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Review Article

An Overview on Prevalence and Detection Approaches of BRAF V600E Mutation in Anaplastic Thyroid Carcinoma: A Systematic Review and Meta-Analysis

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Abstract

Background: BRAF V600E mutation is proved critical in the progression and invasion of thyroid cancer, and as a prognostic biomarker. As anaplastic thyroid cancer (ATC) is a rare and aggressive form of thyroid cancer, this study was conducted to provide a view on prevalence of BRAF V600E as well as the best molecular diagnostic method in ATC patients.

Methods: A comprehensive literature search was performed from their inception to Oct 2022 in PubMed, Scopus, Google Scholar, and Web of Science (WoS). The data of the prevalence of ATC were extracted. Moreover, the diagnostic feature of the available diagnostic tools was extracted to measure the sensitivity and specificity. To pool the prevalence data, we used meta-proportion analysis and diagnostic meta-analysis was conducted to determine the specificity and sensitivity of the immunohistochemistry method in detecting BRAF V600E mutation among patients with ATC.

Results: Overall, 34 studies were included in this meta-analysis. The incidence of BRAF V600E was shown 33% in the 978 patients. The sensitivity and specificity of IHC in detecting BRAF V600E were detected 78.9% (95%CI: 60.1-97.2), and 69.7% (95%CI: 41.2-98.1), respectively.

Conclusion: IHC had an acceptable prognostic profile for detecting BRAF V600E in ATC patients. The diagnosis of BRAF mutation is critical in clinical trials and may be helpful for choosing proper-targeted therapy strategies in ATC patients.

Keywords: Anaplastic thyroid carcinoma; Diagnostic methods; B-type Raf kinase V600E mutation; Systematic review

Introduction

Anaplastic thyroid cancer (ATC) represents a rare form of poorly differentiated thyroid cancer accounting for about 2% of all thyroid cancers. However, due to its rapid progression, aggressive

behavior and short median survival of patients (almost 3 to 6 months), ATC is responsible for over 30%–40% of total thyroid cancer deaths (1-3). ATC is derived from follicular cells of thyroid cancers and could develop and progress in a stepwise fashion from well-differentiated thyroid cancers such as papillary thyroid carcinomas (PTCs) and follicular thyroid carcinomas (FTCs) to poorly-differentiated thyroid carcinomas and then to ATC (4-7).

Through the dedifferentiation and anaplastic transformation of PTC or FTC to ATC, genomic alterations in some oncogenic and tumor suppressor genes, including RET rearrangements, BRAF, RAS, TERT, TP53 genes, accumulate involved in thyroid tumorigenesis (8, 9). Besides, additional alterations in several pathways, including the Wnt-β-catenin, the PTEN-AKT, the PI3/AKT/mTOR pathways, and mutations in the SWI/SNF complex, AID/APOBEC family of cytidine deaminases, histone modification genes, cell cycle genes and loss of function mutations in DNA repair genes have been reported in ATC (10-14). Acquisition of various genetic aberrations in the multi-step progression of ATC causes high rates of metastasis and mortalities, resistance to RAI and conventional therapies, so understanding the genetics involved in the process of development of ATC provides identifying novel targeted therapies and methods for therapeutic options in ATC patients (2, 15, 16).

Among the genetic changes involved in thyroid tumorigenesis, BRAF V600E mutation, a common driver variant in in PTC (18%–87%) and in poorly differentiated thyroid carcinoma (PDTC), ATC, and Hürthle cell thyroid carcinoma, presents a beneficial therapeutic effect from targeting BRAF. The diagnosis of ATC is based on clinical suspicion, neck ultrasound, fine needle aspiration (FNA) biopsy, and by light microscopy and immunohistochemistry to discover anaplastic thyroid tumors (17-19). The diagnosis of ATC is based on clinical suspicion, neck ultrasound, FNA biopsy, and by light microscopy and immunohistochemistry to discover anaplastic thyroid tumors (9, 20). However, the discrimination of ATC from other poorly differentiated carcinomas in the neck is laborious and not definitely confirmed by typical diagnostic methods (19, 21, 22).

Because of the frequency (10%-50%) of BRAF V600E mutation and consistency with poor prognosis in ATC (17, 18, 23), applying the genetic analysis for this mutation proffer a straightforward and useful tool in addition to conventional diagnostic procedures. Numerous molecular techniques including PCR-based techniques and sequencing and immunohistochemistry methods adopted to identify the presence of BRAF V600E mutation.

On account of restricted and controversial studies addressing the frequency of BRAF mutations (24, 25), we performed this systematic review and meta-analysis to investigate the prevalence of BRAF V600E mutation and to consider the methods, such as immunohistochemistry (IHC), for molecular detection of BRAF V600E mutation in ATC patients.

Methods and Materials

The latest version of the PRISMA statement was used to conduct this review. All the studies investigating the BRAF V600 were included if eligible for the study (26). The protocol of this study was registered prospectively in PROSPERO (CRD42022385426).

Literature Searches, Search Strategies, and Eligibility Criteria

An expert librarian (OA), a specialist in design search queries was responsible for the literature search. In brief, a search strategy aimed to retrieve all related documents from human studies published up to Jun 2022 that explore the incidence of BRAF V600E mutation among patients with ATC. Citations were found by searching the following sources: PubMed, Scopus, Google Scholar, and Web of Science (WoS). Combinations of subject headings, keywords, and synonyms used included all two key terms: 1) BRAF V600E and 2) anaplastic thyroid carcinoma.

Study Selection

We included all the studies that specifically determined the mutation of BRAF V600E in patients with ATC with any molecular method. Besides, non-English records, case reports and case series with less than 5 patients were excluded. However, we included one study with less than five patients; since in this particular study diagnostic function of different molecular method were assessed among patients with different thyroid lesions (27). After duplicates were removed, two reviewers (FA, PR) independently screened retrieved publication. The initial screen of the title & abstract for full-text assessment was determined based on the mention of thyroid cancer and consideration of possible mutations. An additional 7 articles were added from other sources especially from the screening of related reviews of the topic. These sources include a review of references in published reviews and included articles and additional articles recommended by expert researchers and clinicians in the field. Case reports and review articles studies were excluded. The reviewers independently determined if studies met inclusion and exclusion criteria. Discrepancies were settled by a third reviewer (MA).

Data extraction

Three reviewers (ME, SC, AKB) were independently extracted the data. Among the articles that met inclusion and exclusion criteria for analysis. We included studies that exclusively report the number of ATC patients with BRAF V600E mutation using different detection methods. Case reports, very low sample sized case series (n <5), pre-clinical studies, and any type of reviews were excluded. In brief, quantitative measures included, sample size, number of ATC cases with BRAF mutation, and diagnostic profile of detection method. Binary measures included the presence of genetic mutations. Data management was performed with Microsoft Excel. Any discrepancy in this stage were resolve by the re-evaluation of the third reviewer (AKB).

Statistical Analysis

The data entry and analysis were conducted for the meta-analysis using Stata 14.0 (Stata Corporation, College Station, Texas, USA) software. We used the "metaprop" command to estimate the pooled incidence of BRAF V600E mutation in ATC. Heterogeneity was assessed by I2 values. If I2<50% and P>0.1 between studies, the fixed-effects model was used, and if I2>50% and P<0.1, the chi-square analysis indicated study heterogeneity, and the random-effects model was used. All the analysis was performed using Freeman–Tukey Double arcsine transformations. Doi plot were made to assess publication bias in the included studies, and if large, it was further assessed using Egger's test. Moreover, "midas" and metandi commands were used to performed diagnostic meta-analysis. Firstly, we performed a univariate analysis to calculate weighted mean sensitivity and specificity with regarded 95% confidence intervals (CIs). Furthermore, a bivariate random-effects model was employed for the analysis and pooling of diagnostic performance measurements, in regards to both sensitivity and specificity. *P*-value < 0.05 was considered as statistically significant.

Results

Study Selection and Study Characteristics

Overall, 2241 citations were retrieved according to our systematic search. After removing of duplicates and screening of title and abstract of the retrieved studies, 78 records were remained for full text assessment. Finally, 34 articles published up to Oct 2022 were included in the pool study. Figure 1 shows the flow diagram of the selection process. Each study included 5-126 patients, with 978 patients in total. In these studies, 15 were performed in the USA, 4 in Korea, 3 in China and Japan each, and one in each of Italy, India, Russia, Ukraine, UK, France and Spain. The characteristics of the included studies were summarized in Table 1.

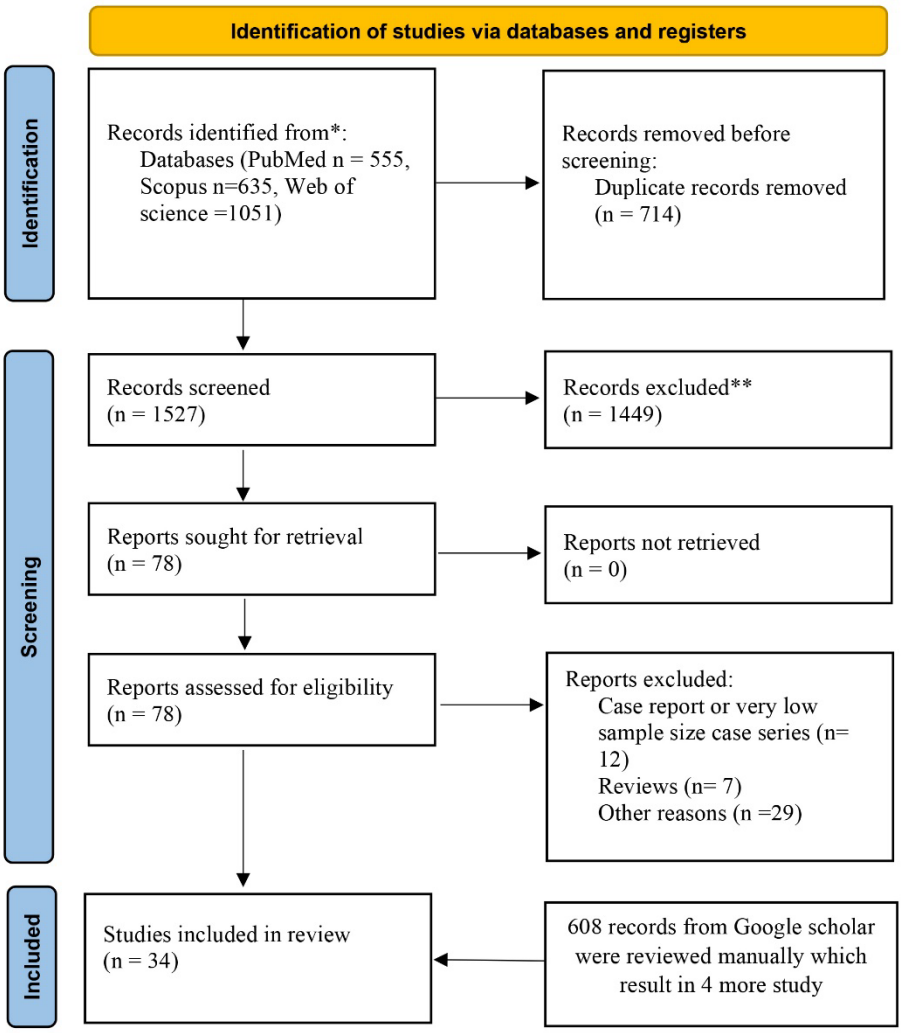

Fig. 1: The PRISMA flow-chart shows the flow of study selection through the different phases of a meta-analysis

Table 1: The characteristics of the included studies

Author	*Year*	*Country*	*Total Population*	*Sample*	*Diagnostic Tool*	*BRAF Positive (No.)*
Choi et al. (23)	2016	USA	15	FFPE	sequencing	6
Nikiforova et al. (25)	2003	Italy	29	FFPE	Light Cycler FMCA, SSCP, and direct sequencing	3
Duan et al. (28)	2019	China	25	FFPE	NGS	14
Begum et al. (19)	2004	USA	16	FFPE	PCR / codon1796	8
Xing et al. (29)	2004	Ukraine	10	FFPE	Sequencing	2

Table 2: Continued …

Iyer et al. (30)	2018	USA	44	cfDNA	NGS	20
Qin et al. (31)	2021	USA	87	cfDNA	Targeted NGS	41
Rashid et al. (32)	2019	India	34	FFPE	RFLP -sequencing	10
Song et al. (33)	2020	Korea	16	Tissue	NGS	6
Jeon et al. (34)	2016	Korea	11	FFPE	NGS	10
Chen T et al.(35)	2020	USA	28	FFPE	IHC	10
Shi et al. (36)	2015	China	106	FFPE	Sequencing	16
Titov et al. (37)	2021	Russia	10	FNA	AS-PCR	4
Nakamura et al. (38)	2005	USA	10	FFT/FFPE	Sequencing	1
Sandulache et al. (39)	2016	USA	23	cfDNA	NGS	9
Mitsiades et al. (40)	2007	USA	7	FFPE	Sequencing	1
Ronald et al. (41)	2013	USA	22	FFPE	IHC	15
Bae et al. (42)	2016	Korea	5	FFPE	Sequencing	4
Kim et al. (43)	2004	USA	37	NR	NR	8
Rushton et al. (44)	2016	UK	53	FFPE	IHC	7
Xu et al. (45)	2020	USA	126	FFPE	Mix	57
Costa et al. (46)	2008	Spain	36	FFPE	Sequencing	11
Zhu et al. (47)	2015	China	10	FFPE	IHC	1
Deeken-Draisey et al. (48)	2018	USA	9	FFPE	Sequencing	5
Na et al. (49)	2015	Korea	9	FFPE	IHC	6
Romei et al. (13)	2018	Italy	21	Fresh frozen tissue	sequencing	4
Quiros et al. (50)	2005	USA	8	NR	sequencing	5
Gauchotto et al. (51)	2011	France	14	FFPE	sequencing	2
Takano et al. (52)	2007	Japan	20	FFPE	sequencing	4
Ricarte-Filho et al. (53)	2009	USA	18	FFPE	sequencing	7
Fukushima et al. (54)	2003	Japan	7	NR	sequencing	0
Namba et al. (55)	2003	Japan	6	FFPE	sequencing	2

NGS: next generation sequencing, FFPE: formalin-fixed, paraffin-embedded tissue, cfDNA: cell free DNA, IHC: immunohistochemistry, AS-PCR: Allele-specific polymerase chain reaction, SSCP: single-strand conformation polymorphism, FMCA: fluorescence melting curve analysis, NR: not reported

Prevalence of BRAF V600E mutation among patients with ATC

The pooled incidence of BRAF V600E was 33% (95%CI: 0.26-0.40, I^2=77.55%, *P*=0.001) in the 884 patients (Fig. 2). Besides, in two steps we exclude low sample size studies, to see the effect of low sample size studies on the pooled prevalence. In this regard we observed that if we only considered the studies with sample size above 20 patients, the pooled estimate will become 31% (95%CI: 0.26-0.40) and if the studies with more than 30 participants are considered the estimate will remain similar, 31% (95%CI: 0.22-0.40) (Fig. 3). Moreover, if we exclude the patients with the accompanying differentiated lesion this estimate would reduce to 20% (95%CI: 0.06-0.40, I^2=83.4%, *P*=0.001) (Fig. 3). According to our analysis, we observed LFK index value of -0.66 which indicates no asymmetry in the effect of included studies. Besides, the egger test also rejected the presence of publication bias (P-Egger=0.657). Upon existence of data on different countries we depict the prevalence of BRAF mutation in different region of the world (Fig. 4). In this regard, the pooled prevalence of BRAF V600E mutation in USA is 40% (95%CI: 0.33-0.47, ranged from 14% to 68%). Furthermore, in the South Korea the similar parameter is about 70% which is the highest among all included countries.

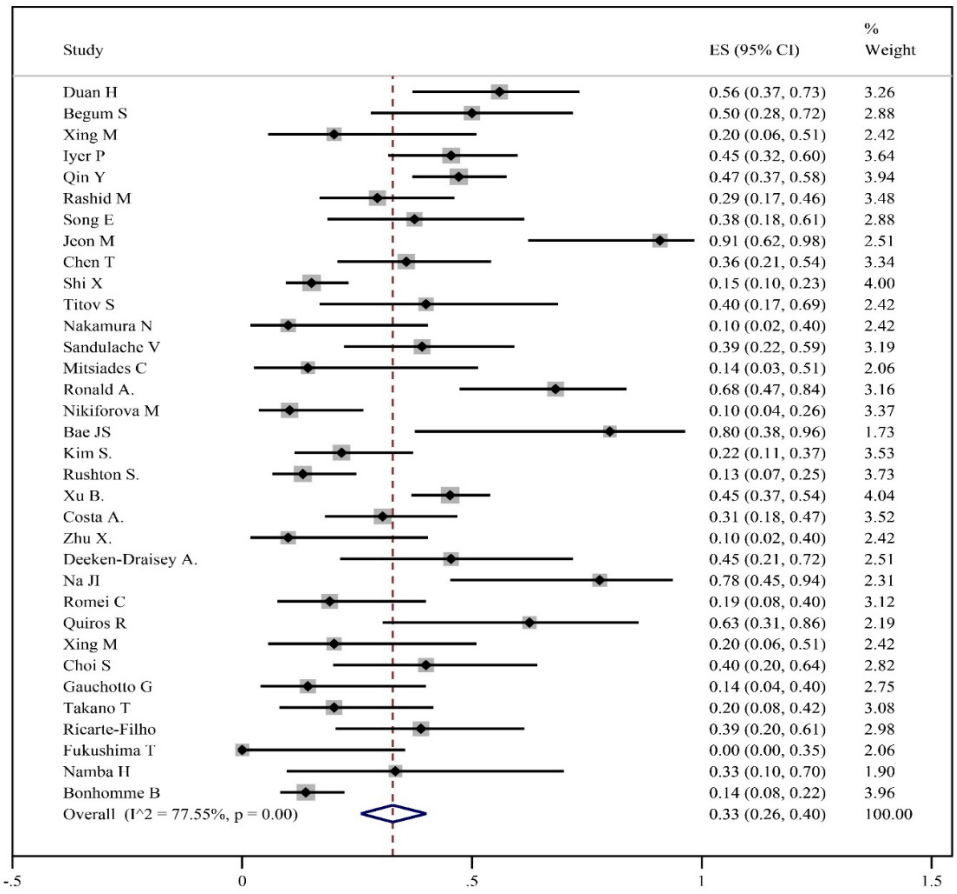

Fig. 2: The forest plot of the prevalence of BRAF V600E mutation among the ATC patients

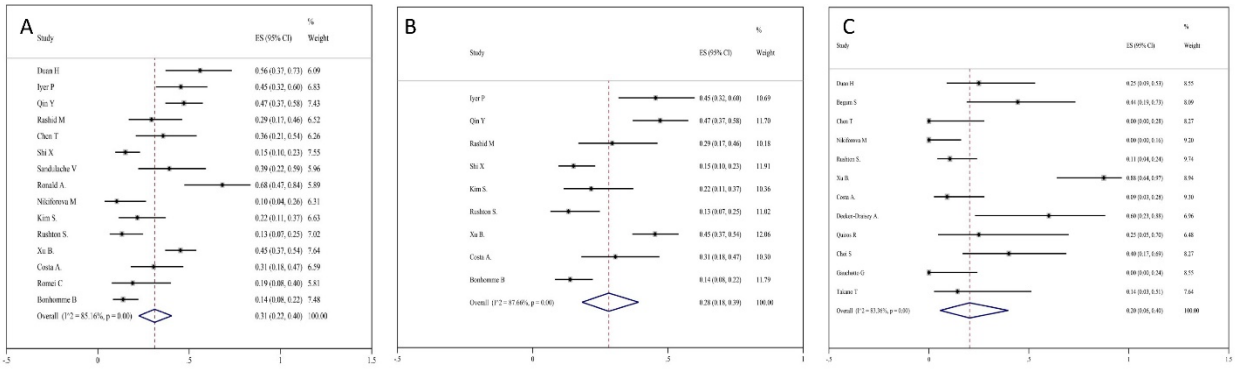

Fig. 3: The forest plot of the prevalence of BRAF V600E mutation A) In studies with more than 20 patients B) In studies more than 30 patients C) The prevalence of BRAF V600E mutation among the ATC patients without accompanying differentiated component

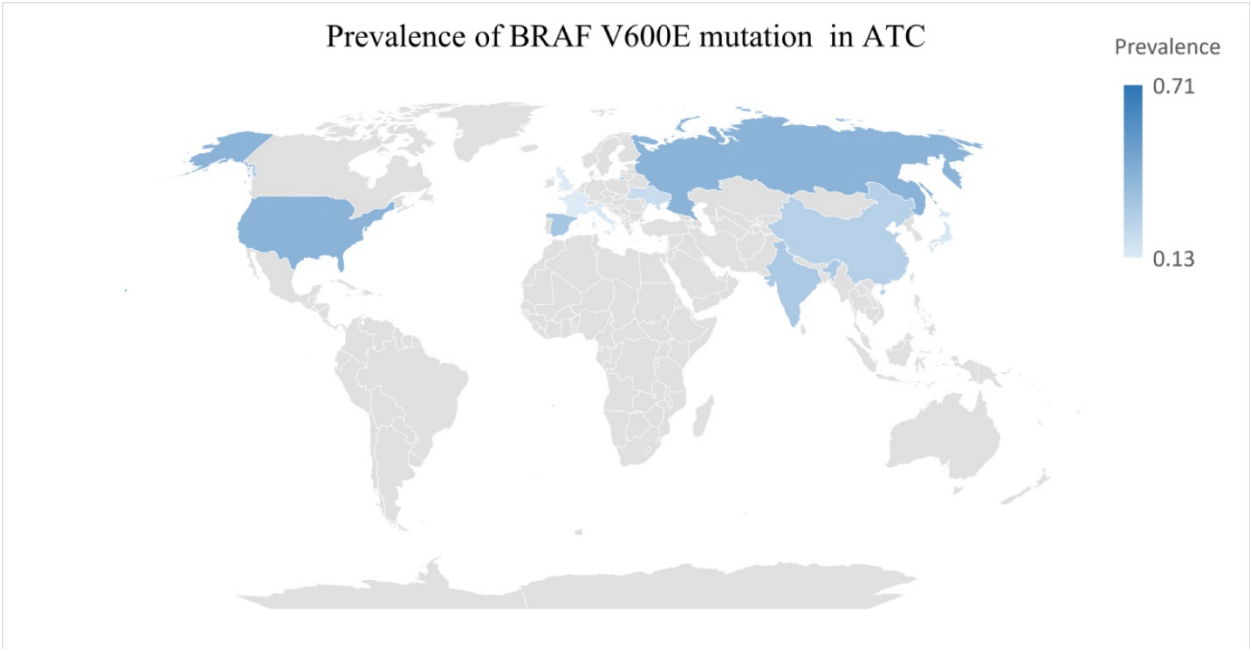

Fig. 4: The prevalence of BRAF V600E mutation among the ATC patients in different countries

Diagnostic accuracy of IHC

Based on our search, only seven studies reported the diagnostic profile of sequencing and IHC in detecting BRAF V600E mutation in patients with ATC. Among these seven studies, five assessed (27, 35, 41, 44, 49) the IHC and two evaluated the diagnostic function of sequencing in liquid base samples (30, 31). Iyer et al. used two different diagnostic methods to detect the BRAF mutation in cell free DNA sample of patients with ATC (30). Based on their report, next generation sequencing had the sensitivity and specificity of 79% and 100%, respectively. Furthermore, the same parameters for the ddPCR method were 85% and 100% respectively (30). Besides, Qin et al used the targeted NGS as the detection route, which provided the better sensitivity (88%) and similar specificity (100%) (31). Since the data on sequencing methods was limited, we performed the diagnostic meta-analysis only for the IHC method. Based on our analysis the pooled sensitivity and specificity of IHC in detecting BRAF

V600E were 94% (95%CI: 73-99) and 88% (95%CI: 46-98), respectively. During this analysis, we added a one value to the number of false negatives in the study by Na J, et al (49). This was inevitable since there were some mathematical problems that prevented the conduction of the analysis. Moreover, the HSROC of the IHC is demonstrated in Fig. 5.

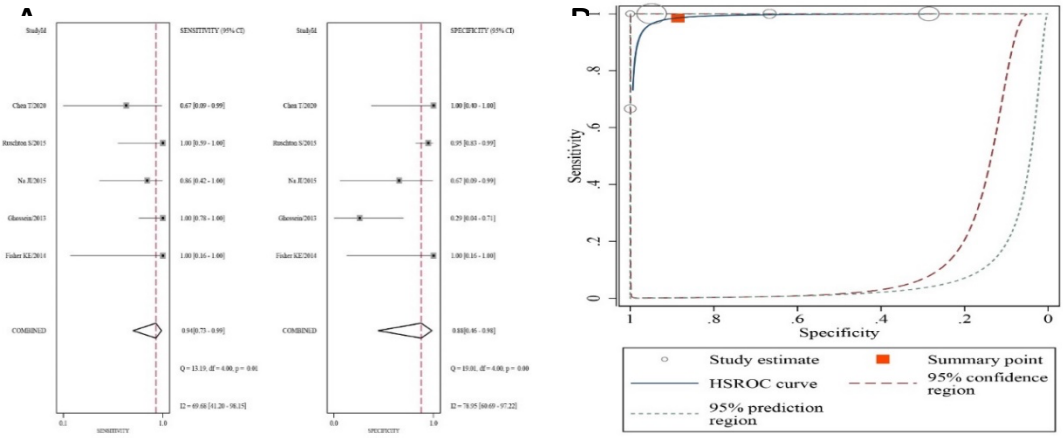

Fig. 5: A) Forest plot from a random effect meta-analysis examining specificity of BRAF V600E immunohistochemistry in anaplastic thyroid cancer B) The hierarchical summary receiver operating characteristic (HSROC) curve shows the summary of the sensitivity and specificity of the immunohistochemistry for diagnosis of BRAF V600E in patients with anaplastic thyroid carcinoma

Discussion

Thyroid cancers are counted as the most common neoplasms of endocrine system. The aberrancy of genetic regulation leads to abnormal gene expression and dysfunction. In thyroid cancer particular genes such as BRAF V600E has been extensively studies and considering the promising results this gene found its route to the clinics and currently used as a biomarker in management of thyroid cancer. Despite the very low incidence rate of ATC, several studies from the bench revealed the possible role of this gene mutation in pathogenesis of ATC. In this study we demonstrate the prevalence of BRAF V600E mutation among patients with ATC. Actually, this disease is considered as a very rare disease with an age-adjusted incidence rate of 0.2 per 1 million people (56). Despite the low incidence rate, the trend shows inflation since the early 70s (56). We observed that 33% of patients with ATC had a BRAF V600E mutation. This measure belongs to patients who simultaneously have differentiated thyroid cancer. Previously it has been observed that the presence of BRAF is associated with poor prognosis and worse outcomes in thyroid cancer patients with this mutation (57). Therefore, the targeted therapy against BRAF mutation in ATC has been studied in both experimental and clinical trial settings.

Regarding the anti-BRAF targeted therapies, several phase I and II clinical trials have been conducted. In a phase I trial on 14 patients with ATC, it was shown that dabrafenib single therapy led to a partial response in one participant and stable disease in two patients (58). Moreover, BRAF/MEK targeted therapy combination of dabrafenib and trametinib in 36 patients with ATC. According to their report, 17 patients yielded partial responses besides 3 had complete responses (59). This combination has been approved for the treatment of locally advanced or metastatic ATC patients who carried $BRAF^{V600E}$ mutation and had no suitable treatment options (60). The results of these trials lead these agents to get FDA approval for treatment of ATC and

suggested as a treatment option in the current guidelines (4).

In our country-based analysis of BRAF mutation in ATC patients revealed that there was a disequilibrium on the prevalence of BRAF mutation in different geographical regions. As an instance, the prevalence of this mutation in the Asian population is rather higher compared to the rest of world. One reason for this observation can be the fact that in the majority of the reports from this area, it was not disclosed if the samples of ATC have the simultaneous accompanying PTC. The other reason is the small sample size of the Asian studies. As so, there was only one study with 57 sample numbers of ATC patients (36). Besides, the US population with ATC have higher mutation rate compared to European country. The heterogeneous population of the USA from ethnicity perspective can be somehow justifying for this finding although low sample size of studies may have effect on accuracy of this finding.

DNA-based and genomic methods are still considered as the gold standard for detection of the *BRAF* V600E mutation clinical samples (4, 61). However, IHC is also recommended as valid diagnostic method in detecting this mutation in ATC patients (4). Immunohistochemistry using the VE1 antibody was developed to detect the *BRAF* V600E mutation. Nevertheless, it was uncertain whether it could switch molecular testing in clinical practice. False-negative/-positive results might occur while IHC performed to detect BRAF V600E mutation due to unsatisfactory specificity of the antibody, or suboptimal IHC procedure (62). However, other reports indicated that immunohistochemistry with Anti-BRAF V600E antibody is a Sensitive method in Cancer patients (63). In this study, IHC had an acceptable diagnostic method in detecting BRAF V600E. Although the IHC method has shown acceptable accuracy results, the number of included studies was limited. Hence, more studies are needed to produce a strong standpoint about the IHC diagnostic accuracy. We recommend that performing additional molecular tests may help to evaluate *BRAF* mutational status in IHC samples with equivocal staining pattern results. Besides, introducing a specific panel of biomarkers and therapeutic targets may enlighten the clinical teams in both disease progression and management of ATC. Similar strategy has been already applied for other thyroid and endocrine tumors (64, 65).

The study limitation is that different diagnostic molecular methods were used in the included studies, which limited the number of pooled studies.

Conclusion

The prevalence of BRAF V600E mutation is considerable among the ATC patients. Therefore, diagnosis of the BRAF mutation is critical in clinical and targeted therapy. Based on our study, IHC was accepted as a proper diagnostic method for detecting BRAF V600E.

Journalism Ethics considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

Data availability

Part of the datasets generated during and/or analyzed during the current study are represented in Table 1. Full access to datasets is available from the corresponding author upon reasonable request.

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Conflict of interest

The authors declare that there is no conflict of interests.

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