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RESEARCH ARTICLE

Molecular Genetics

BRAF (V600E) gene mutation in patients with thyroid cancer in Sri Lanka: A pilot study

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Abstract: Thyroid cancer (TC) is the most common endocrine malignancy worldwide. Occasionally, inadequate treatment of malignancies and overtreatment of benign conditions result due to inconclusive diagnosis, while poor clinical outcomes occur due to cancer recurrence or resistance to existing therapies. Elucidation of the molecular genetic basis of TC potentiates its diagnosis and treatment. BRAF(V600E) is a commonly occurring mutation in TC. This retrospective cross-sectional study was undertaken to determine the prevalence of this mutation in a cohort of Sri Lankan TC patients. DNA was extracted from formalin-fixed paraffin-embedded (FFPE) thyroid tissue samples (malignant subtypes, n = 50 (papillary, follicular, medullary, anaplastic, and oncocytic thyroid carcinomas); benign type, follicular adenoma, n = 26) using the phenol-chloroform method. Mutant allele specific PCR (sensitive to 1-5% mutant allele detection in a wild-type background) amplified BRAF wild-type, and mutant alleles. Genotypes were established by visualizing PCR amplicons (125 bp) of each allele type on agarose gels. Based on the allele specific PCR used for molecular diagnosis, most of TC patients in the study population (n = 68) were of the heterozygous genotype. One individual with papillary TC was homozygous for the mutant allele, and the remaining seven were homozygous for the wild-type allele. As this mutation was not restricted to malignant subtypes in comparison to the benign subtype ($p > 0.05$), its applicability as a diagnostic marker remains contentious. These preliminary findings underpin the potential for optimizing TC management through molecular diagnosis using available targeted therapy.

Keywords: BRAF(V600E) gene mutation, malignant types, molecular marker, targeted therapy, thyroid cancer.

INTRODUCTION

Thyroid cancer (TC) is the most common endocrine malignancy, with a continuously increasing disease burden, with an exponential rise in cases both globally and locally. TC is described under four main pathological types; papillary thyroid carcinoma (PTC), follicular thyroid carcinoma (FTC), medullary thyroid carcinoma (MTC), and anaplastic thyroid carcinoma (ATC) (Ge *et al.*, 2020). Other rare types include oncocytic carcinoma (OC, previously known as Hürthle cell carcinoma), and insular cancer (Liska & Galbavy, 2005). The most prominent subtype (80%) is PTC (Ito *et al.*, 2014).

TC has a higher global mortality rate than other endocrine malignancies (Kobawala *et al.*, 2016). Currently, TC represents approximately 2.1% of all newly diagnosed tumours worldwide (Crispo *et al.*, 2019), with an unprecedented rise in the cumulative number of living cases of TC (Xing, 2019). This disease affects women threefold more frequently than men (Warakomski *et al.*, 2018). Since the latter part of the last century, TC has increased at a rapid rate, especially among women in developed countries (Jayarajah *et al.*, 2018). In countries such as the United States (Jung *et al.*, 2014), Canada (Liu *et al.*, 2001) and Australia (Grodski *et al.*, 2008), the incidence of TC has more than doubled over the last few decades; developing nations in the Asian region recorded

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a greater incidence of TC, compared to the West (Kilfoy *et al.*, 2009). China has witnessed a four-fold increase in TC over 25 years (1983 - 2007) (Wang & Wang, 2015). The increasing incidence rate has evoked much interest and concern towards the management of TC.

The National Cancer Control Programme of the Ministry of Health, Sri Lanka maintains the most comprehensive database on information regarding TC in Sri Lanka. Accordingly, the local incidence of TC has increased significantly, as in other parts of the world (Jayarajah *et al.*, 2018). In 2014, the thyroid gland became the fifth leading cancer site among Sri Lankans *(Cancer Incidence Data Sri Lanka, 2014)*. Currently, it is the third most common cancer among Sri Lankan women, having accounted for 8.7% of the new female cancer cases in 2020 (International Agency for Research on Cancer, 2020).

Although TC elicits excellent prognosis, conventional diagnostic tests occasionally lead to inconclusive results, that may either cause inadequate treatment of aggressive, high-risk thyroid malignancies or overtreatment of low-risk, benign conditions (Xing, 2019). In addition, despite large costs and resources incurred on prevailing therapeutic methods, some patients manifest poor clinical outcomes due to resistance to existing treatment options, and cancer recurrence. These complications contribute to the continuously increasing disease burden and associated cost of treatment, highlighting the need for novel diagnostics and treatment modalities.

Molecular targeted therapies for TC are based on the concept of precision medicine. A major challenge faced by clinicians is balancing the therapeutic approach towards patients with different types of TC (Gil *et al.*, 2020), which can be resolved using molecular markers for efficient TC diagnosis, and molecular targeted therapy.

Most of the genetic alterations implicated in TC lead to the aberrant activation of the RAS–RAF–MEK–MAP kinase cell signaling pathway (Frasca *et al.*, 2008). Four types of somatic genetic alterations are extensively studied due to their potential in the diagnosis and prognosis of follicular cell-derived thyroid carcinomas (TCs). These are BRAF mutations, RAS mutations, RET/PTC, and PAX8/PPARγ rearrangements (Bozec *et al*., 2013). In addition, TERT promoter mutations, TP53 mutations, and NTRK fusions have also been reported in TC (Prasad *et al.*, 2016; Volante *et al.*, 2021). RAS mutations occur in about 21% of PTC and 57% of FTC (Howell *et al.*, 2013). RET/PTC rearrangement is the second most common genetic alteration in PTC, accounting for a prevalence of approximately 20% of PTC cases (Bozec *et al.*, 2013). PAX8/PPARγ rearrangements occur in approximately 30 – 35% of FTC cases (Raman & Koenig, 2014). In familial MTC, germ-line mutations in the RET gene have been reported while the inactivation of the p53 tumor-suppressor gene occurs in ATC (Xing, 2005). The prevalence of TERT promoter mutation is lower in PTC (~ 12%), but higher in poorly differentiated or undifferentiated types of TC such as ATC, accounting for about 50% in ATC cases (Soares *et al.*, 2021).

BRAF (B-type Raf kinase protein) gene mutations are known to play a significant role in the manifestation and progression of thyroid tumours. BRAF is an integral part of the RAS/MAP kinase pathway (Xing, 2005). The most common BRAF mutation observed in TC, especially the PTC subtype, is the BRAF (V600E) mutation (Santonastaso *et al.*, 2019) which causes a valine (V) to glutamate (E) substitution at the 600th amino acid residue of the protein (Bozec *et al.*, 2013). Upon mutation, the V600E substitution disrupts the hydrophobic interactions and retains the protein in a catalytically active conformation (Moulana *et al.*, 2018), causing elevated kinase activity and tumorigenesis (Lassalle *et al.*, 2010). It is considered as an "early driver" mutation in TC (Volante *et al.*, 2021). The reported prevalence of BRAF V600E mutation in PTC ranges from 40 – 80%, while it is less prevalent (1.4%) (Kebebew *et al.*, 2007) or absent (Tennakoon *et al.*, 2017) in the FTC subtype, which is mostly associated with RAS mutations. In poorly differentiated TC subtypes such as ATC, BRAF V600E prevalence ranges from 10% - 50% (Volante *et al.*, 2021). Its presence has also been reported in oncocytic carcinoma (Hürthle cell carcinoma), but within a small fraction of cells within the tumour area (McFadden & Sadow, 2021).

BRAF mutational analysis has been established as part of the diagnostic protocol for TC in Italy (Santonastaso *et al.*, 2019), while revised guidelines of the American Thyroid Association recommend the use of molecular markers including BRAF during cases of indeterminate fine needle aspiration (FNA) test results (Haugen *et al.*, 2016).

This pilot study aims to assess the prevalence of the BRAF(V600E) mutation in a cohort of thyroid cancer patients in Sri Lanka, highlighting the need to investigate its prognostic impact for effective molecular diagnosis, and management in the local context.

MATERIALS AND METHODS

Study population

The study was based on patients reported to the National hospital, Kandy, the second largest tertiary care state medical institution in Sri Lanka. Formalin-fixed paraffin-embedded (FFPE) cancerous thyroid tissue samples of patients who had been initially diagnosed with thyroid cancer by histopathological examination by an experienced histopathologist, were included in the study. Those with co-morbidities and indistinct diagnosis were excluded. Prior to the acquisition of samples, inclusion of the tumour area in the FFPE thyroid tissue was confirmed by the same histopathologist who confirmed the initial diagnosis.

Thus, FFPE samples of patients with thyroid cancer sub-types of PTC, FTC, MTC and ATC were included in the study. Samples of patients diagnosed with follicular adenoma (FA) were selected as controls due to the reported absence of BRAF V600E mutation (0 %) in FFPE samples of this benign tumour type (Sapio *et al.*, 2006; Yoo *et al.*, 2016). Accordingly, FFPE samples were retrospectively selected from medical records, reported within the year 2020.

As advocated by WHO guidelines in 1991, the sample size calculation was carried out using the following formula (Charan and Biswas, 2013):

$$\text{Sample size} = \frac{Z_{1-\alpha/2}^{2}\, p\,(1 - p)}{d^2}$$

where,

n = Desired sample size population <10,000
Z = Standard normal deviate – set at 1.96 at 95% confidence level
p = Proportion of subjects (considering the 5-year prevalence proportion) – 0.02492%
d = absolute precision or sampling error tolerated = 5%

A sample size of 37 was obtained when the formula was applied based on the island-wide 5-year prevalence data of thyroid cancer patients.

PCR analysis of samples for BRAF(V600E) mutation

DNA was extracted using the phenol-chloroform method, from 10 μm thick microtome sections of FFPE thyroid tissue samples (Sambrook & Russel, 1982). The quality of the extracted DNA was determined by agarose gel electrophoresis. Two separate allele-specific PCRs (targeting the wild type and the mutant alleles) were performed on each DNA sample, on Mutant allele specific PCR amplification (MASA) to detect the presence of the BRAF(V600E) gene mutation (Sapio *et al.*, 2006). The wild type allele of the BRAF gene was amplified using a forward primer specific for the wild type allele (5'GTGATTTTGGTCTAGCTACAGT-3'), with the common reverse primer (5'-GGCCAAAATTTAATCAGTGGA- 3') (Davies *et al.*, 2002). The mutant allele of the BRAF gene, with the transversion mutation, was amplified using the forward primer specific for the mutant allele (5'-GTGATTTTGGTCTAGCTACAGA-3') and the common reverse primer.

PCR amplicons were visualized on 2% agarose gels (Santonastaso *et al.*, 2019). A band corresponding to 125 bp indicated the presence of each allele. A DNA sample confirmed by sequencing to contain the mutant allele was used as the positive control. The genotype of the BRAF gene of each study subject was thus established.

Clinical and socio-demographic information of study subjects

Clinical information such as the histological TC subtype diagnosis, age at incidence, and gender were obtained from the clinical records of the patient. In addition, information on the diet (daily dietary intake patterns based on the types of food) and habits (smoking, alcohol consumption, exercise) associated with the day-to-day lifestyle of the study population were collected using an interviewer-administered questionnaire. For this purpose, the post-surgery patients whose FFPE samples were selected for the study were interviewed on their subsequent visits to the hospital clinic for routine checkups.

Statistical analysis

SPSS version 26.0 (IBM, USA) software for Microsoft Windows was used for the statistical analyses of data. The Chi-square test was used to compare categorical variables. The Mann-Whitney U test and Kruskal-Wallis test were used to assess the significance of the difference of a variable among groups, where appropriate. The significance level was set at $p < 0.05$.

Ethical clearance

Ethical approval for the study was granted by the Ethics Review Committee of the Institute of Biology, Sri Lanka (Application No: ERC IOBSL 224 10 2020).

RESULTS AND DISCUSSION

Prevalence of TC types, demographic, and clinical data of the study cohort

The cohort of malignant TC samples (n = 50) of the five TC sub-types (PTC, FTC, MTC, ATC, OC) were representative of the prevalence of each sub-type in the country (Jayarajah *et al.*, 2018). A comparison between the percentages of samples collected for each malignant sub-type, against its prevalence is presented in Table 1. The study group comprised a total of 76 individuals (67 females and 9 males); the selected samples represented all malignant types of TC present in Sri Lanka (a total of 50; n = 33 of PTC, 11 of FTC, 02 of ATC, 03 of OC, and 01 MTC), while follicular adenoma (FA) samples (n = 26) represented a benign thyroid tumour condition. Of the 33 PTC samples, 15 were papillary microcarcinomas.

Demographic details of the study subjects are presented in Table 2. An overall female preponderance was evident, both with the malignant and the benign tumor conditions. Yet, the proportion of females and males with malignant tumours did not significantly differ from the benign tumor condition ($p > 0.05$). Though not statistically significant, the mean age of males at the onset of thyroid tumour was higher than that of females ($p > 0.05$).

Table 1: Percentage distribution of each malignant thyroid cancer sub-type within the study cohort and the respective island-wide prevalence

	Subtype of Thyroid Cancer				
	PTC	FTC	MTC	ATC	OC
Prevalence within the study cohort	66%	14.47%	1.31%	2.63%	3.94%
Local prevalence[a]	69%	18.4%	1.8%	3.71%	7.1%

[a] (Jayarajah *et al.*, 2018); PTC- Papillary thyroid cancer; FTC - Follicular thyroid cancer; MTC – Medullary thyroid cancer; ATC – Anaplastic thyroid cancer; OC- oncocytic carcinoma.

Table 2: Summary of demographic and clinical data of study subjects

	Diagnosis					
	PTC	FTC	MTC	ATC	OC	FA
Number of patients	42	12	01	02	03	45
Number of females	35	11	1	2	3	36
Number of males	7	1	-	-	-	9
Number of FFPE tissue samples obtained	33	11	01	02	03	26
Mean age (years) (± SD)	45.8 (±13.5)	52.7 (±16.1)	73	66 (± 4.00)	52.3 (± 11.5)	46.4 (± 13.0)
Mean age of females (years) (± SD)	43.6 (±12.4)	49.7 (±14.5)	73	66 (± 4.00)	52.3 (± 11.5)	44.8 (± 12.7)
Mean age of males (years) (± SD)	49.6 (± 9.4)	73	-	-	-	52.9 (± 11.6)

Values are presented as mean ± SD. PTC – Papillary thyroid cancer; FTC - Follicular thyroid cancer; MTC – Medullary thyroid cancer; ATC – Anaplastic thyroid cancer; OC- Oncocytic carcinoma; FA – Follicular adenoma

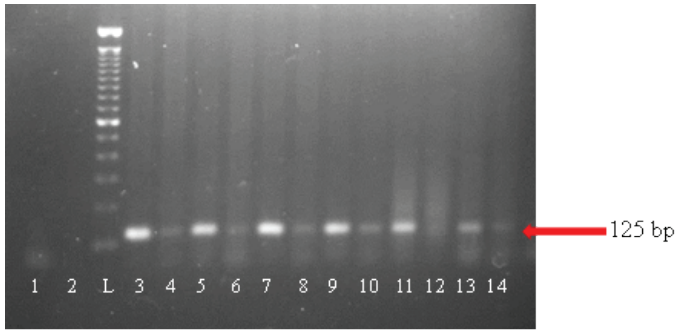

Figure 1: Visualization of BRAF wild-type and mutant allele-specific PCR products of 6 DNA samples. Lanes 1 and 2 - Reagent controls, for the wild type (WT) and the mutant allele, respectively; L – 100 bp ladder; 3, 5, 7, 9, 11, 13 – PCR products of WT alleles and 4, 6, 8, 10, 12, 14 - Mutant alleles of the identical 6 samples.

Table 3: BRAF genotype distribution of study subjects

Type of thyroid tumour	Homozygous for wild-type allele	Homozygous for mutant allele	Heterozygous
PTC (n = 33)	04	01	28
FTC (n = 11)	-	-	11
MTC (n = 01)	-	-	01
ATC (n = 02)	-	-	02
OC (n = 03)	-	-	03
FA (n = 26)	03	-	23

PTC – Papillary thyroid cancer; FTC – Follicular thyroid cancer; MTC – Medullary thyroid cancer; ATC – Anaplastic thyroid cancer; OC – oncocytic carcinoma; FA – Follicular adenoma.

BRAF genotype of the study subjects

The molecular analysis (Figure 1) established that a majority of the study group were heterozygous (n = 68) for the BRAF gene, while one individual diagnosed with PTC, exclusively carried the mutant allele. The rest of the study participants harbored only the wild type allele (n = 4 of PTC; n = 3 of FA). The BRAF genotype of the study participants are presented in Table 3.

Positivity for the BRAF(V600E) mutation was not significant in malignant subtypes, when compared with the benign subtype ($p > 0.05$). The frequency of the BRAF(V600E) mutation among the thyroid tumor subtypes were 87.88% of PTC, 100% of FTC/ MTC/ ATC/ OC, and 88.46% of FA.

As the genetic constitution of a population varies from region to region, and Sri Lanka has a higher degree of endemism than non-island nations, it was essential to determine the prevalence of the BRAF(V600E) mutation in a cohort of Sri Lankan TC patients. In the current study, of the individuals diagnosed with the conventional PTC (n = 18), only 4 lacked the mutant allele. However, all papillary microcarcinomas (n = 15), a subcategory of PTC, were positive for the mutation. The overall BRAF positivity of PTC subtype (~88%) accounted for a higher proportion than previously reported (Xing, 2005; Silver *et al.,* 2021).

In addition to PTC, positivity for the BRAF V600E mutation was also observed in all FFPE samples of other TC subtypes (FTC, ATC, MTC, and OC) and in most FA samples. Existing studies which report the prevalence of BRAF V600E mutation include study cohorts from Chinese (Xing, 2005; Cong *et al.*, 2024) or European populations (Sapio *et al.*, 2006).

A significant difference was not observed when BRAF positivity in malignant TC subtypes was compared with that of the benign category, FA ($p > 0.05$). While most studies that checked for the presence of BRAF V600E in FA had reported a 0% frequency (Sapio *et al.*, 2006; Yoo *et al.*, 2016), conversely, in a study based on BRAF V600E detection in FNA biopsies, Chen *et al.* (2018) reported that of 36 samples positive for BRAF V600E mutation, 5 were confirmed benign, indicating a prevalence of approximately 14% (Chen *et al.*, 2018). Therefore, although execdingly rare, BRAF V600E mutation may be present in the benign tumour type FA. It is important to note that the FA samples were not reassessed by histopathology to exclude the missed possibility of the samples carrying the encapsulated follicular variant of PTC. In TC pathogenesis, there is a possibility for the encapsulated variant of PTC to arise in FA. This remains a challenge in FA management and TC diagnosis (Singh *et al.*, 2018). The FA patients, whose FFPE samples harboured the mutant allele, could have a predisposition to malignancy, which could lead to the follicular variant of PTC (Tennakoon *et al.*, 2017; Singh *et al.*, 2018). Longitudinal studies of FA patients are necessary to confirm this possibility.

Considering the genotypes, a single PTC patient sample was homozygous for the mutant allele, while all other BRAF-positive samples were heterozygous, carrying only one mutant allele. Based on the available literature, the presence of one mutant allele is sufficient to induce phenotypic alterations compared to the homozygous wild-type genotype (Sapio *et al.*, 2006). Studies based on PTC have indicated that the percentage of BRAF V600E positive alleles in a tumour area, which mostly consists of a mixture of tumour cells carrying both wild-type and mutant BRAF, is positively correlated with the tumour burden (Guerra *et al.*, 2012; Cheng *et al.,* 2014).

This study was based on the mutant-allele-specific PCR method (Sapio *et al.*, 2006), which has a high sensitivity of detection of 1-5% BRAF V600E mutation in a wild-type background (Yang *et al.*, 2017), comparable to other available, more sensitive, testing methods such as real-time PCR (1-2% mutation detection) with hybridizing probes, and amplification refractory mutation system-PCR (0.5% mutation detection). Other molecular methods such as Sanger sequencing (15 – 20%), high-resolution melting analysis (5-10%), *etc*. have relatively lower sensitivities (Sapio *et al.*, 2006; Huang *et al.*, 2013; Pisareva *et al.*, 2014). Therefore, varying sensitivity of the methods used to detect the mutation may yield incongruent results.

Risk factors of TC

Risk factors assessed in relation to thyroid tumour onset were family history, gender, age, dietary intake, habits, and body mass index (BMI) of which none significantly correlated with thyroid tumour onset ($p > 0.05$). Most study subjects were between 31–50 years old, at the diagnosis of thyroid tumour. Obesity was prominently observed in the current study group. A significant increment in the mean age of BRAF(V600E) positive individuals, compared to BRAF(V600E) negative individuals, was observed in the PTC subtype ($p \leq 0.05$).

The onset of thyroid tumours in females at an earlier age is speculated to be a result of the sex hormones and pregnancy-related hormones of females at this age, which are clear distinctions from the male physiology (Rahbari *et al.*, 2010). Overall, most patients were aged between 31 and 50 years. When considering the BMI status of study subjects, most individuals belonged to the obese category. The outcome of this pilot study warrants to be confirmed via an in-depth investigation using a larger cohort of TC patients.

The observed female-to-male ratio (6.5:1) was comparable with the age-standardized global incidence rate of TC between the two genders (International Agency for Research on Cancer, 2020). The mean age of onset of TC in males was slightly higher than that of females. This observation was in agreement with the trends observed in previous studies conducted using the data of the Surveillance, Epidemiology and End Results (SEER) Program of the US (Rahbari *et al.*, 2010).

The high disease burden of TC has significant implications for a nation's healthcare system and resource allocation. Surgical facilities and radioactive iodine, the available therapeutic modalities for TC, are in short supply in many developing countries, including Sri Lanka (Jayarajah *et al.*, 2018). Hence, application of molecular markers in TC diagnosis and treatment could benefit the free healthcare system and therefore, the patients of any developing nation.

Oncogenic BRAF gene mutations are significant in TC-based research due to their association with the onset and progression of the disease (Xing, 2005; Wang *et al.*, 2018). Of these, the most commonly reported and extensively studied mutation concerning TC is the BRAF(V600E) mutation (Xing, 2005; Silver *et al.*, 2021). The prevalence and potential of this gene mutation to serve as a molecular marker for TC have

been assessed in several countries. Subsequently, this has led to FDA-approved genetic screening tests for BRAF(V600E) mutation before treatment; drugs which target this mutation and its aberrant products are currently under clinical trials (Crispo *et al.*, 2019). In Sri Lanka, although the clinical significance of molecular markers of TC, including the BRAF(V600E) mutation, has been acknowledged in few recent publications (Tennakoon *et al.*, 2017; Moulana *et al*., 2018), experimental studies have not been conducted to assess the prevalence of this mutation. Genetic screening is not yet routinely performed on TC patients, locally.

Based on this study, although the applicability of BRAF (V600E) as a prognostic or diagnostic marker for TC remains ambiguous, its potential to serve as a therapeutic marker remains significant. There are small-molecule drugs, recently approved by the FDA, designed to specifically target this mutation's aberrant protein product, such as "vemurafenib" and "dabrafenib" (Crispo *et al.*, 2019). These may have a positive impact on the subgroup of TC patients harboring the mutation. In Sri Lanka, these drugs are yet to be approved for clinical use. Preclinical trials performed elsewhere have indicated that these drugs are capable of restoring the radioactive iodine (RAI) uptake in BRAF (V600E) mutant cells which are resistant to RAI therapy (Crispo *et al.*, 2019). Currently, the FDA has approved the use of the targeted BRAF inhibitor, dabrafenib, along with the MEK inhibitor trametinib, for patients with any type of advanced solid tumours carrying the BRAF V600E mutation. This combination of targeted drugs led to highly promising results in a Phase II clinical trial based on patients (n = 16) with ATC carrying the BRAF V600E mutation, reporting an overall response rate of 69% in patients (Zhang et al., 2023). The study concluded that screening for the mutation should be performed in ATC patients due to the potential of targeted therapy to improve the clinical outcomes of the patients (Subbiah *et al.*, 2018). Hence, screening for the BRAF mutation holds the promise of facilitating more informed therapeutic decisions, especially for patients who do not respond to RAI therapy (Crispo *et al.*, 2019).

Analysis of additional mutations is increasingly utilized for TC prognosis (Xu, 2023; Chindris *et al.*, 2024). The presence of BRAFV600E alongside mutations in other genes such as PIK3CA, the TERT promoter, or TP53 is linked to poorer clinical outcomes. Emerging data indicate that the combination of specific genetic alterations with BRAF mutations could serve as better prognostic tools and predictors of TC aggressiveness (Ahmadi & Landa, 2024).

This pilot study investigated the prevalence of the BRAF V600E mutation among TC patients in Sri Lanka, motivated by its established prognostic and therapeutic significance. A notable limitation of this pilot study is the absence of mutation burden quantification, which may provide a better understanding of the prevalence of BRAF V600E mutation within the tumour area of each TC subtype and the benign tumour, FA. Detailed analysis of PTC subtypes and longitudinal studies focusing on follicular adenoma (FA) patients harboring the BRAF mutation to assess potential predisposition to malignancy in Sri Lankan patients are potential areas of future study. Additionally, the use of fine-needle aspiration (FNA) biopsy samples, alongside formalin-fixed paraffin-embedded (FFPE) tissues, could offer a complementary approach to assess the robustness of diagnostic methodologies of thyroid cancer.

CONCLUSION

BRAF(V600E) mutation is prevalent in a high proportion of Sri Lankan patients (91%) with thyroid cancer subtypes, and is also mirrored in the benign thyroid tumour, follicular adenoma (FA). However, as the frequency of BRAF(V600E) mutation was not distinct in TC when compared with the benign tumour, its applicability as a diagnostic marker is contentious. Yet, if present, it may serve as an effective therapeutic marker targeted in TC therapy. This can facilitate alternative therapeutic options, especially for TC patients who are resistant to RAI therapy, and for those who manifest cancer recurrence.

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