ORIGINAL RESEARCH

A Study of *RET*/PTC1 and *RET*/PTC3 Gene Rearrangement in Papillary Thyroid Carcinoma with Clinico-Pathological Association in a Tertiary Care Centre of Karnataka

[1]Dr. B R Ashwini, [2]Dr. Nirmala C, [3]Dr. Subhashini H Bevinakatti, [4]Dr. M Natarajan, [5]Dr. Dayananda S Biligi

[1]Assistant Professor, [2]Senior Resident [3,4]Professor, [5]Professor and Head, Department of Pathology, Bangalore Medical College and Research Institute, Bengaluru, Karnataka, India

Corresponding Author
Dr. B R Ashwini
Assistant Professor, Department of Pathology, Bangalore Medical College and Research Institute, Bengaluru, Karnataka, India
Email: dr_ashwinibr@yahoo.co.in

Received: 14 February, 2023 Accepted: 16 March, 2023

ABSTRACT

Introduction: Papillary carcinoma of thyroid (PTC) is the commonest malignancy of thyroid. Various genetic alterations like gene mutation, gene amplification, gene translocation and gene methylation results in PTC. The prevalence of *RET*/PTC in papillary carcinomas shows significant geographic variation between 2.5% to 67%. There are more than 13 different types of *RET*/PTC translocations with *RET*/PTC1 and *RET*/PTC3 being commonest, accounting for >90% of all rearrangements. Many studies have correlated *RET*/PTC1 and *RET*/PTC 3 with clinicopathological features and found varying results. Thus, we intend to study the frequency of *RET*/PTC gene rearrangement among PTC at our institute and see the association of *RET*/PTC1 and *RET*/PTC3 gene rearrangement with various histomorphological features. **Methods:** The study was conducted on histopathologically proved PTC cases. The Hematoxylin and eosin (H&E) stained slides prepared from formalin fixed paraffin embedded (FFPE), tissue blocks were evaluated and the area of interest with presence of >50% tumour area and absence of haemorrhage, necrosis and stromal desmoplasia was selected for PCR. The RNA was extracted followed by singleton Taqman gene expression assay for *RET*/PTC1 and *RET*/PTC3 rearrangement using Ag-PATH one step RT-PCR. *ACTB* gene was used as internal control. The results were correlated with various clinicopathological features for statistical significance. **Results**: A total of 18 PTC cases were included in the study. There were 6 (33.3%) males and 12 (66.6%) females with age range from 21 years to 68 years. On microscopic examination, conventional PTC, were seen in 10 cases (55.6%) and follicular variant seen in 8 cases (44.4%). The *RET*/PTC gene arrangement was seen in 3 out of 18 cases (16.6%) with *RET*/PTC1 gene arrangement seen in 1 cases (5.6%) and *RET*/PTC3 gene arrangement seen in 2 cases (11.1%). No statistically significant association of *RET*/PTC gene arrangement with age, gender, size of the lesion and microscopic type were noted.

Key words: PTC, *RET*/PTC1, *RET*/PTC3, Clinocopathological Correlation, No statistically significant association

INTRODUCTION

Thyroid cancer is a common endocrine malignancy that has shown a rapid increase in the global incidence in recent decades. The incidence rates have increased by about 58.1% between 1970 to 2003 [(1).] In India, over the past decade there is a significant raise in the incidence of PTC by about 62% in females and 48% in males[(2)]. Papillary carcinoma of thyroid (PTC) is the most common thyroid malignancy seen between third to fourth decades of life accounting for 70% of the thyroid carcinomas [(3)]. Various types of genetic alterations like gene mutation (BRAF gene), gene translocation (RET/PTC rearrangement) and gene methylation has been identified as driver genetic alteration resulting in PTC. [(4, 5)]

The prevalence of *RET*/PTC rearrangement in PTC has shown significant geographic variations of 20-70 %[(6)]. In India, there are no larger studies to know the prevalence of *RET*/PTC rearrangement in PTC across

the country. Some of the studies have shown the prevalence rate to range between 25-86.6% [7, 8]

There are more than 13 different types of *RET*/PTC translocations, all resulting from the fusion of the tyrosine kinase domain of *RET* to the 5' portion of different genes [6]. *RET*/PTC1 and *RET*/PTC3 are the most common types, accounting for >90% of all rearrangements [9, 10].

There is some evidence that different types of *RET*/PTC may be associated with distinct biologic properties of papillary carcinomas. Some of the studies have shown that *RET*/PTC1 tends to be more common in tumours with typical papillary growth and micro-carcinoma with a more benign clinical course, whereas *RET*/PTC3 shows a strong correlation with the solid variant of papillary carcinoma with more aggressive tumour behaviour [9]. Thus, we intend to study the frequency of *RET*/PTC gene rearrangement among PTC at our institution and see the association between *RET*/PTC1 and *RET*/PTC3 gene rearrangement with various histomorphological features of PTC.

METHODOLOGY

The study was conducted retrospectively on histopathologically proved PTC cases over a period of two years after obtaining institutional ethical committee clearance. The patient's demographic details and gross specimen details were documented from hospital records. The Hematoxylin and eosin (H&E) stained slides prepared from formalin fixed paraffin embedded (FFPE) tissue blocks were evaluated for various histopathological features like pattern of tumour cell arrangement, extra thyroidal extension and Lymph node involvement. The area of interest from the tumour block, with presence of >50% tumour cellularity and absence of haemorrhage, necrosis and stromal desmoplasia were selected for Polymerase chain reaction (PCR). The RNA was extracted with RNeasy FFPE Kit (Qiagen), and the quality of the RNA extracted was checked using multiskan Skyhigh spectrophotometer. The RNA was subjected to single step cDNA conversion and Taqman gene expression assay using Ag-Path one step RT-PCR Reagent (Applied biosystems). A singleplex RT-PCR was performed using 2x RT-PCR Buffer of 12.5 µL, 25x RT-PCR Enzyme Mix 1 µL, RNA sample of 2 µL, Forward and reverse Taqman primer –probe mixture of 2 µL, nuclease free water of 7.5 µL with the total reaction volume of 25 µL. *ACTB* gene (Thermoscientific) was used as internal control for the PCR. The primer – probes [7] used in the reaction are as shown in table 1.

TABLE 1: shows the primer - probe used in the Taqman gene expression assay:

GENE	PRIMER/PROBE TYPE	SEQUENCE
RET/PTC1	FORWARD PRIMER	CGCGACCTGCGCAAA
	REVERSE PRIMER	CAAGTTCTTCCGAGGGAATTCC
	PROBE	6**FAM**CAAGCGTAACCATCGAGGATCCAAAGT TAMRA
RET/PTC3	FORWARD PRIMER	CCCCAGGACTGGCTTACCC
	REVERSE PRIMER	CAAGTTCTTCCGAGGGAATTCC
	PROBE	6**FAM** AAAGCAGACCTTGGAGAACAGTCAGGAGGTAMRA
ACTB	FORWARD PRIMER	TGACGGGGTCACCCACACTGTGCCCATCTA
	REVERSE PRIMER	CTAGAAGCATTGCGGTGGACGATGGAGGG
	Probe	6**FAM**CCG GCT TCG CGG GCG AC - TAMRA

The reaction was performed on Quantstudio 5 with the PCR condition as mentioned in Table 2. The results of RET/PTC1 and RET/PTC3 gene expression assay was correlated with various clinicopathological features using descriptive statistics.

Table 2: Shows PCR conditions used for *RET*/PTC 1 and 3 and *ACTB* gene Taqman gene expression assay.

Settings	Step 1	Step 2	Step 3	
stage	Reverse transcription	Denaturation	PCR (Denature)	PCR (anneal /extend)
temperature	45^0	95^0	95^0	60^0
time	10 mins	10 mins	15 sec	45

The results were looked for association with various clinico-pathological features for statistical significance. The data was analyzed using descriptive statistics and association between RET/PTC rearrangement and various histomorphological features were assessed using Fisher's Exact Test,

Likelihood ratio and Liner – by linear association tests.

RESULTS

A total of 18 PTC specimens were included in the study. There were 6 (33.3%) males and 12 (66.7%) females with age range from 21 years to 68 years and mean age of 38.5 yrs. Majority of the cases were between 30-39 years of age (55.6%).

On gross examination 5 cases (27.8%) had tumour size < 2 cms, 8 cases (44.4%) had tumour size of 2 to 4 cms and 5 cases (27.8%) had tumour size of > 4 cms. On microscopic examination conventional PTC with papillary pattern was seen in 10 cases (55.6%) and follicular variant of PTC seen in 8 cases (44.4%).

RET/PTC rearrangement was seen in 3/18 cases (16.7%) with *RET*/PTC1 rearrangement being positive in 1/18 case (5.6%) and *RET*/PTC3 rearrangement being positive in 2/18 cases (11.1%) as seen in **figure 1**.

Figure 1: showing RT-PCR amplification plot for *RET*/PTC gene rearrangement with *ACTB* (β actin) as internal control.

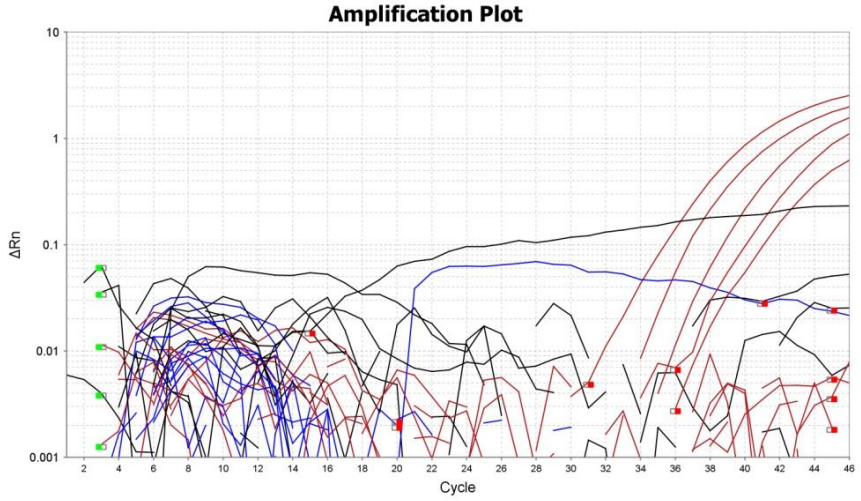

Image shows amplification plot of RET/PTC in (black) and ACTB gene (red) as internal control.

The association of *RET*/PTC rearrangement with various histomorphological features are as shown in table 3.

Table 3: shows association of *RET*/PTC rearrangement with various histomorphological features

Sl No	VARIABLE	No of cases with percentage	RET/PTC (type)	
1.	Male	6 (33.3%)	1(5.6%)	RET/PTC1
	Female	12 (66.7%)	2 (11.1%)	RET/PTC1 RET/PTC3
2	Age	21 - 68 years	21, 40 yrs	RET/PTC3
			60 yrs	RET/PTC1
3. Gross	<2 cms	5 (27.8%)	1 (5.6%)	RET/PTC3
	2-4 cms	8 (44.4%)	1 (5.6%)	RET/PTC3
	>4 cms	5 (27.8%)	1 (5.6%)	RET/PTC1
4. microscopy	Papillary	10(55.6%)	1 (5.6%)	RET/PTC3
	follicular	8(44.4%)	2 (11.1%)	RET/PTC1, RET/PTC3
5.	Multi-focality	4/18 (22.2%)	No statistically significant association noted	
6.	Lymph node involvement	2/18 (13.3%)	No statistically significant association noted	
7.	Extra-thyroidal extension	3/18 (20.0%)	No statistically significant association noted	

DISCUSSION

Thyroid is an endocrine organ with malignancy rate accounting for approximately 1% of all newly diagnosed cancer cases. The most frequent type of thyroid malignancy is PTC, which constitutes for more than 80% of all cases [(12).]

The incidence of PTC increases with age, and women are more frequently affected than men, in the ratios of 2:1 to 4:1[(12)]. The female to male ratio in our study was 2:1, which is similar to another study done in Italy by Basolo et al which showed male : female ratio of 2.2:1[(13)]

Various genetic alterations that can be seen in PTC are *BRAF* mutation, *RET*/PTC translocation, *RAS* gene mutation, mutation in *TERT* promoter gene and *ALK* translocation [(4)]

In a study conducted by George N at al in North India to study the prevalence of genetic alterations in Endemic goitre population found no cases with *RET*/PTC translocation out of 109 cases that they studied[(14)]

Among the 13 different types of *RET*/PTC rearrangements, the *RET*/PTC1 and *RET*/PTC3, are the commonest type of rearrangements seen in PTC. The most common *RET* fusions in PTC are CCDC6-*RET* (also named *RET*/PTC1) and NCOA4-*RET* (also named *RET*/PTC3), accounting for about 90% of all the *RET* gene fusion-positive cases [(15)] . Both *RET*/PTC1 and *RET*/PTC3 are intrachromosomal paracentric inversions as both the genes participating in the fusion are located on chromosome 10q [(16, 17,18)]

There is paucity of literature in studies of *RET*/PTC translocation in PTC in India. A study conducted by Mishra A et al.[(19)] studied the prevalence of *RET*/PTC translocation in PTC by immunohistochemistry in endemic goitre areas of India and found the prevalence to be 44%.

The study by Liu RT et al showed *RET* rearrangements in 8 of 105 (8%) sporadic PTCs in Taiwan, which was a much lower prevalence than previously reported for this population but comparable to those reported in other nations using similar methodology [(20)]. Whereas according to a study conducted by Zhu Et al the prevalence of RET/PTC translocation varied from 17-40% based on the technique used for detection of RET/PTC activation [(21)]

The molecular genetic study by Puxeddu et al demonstrated, positivity of *RET*/PTC1 and *RET*/PTC3 in 13 of 48 tumors (27.1%), and an exclusive or preferential RET-TK expression was seen in 17 cases (35.4%) [(22)].

In our study 3/18 cases (16.7%) showed *RET*/PTC rearrangement, with *RET*/PTC1 seen in 5.6% and *RET*/PTC 3 seen in 11.1% cases.

Fenton et al showed that among adults, *RET*/PTC1 was common and may be associated with an aggressive clinical course. They described that the incidence and significance of *RET*/PTC rearrangements were less well understood among children. In their study out of 74 children with PTC, no correlation between the *RET*/PTC rearrangement with age, tumor size, focality, and the extent of disease at the time of diagnosis, or recurrence were noted [(23)]

In the study by Erdogan M et al, on 101 patients with PTC, showed *RET*/PTC positivity in 67 (66.3%) cases with p value of <0.001. *RET*/PTC1 was positive in 32(31.7%), and *RET*/PTC3 in 21(20.8%) cases, with both rearrangements being positive in 10 (9.9%) cases. Their study showed no significant association between *RET*/PTC expression and the clinical and pathological features[(24)].

In 2018, Khan et al studied *RET*/PTC rearrangement among 40 cases of PTC in Kashmiri's and observed that *RET*/PTC rearrangements were confined to 10/40 (20.83%) cases. Presence of *RET*/PTC rearrangement significantly correlated with gender, elevated TSH levels and lymph node metastasis. They concluded that *RET*/PTC3 rearrangement was a frequent event in the oncogenesis of thyroid[(7)]

In 2014, Rao et al did quantitative estimation of *RET*/PTC1 and *RET*/PTC3 gene rearrangements by real-time PCR on 30 cases of PTC. They showed no statistically significant differences between positive and negative RET/PTC3 mRNA expression with clinico- pathological features. They concluded that *RET*/PTC3 gene rearrangements were the most prevalent form of rearrangements in PTCs among Chennai population[(8)].

In the study by Lee et al, on 11 PTC patients, two PTC had *RET*/PTC1, 3 had *RET*/PTC2, and 1case had *RET*/PTC3 rearrangement. Although the cause of high frequency of *RET*/PTC rearrangement in Chinese papillary thyroid carcinomas was unknown, their study showed that *RET* rearrangement is an important genetic lesion underlying the development of thyroid papillary carcinoma in Taiwan [(25)].

Our study showed no statistically significant association between sex, gross size of PTC, microscopic pattern, extra thyroidal extension, lymph node metastasis and multifocality of the lesion with RT/PTC translocation which might translate that RET/PTC may not be implicated in prognosis or the outcome of the disease process. The findings of our studies are similar to another study done in Italy by Basolo et al [(13)] .

CONCLUSION

In our study, the frequency of *RET*/PTC gene translocation in PTC was 16.7%, with RET/PTC1 and RET/PTC3 gene rearrangement being 5.6% and 11.1% respectively. No statistically significant association of *RET*/PTC gene translocation with various clinico-pathological features were noted. To the best of our knowledge, this is a first study from Karnataka on prevalence and clinico-pathological association of PTC with RET/PTC gene rearrangements. Larger studies in this regard are essential to confirm whether *RET*/PTC is a driver

genetic alteration in PTC and if there are other genetic events in addition to *RET*/PTC activation responsible for malignant phenotype and cancer progression.

REFERENCES

1. Du L, Wang Y, Sun X, Li H, Geng X, Ge M, Zhu Y. Thyroid cancer: trends in incidence, mortality and clinical-pathological patterns in Zhejiang Province, Southeast China. BMC Cancer. 2018 Mar 15;18(1):291. doi: 10.1186/s12885-018-4081-7. PMID: 29544469; PMCID: PMC5856225.
2. Veedu JS, Wang K, Lei F, Chen Q, Huang B, Mathew A. Trends in thyroid cancer in India. J Clin Oncol 2018;36:15.
3. Rao R, Giriyan SS, Rangappa PK. Clinicopathological profile of papillary carcinoma of thyroid: A 10-year experience in a tertiary care institute in North Karnataka, India. Indian J Cancer. 2017 Jul-Sep;54(3):514-518. doi: 10.4103/ijc.IJC_337_17. PMID: 29798949.
4. Rosai J, Albores JS, Asioli S, Baloch WZ, Bogdanova T, Chen H, et al. Papillary thyroid carcinoma. In: WHO Classification of Tumors Of Endocrine origin. 4th ed. Lyon; 2017. p. 81–91.
5. Prete A, Borges de SouzaP, Censi S, Muzza M,Nucci N and Sponziello M. Update on Fundamental Mechanisms of Thyroid Cancer.Front Endocrinol.2020 March; 11:102
6. Romei C, Elisei R. RET/PTC Translocations and Clinico-Pathological Features in Human Papillary Thyroid Carcinoma. Front Endocrinol (Lausanne). 2012 Apr 11;3:54. doi: 10.3389/fendo.2012.00054. PMID: 22654872; PMCID: PMC3356050.
7. Khan MS, Qadri Q, Makhdoomi MJ, Wani MA, Malik AA, Niyaz M, Masoodi SR, Andrabi KI, Ahmad R, Mudassar S. RET/PTC Gene Rearrangements in Thyroid Carcinogenesis: Assessment and Clinico-Pathological Correlations. Pathol Oncol Res. 2020 Jan;26(1):507-513. doi: 10.1007/s12253-018-0540-3. Epub 2018 Nov 22. PMID: 30467698.
8. Rao PJ, Vardhini NV, Parvathi MV, Murthy PB, Sudhakar G. Prevalence of RET/PTC1 and RET/PTC3 gene rearrangements in Chennai population and its correlation with clinical parameters. Tumour Biol. 2014 Oct;35(10):9539-48. doi: 10.1007/s13277-014-1909-x. Epub 2014 Jun 24. PMID: 24957039.
9. Nikiforov YE. RET/PTC rearrangement in thyroid tumors. Endocr Pathol. 2002 Spring;13(1):3-16. doi: 10.1385/ep:13:1:03. PMID: 12114746.
10. The cancer genome research network. Integrated genomic characterisation of papillary thyroid carcinoma. Cell. (2014) 159:676-90. doi: 10.1016/j.cell. 2014.09.050
11. Rhoden KJ, Johnson C, Brandao G, Howe JG, Smith BR, Tallini G. Real-time quantitative RT-PCR identifies distinct c-RET, RET/PTC1 and RET/PTC3 expression patterns in papillary thyroid carcinoma. Lab Invest. 2004 Dec;84(12):1557-70. doi: 10.1038/labinvest.3700198.
12. Raffaele Ciampi, Yuri E. Nikiforov, RET/PTC Rearrangements and BRAF Mutations in Thyroid Tumorigenesis, Endocrinology, Volume 148, Issue 3, 1 March 2007, Pages 936–941, https://doi.org/10.1210/en.2006-0921
13. Basolo et al RET protein expression has no prognostic impact on the long-term outcome of papillary thyroid carcinoma. Eur. J. Endocrinol. 2001
14. George N, Agarwal A, Kumari N, Agarwal S, Krisnani N, Gupta SK. Mutational profile of papillary thyroid carcinoma in an endemic goiter region of North India. Indian J Endocr Metab 2018;22:505-10
15. Santoro M, Moccia M, Federico G, Carlomagno F. RET Gene Fusions in Malignancies of the Thyroid and Other Tissues. Genes (Basel). 2020 Apr 15;11(4):424. doi: 10.3390/genes11040424. PMID: 32326537; PMCID: PMC7230609.
16. Abdullah MI, Junit SM, Ng KL, Jayapalan JJ, Karikalan B, Hashim OH. Papillary Thyroid Cancer: Genetic Alterations and Molecular Biomarker Investigations. Int J Med Sci. 2019 Feb 28;16(3):450-460. doi: 10.7150/ijms.29935. PMID: 30911279; PMCID: PMC6428975.
17. Grieco M, Santoro M, Berlingeri MT et al. PTC is a novel rearranged form of the ret proto oncogene and is frequently detected in vivo in human thyroid papillary carcinomas. Cell 1990 60 557-563.
18. Jhiang SM, Smanik P & Mazzaferri EL. Development of a single step duplex RT-PCR detecting different forms of ret activation and identification of the third form of in vivo ret activation in human papillary thyroid carcinoma. Cancer Letters 1994 78 69-76.
19. Mishra A, Agrawal V, Krishnani N, Mishra S K. Prevalence of RET/PTC expression in papillary thyroid carcinoma and its correlation with prognostic factors in a north Indian population.J Postgrad Med 2009;55:171-175
20. Liu RT, Chou FF, Wang CH, Lin CL, Chao FP, Chung JC, Huang CC, Wang PW, Cheng JT. Low prevalence of RET rearrangements (RET/PTC1, RET/PTC2, RET/PTC3, and ELKS-RET) in sporadic papillary thyroid carcinomas in Taiwan Chinese. Thyroid. 2005 Apr;15(4):326-35. doi: 10.1089/thy.2005.15.326. PMID: 15876154.
21. Zhu Z, Ciampi R, Nikiforova MN, Gandhi M, Nikiforov YE. Prevalence of RET / PTC rearrangements in thyroid papillary carcinomas: Effects of the detection methods and genetic heterogeneity. J Clin Endocrinol Metab 2006;91:3603-10.
22. Puxeddu E, Moretti S, Giannico A, Martinelli M, Marino C, Avenia N, Cristofani R, Farabi R, Reboldi G, Ribacchi R, Pontecorvi A, Santeusanio F. Ret/PTC activation does not influence clinical and pathological features of adult papillary thyroid carcinomas. Eur J Endocrinol. 2003 May;148(5):505-13. doi: 10.1530/eje.0.1480505. PMID: 12720532.
23. Fenton CL, Lukes Y, Nicholson D, Dinauer CA, Francis GL, Tuttle RM. The RET/PTC mutations are common in sporadic papillary thyroid carcinoma of children and young adults. J Clin Endocrinol Metab. 2000 Mar;85(3):1170-5. doi: 10.1210/jcem.85.3.6472. PMID: 10720057.
24. Erdoğan M, Berdeli A, Karadeniz M, Ertan Y, Cetinkalp S, Saygili F, Tuncyurek M, Yilmaz C, Tuzun M, Kabalak T, Uluer H, Ozgen AG. The prevalence of RET/PTC mutations in papillary thyroid cancers in Turkish population and its relation between tumor histopathology and prognostic factors. Exp Clin Endocrinol Diabetes. 2008 Apr;116(4):225-30. doi: 10.1055/s-2007-992153. PMID: 18393128.

25. Chen-Hsen Lee, Li-Sung Hsu, Chin-Wen Chi, Gen-Der Chen, An-Hong Yang, Jeou-Yuan Chen, High Frequency of Rearrangement of the *RET* Protooncogene (*RET/PTC*) in Chinese Papillary Thyroid Carcinomas, *The Journal of Clinical Endocrinology & Metabolism*, Volume 83, Issue 5, 1 May 1998, Pages 1629–1632, https://doi.org/10.1210/jcem.83.5.4774