTC cases,53 had fibroblasts with 2+ or 3+ membranous positivity for CD 34, while 27 had negative expression (Refer table 6, figure 6).

Table 6: Percentage distribution of the sample according to CD 34 expression.

CD 34 expression	Count	Percent
Positive	53	66
Negative	27	34

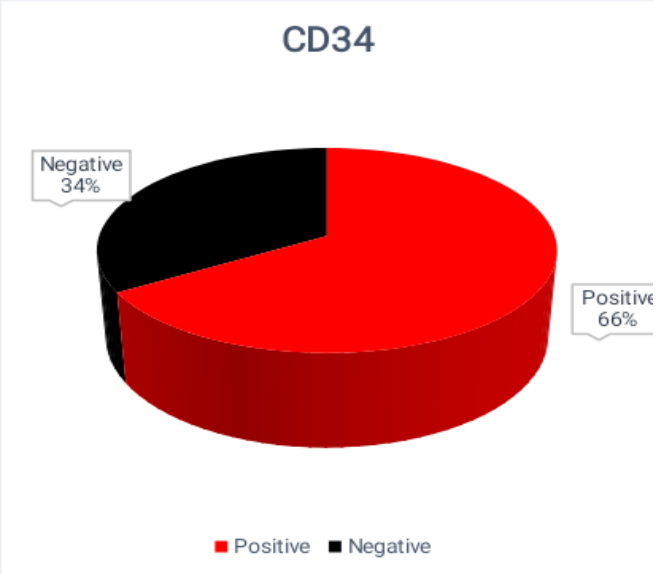


Fig 6: Percentage distribution of the sample according to CD 34 expression.

ASMA in papillary thyroid carcinoma

45 of the 80 papillary thyroid carcinoma cases were positive for ASMA expression, accounting for 56 % of the total. ASMA was found to be negative in 35 of the cases. In addition, a positive reaction to ASMA was also identified in the blood vessels. ASMA positive areas were localized in the tumour stroma along the invasive front.(Refer table 7,figure 7)

Table 7: Percentage distribution of the sample according to ASMA expression.

ASMA expression	Count	Percent
Positive	45	56
Negative	35	44

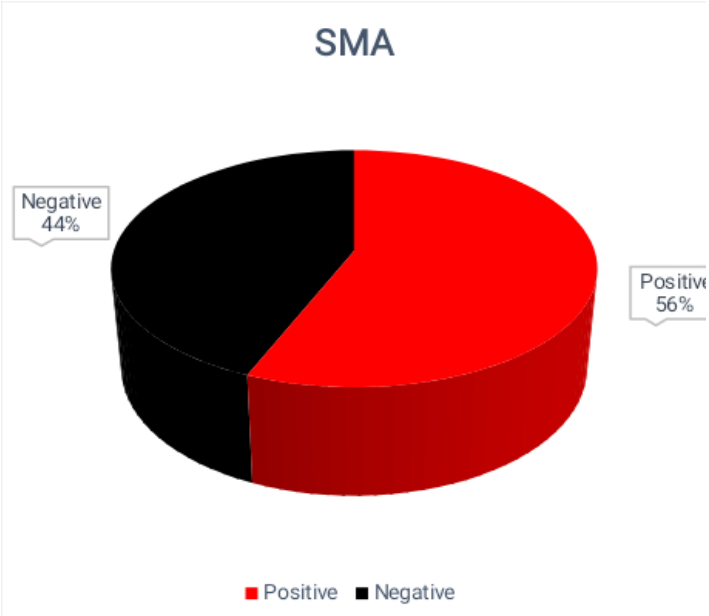


Fig 7: Percentage distribution of sample according to ASMA expression.

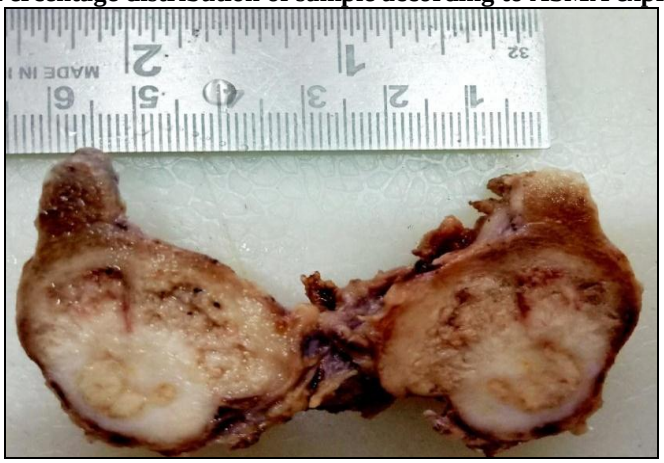


Fig. 8: Macroscopy of papillary carcinoma thyroid.

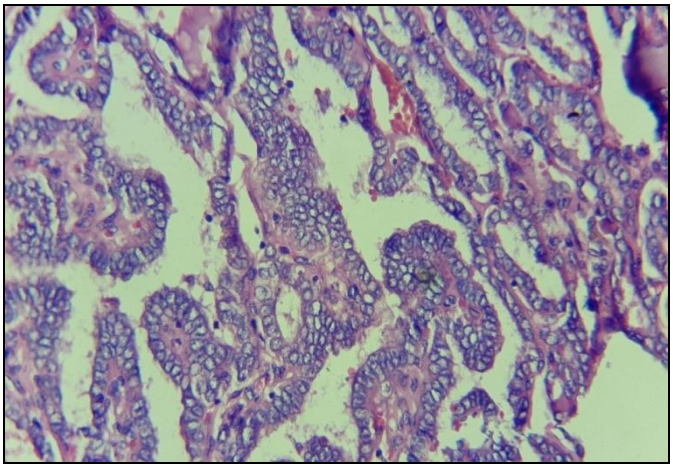


Fig. 9: Microscopy of papillary carcinoma thyroid- classic variant.

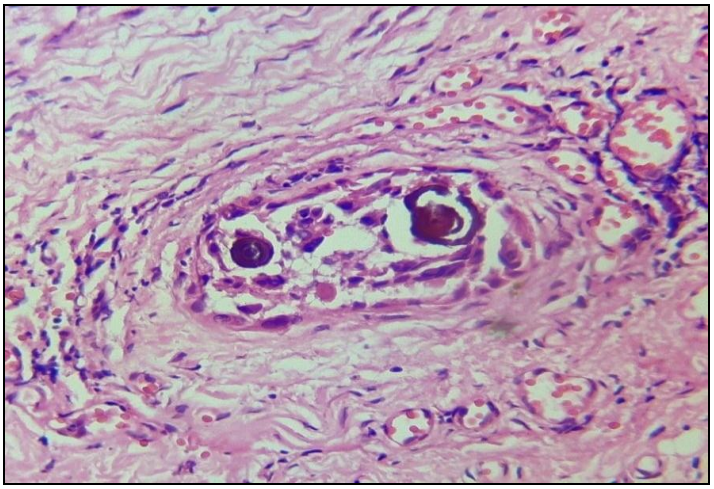


Fig. 10: Psammoma Bodies.

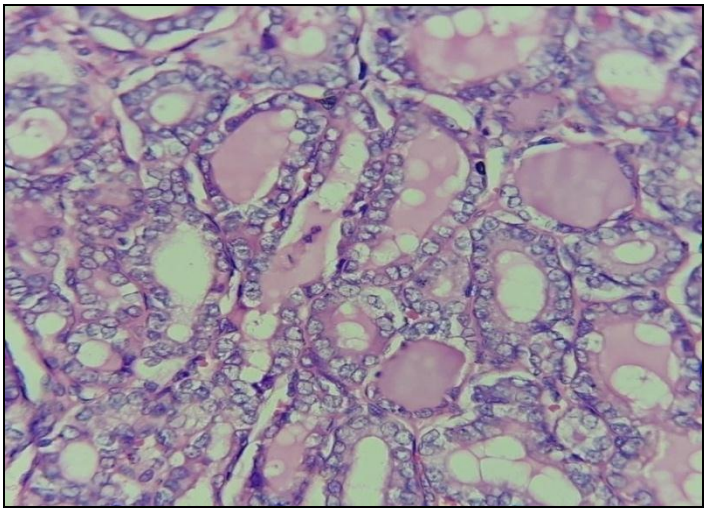


Fig. 11: Microscopy of papillary thyroid carcinoma – Follicular variant.

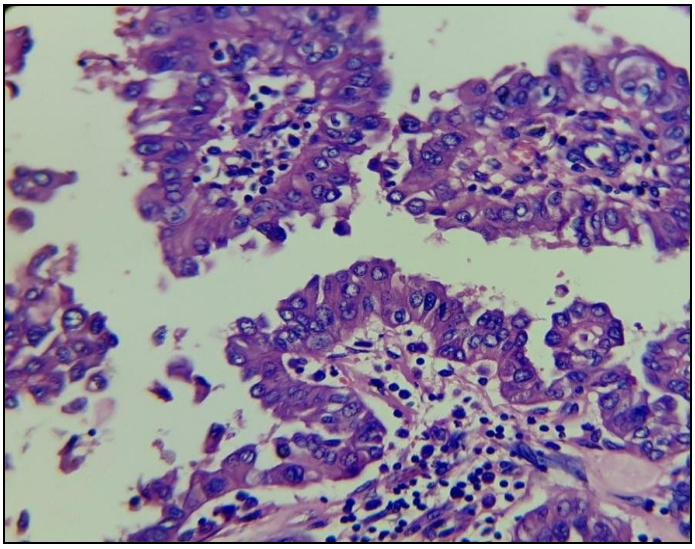


Fig. 12: Microscopy of papillary thyroid carcinoma – Columnar cell variant.

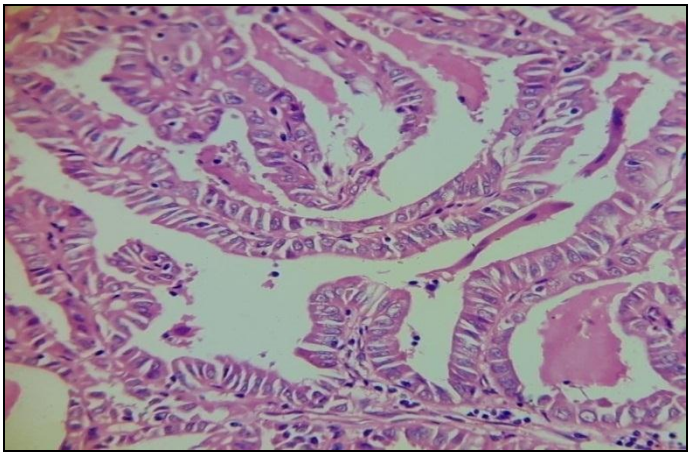
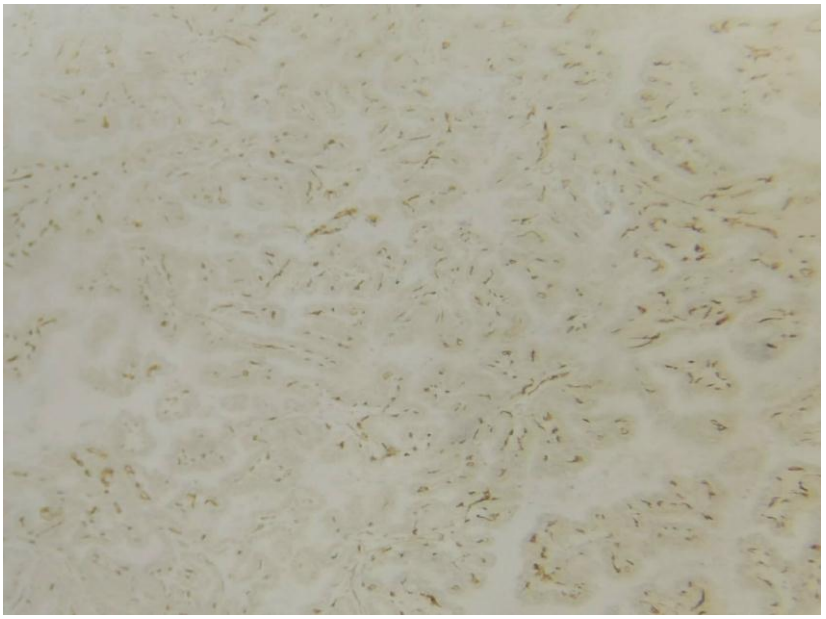
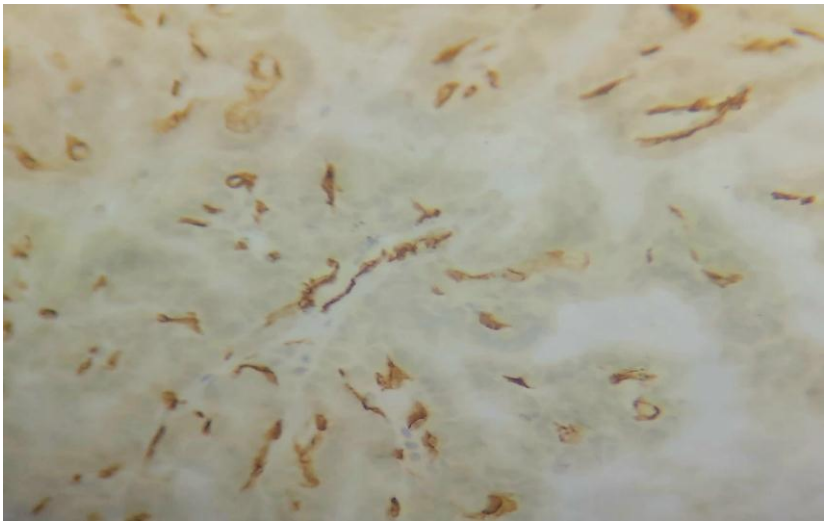


Fig. 13: Microscopy of papillary thyroid carcinoma –Tall cell variant.

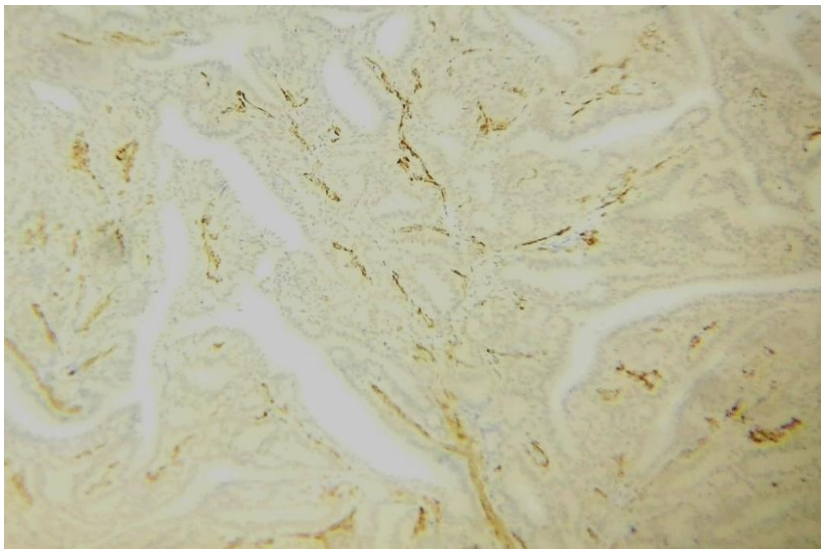


(a) 10x



(b) 40x

Fig. 14: CD 34 expression in papillary thyroid carcinoma.



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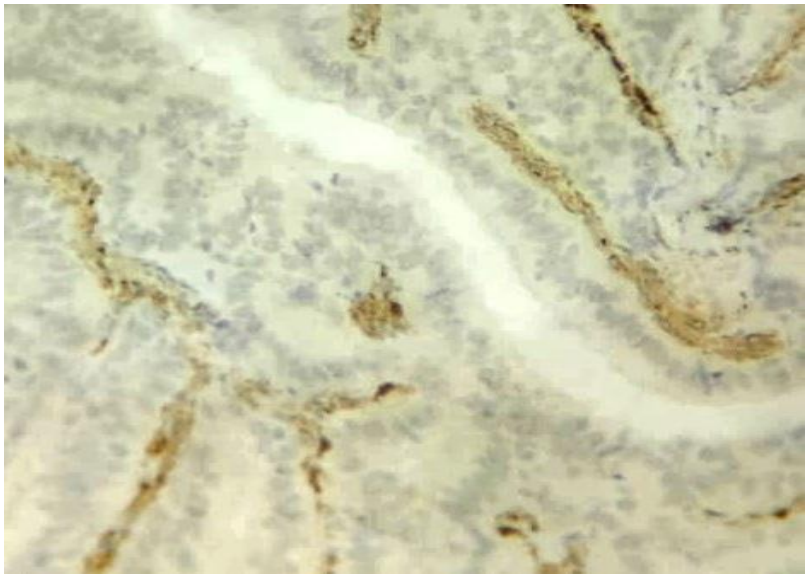

(b) 40x

Fig.15: ASMA expression in papillary thyroid carcinoma.

DISCUSSION

Papillary thyroid carcinoma is one of the most prevalent types of thyroid malignancy, with some cases exhibiting aggressive features. Cancer associated fibroblasts surrounding the cancer cells have been found to have a role in tumor initiation, tumor stimulatory inflammation, metabolism, drug response metastasis, and immune surveillance. Studies are being conducted targeting cancer associated fibroblasts for cancer therapy.

The Present study included 80 histopathologically diagnosed total thyroidectomy cases of papillary thyroid carcinoma that were received in the pathology department from January 2019 to May 2020.Out of 80 cases, most of the cases were seen in the age group less than 45 years and showing a female preponderance. (Refer table 10). Only 26 cases of lymph node metastasis were found , with metastasis occurring slightly more frequently in those over 45 years and in males. The primary objective was to study the expression of CD 34 and Alpha smooth muscle actin- markers of cancer associated fibroblasts in papillary thyroid carcinoma.

When the age group was compared to those of other studies, it was shown that the majority of cases fall in the age group more than 45 years. All of these studies revealed a female preponderance, which is comparable with the findings of the present study.

Table 10: comparison of age group and gender distribution.

Authors	Age group(majority cases)	Gender
Woo Young-Sun et al	>45 years	F>M
Razman simesku et al	>45 years	F>M
Akira oh Shima et al	<45 years	F>M
Kaliszewski et al	>45 years	F>M
Present study	**<45 years**	**F>M**

This difference in the age group as compared to prior studies could be attributable to an increase in USG and cytology studies, as well as health concerns among the younger population, which has led to an increase in health checkups.

Present study showed metastasis to lymph node in 32% of cases, while the studies conducted by Kaliszewski et al showed lymph node metastasis in 26% of cases and Tongtong Li et al found metastasis to lymph node in 33 % of cases, both of which are comparable to our findings (Refer table 11).

Table 11: Comparison of lymph node metastasis.

Authors	LN metastasis(percent)
Tongtong Li et al	33%
Kaliszewski et al	26%
Akira oh Shima et al	43%
Present study	**32%**

The present study found a modest rise in lymph node metastasis in individuals over 45 years old and in males. Regarding CD 34 and SMA expression, 66% of cases were positive for CD 34 and 56% were positive for ASMA, which was slightly lower as compared to the similar studies (Refer table 12).

Table 12: Comparison of CD 34 and ASMA expression.

Authors	CD 34 expression	SMA expression
Jae-Gu Cho et al	80%	65%
Sahar Ali Daoud et al	80%	68%
Jie Zhang et al	78%	69%
Present study	**66%**	**56%**

The following observations were made in the present study:

- The majority of the patients were under 45 years old
- There was a female preponderance.
- 32 % of cases had metastasis to lymph node
- Presence of lymph node metastases were more among males and in age group greater than 45 years.
- The most significant observation was that 66% of cases showed CD 34 positivity, while 56 % showed ASMA positivity.

CONCLUSION

The present study was conducted in the Pathology department, Government Medical College, Thiruvananthapuram. The primary objective was to study the expression of CD 34 and Alpha smooth muscle actin-markers of cancer associated fibroblasts, in papillary thyroid carcinoma cases that had been histopathologically confirmed.

The patients included in the study were separated into two groups: those who were less than 45 years old and those who were more than or equal to 45 years old. The majority of the patients were below 45 years. There is a female predominance. Metastasis to lymph node was found in 32 % of the overall cases studied. On evaluating CD 34 and ASMA expression in papillary thyroid carcinoma, 66% showed CD 34 positivity and 56% showed ASMA positivity.

Through this study, it is concluded that, CD34 and ASMA can both be employed as markers for cancer associated fibroblasts in papillary thyroid carcinoma and that they can be used for targeted drug therapies.

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