RESEARCH ARTICLE

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BRAF V600E and Novel Somatic Mutations in Thyroid Cancer of Libyan Patients

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Abstract

Background: Thyroid cancer (TC) is a common endocrine malignancy that frequently harbours the oncogenic V600E BRAF mutation. This mutation has received considerable attention in recent years for its potential utility in the risk stratification and management of TC. This study aims to investigate BRAF mutational status in thyroid cancer of Libyan patients and their association with clinicopathological factors. **Methods:** 44 thyroid tissue samples were analysed for mutations in exon 15 of the BRAF gene by performing polymerase chain reaction and Sanger sequencing. The results of BRAF mutation screening were correlated to clinical and pathological characteristics of the studied thyroid cancer patients. Statistical analyses were performed using SPSS. **Results:** The BRAF exon 15 mutations were detected in 19 (43.2%) of the thyroid cancer cases. The V600E was the most frequent one found in 15/44 (34.1%) cases. We also detected 6 other variants in 7 patients (15.9%), the S616F, the W619R and the T599S. Three mutations were associated with V600E, the L584I, the D587Y and the synonymous L597L. None of these mutations were reported previously in thyroid cancers. No statistical association was found between BRAF mutations and clinicopathological factors except with papillary thyroid cancer type (p= 0,032). **Conclusions:** Novel BRAF mutations and V600E were frequently detected in thyroid cancer of Libyan patients; this suggests a potential role of these novel mutations in carcinogenesis and in anti-EGFR therapy resistance.

Keywords: BRAF V600E- Non V600E mutations- Thyroid cancer- Libyan patients

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Introduction

Thyroid cancer represents only 1% of all human malignancies. It accounts for more than 90% of all endocrine cancers and is considered among the most curable cancers. It can be classified histologically into follicular epithelial cell-derived papillary thyroid cancer (PTC), follicular thyroid cancer (FTC), anaplastic thyroid cancer (ATC), and para-follicular C-cell derived medullary thyroid cancer (MTC) (Lloyd et al., 2017; Rowe et al., 2007). PTC accounts for more than 90% of thyroid cancer (Kurtulmus et al., 2012; R.V. Lloyd et al., 2017). It tends to remain localised in the thyroid gland, but same times, it may metastasise to regional lymph nodes and less commonly, to the lungs. The high incidence of PTC is in the fifth decade of life and it occurs nearly three times more frequently in women than in men (Hundahl, 1998). A diet low in iodine as well as the radiation sources including certain medical treatments and radiation accidents or nuclear power station arms, may increase the risk of papillary cancer (Abdalla et al., 2015).

BRAF is a serine-threonine kinase member of Raf-kinase family. The BRAF gene on chromosome 7 (7q34) encodes the BRAF protein, which participates in the Mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinase (ERK) signalling pathway. This pathway regulates important cell functions including cellular growth, differentiation, proliferation, senescence, and apoptosis. The mutations are mainly located in the exon 15 in the tyrosine kinase domain. The V600E that results in thymine-to-adenine (T > A) change at nucleotide position 1799 (T1799A) substituting valine by glutamic acid at amino acid position 600 (V600E) (Liu et al., 2016). This leads to an increase in protein expression or activity that can disturb the MAPK signalling pathway, which in turn can result in different developmental disorders such as different types of human cancers (Hussain et al., 2015). BRAF mutation is a common somatic mutation in thyroid cancer. It was identified in 28–83% of PTC specimens, however, no mutations were

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observed in normal thyroid tissue and tissue from patients with benign thyroid disease (Mercer, 2003). When present, this mutation results in constitutive activation of the BRAF protein with consequent activation of the downstream mediators. This study aims to detect mutational hotspot exon 15 status of BRAF gene in thyroid cancer patients from Libya and its association with clinicopathological features to assess its role in cancer progression.

Material and Methods

Patients and tissue samples

Formalin fixed paraffin embedded specimens (FFPE) from 44 patients, undergoing surgical treatment for thyroid cancer in Misurata Cancer Centre (MCC), were obtained. Tissues were collected during the period between January 2016 and June 2017 from the department of histopathology at MCC. We used one case of both adenoma, and Hashimoto and one normal thyroid gland as controls. The ethical approval for the study was provided by the ethical committee of MCC. All the techniques were performed at the department of pathology at Pasteur Institute of Tunis.

Pretreatment of formalin fixed and paraffin embedded samples

Sections of 4µm thick were obtained from FFPE for DNA extraction and histopathological studies. After each specimen, blades were changed to minimize the risk of cross-contamination.

Histopathological study

Hematoxylin-eosin (HE) stained sections were examined by a pathologist to confirm the histological diagnosis and to assess the proportion of tumor cells.

Molecular analysis

DNA extraction and quantification

DNA extraction was conducted using the QIAamp DNA FFPE Tissue kit (Qiagen, Hilden, Germany) according to the manufacturer protocols. DNA concentrations and purities were determined using a NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific, Wilmington, Delaware).

Exon 15 BRAF PCR amplification

According to the Catalogue of Somatic Mutations in Cancer (COSMIC) database, the exon 15 of the BRAF gene is a hotspot pathogenic mutation site. The coding region of this exon, containing the point mutation at position 1799, was amplified using the following forward and reverse primers: F:5`-TCATAATGCTTGCTCTGATAGG-3`and R:5`-GGCCAAAAATTTAATCAGT GG-3' as described in the publication of Arcila et al., (2011). PCR reaction was performed in a 25 µl volume mixture containing 50-100 ng of genomic DNA; forward and reverse primers (20 pmol each) and 10 µl of 2× concentrated AmpliTaq Gold® 360 Master Mix. The PCR cycling parameters were as follow: 5 minutes initial denaturation at 94°C, followed by 35 amplification cycles of 45 seconds at 94°C, 1 minute at 56°C and 1 minute at 72°C, and a final extension step of 10 minutes at 72°C. Amplicons were separated on 1.5% agarose gel and visualized by Syber Green staining.

Exon 15 BRAF sanger sequencing

BRAF gene exon 15 amplicons were subjected to automated DNA sequencing in an ABI PRISM 3130 DNA Analyzer (Applied Biosystems). Each amplicon was sequenced in both directions using Big Dye Terminator Version.3.1 Cycle Sequencing kit (Applied Biosystems) and above-mentioned forward and reverse primers, respectively. All amplicons, before being used as template, were purified by using exonuclease I (EXO I) and shrimp alkaline phosphatase (SAP) to degrade excess primers and nucleotides.

Cycle sequencing reaction and PCR program were performed as per manufacturer's protocol. Sequences were analyzed and compared by Bioedit-software.

Prediction tools

BRAF mutations were analyzed with different computational tools like Catalogue of Somatic Mutations in Cancer (COSMIC): https://cancer.sanger.ac.uk/cosmic,Ensembl, Mutation taster: http://www.mutationtaster.org/, Sorting Intolerant From Tolerant (SIFT): http://sift.bii.a-star.edu.sg/, Effect Analyzer (PROVEAN): http://provean.jcvi.org/protein_batch_submit.php?species=human, Human Splicing Finder (HSF): http://umd.be/Redirect.html,: https://www.ensembl.org/index.html, A mutation pathogenicity prediction system (UMD predictor): http://umd-predictor.eu/analysis.php and ClinVar: https://www.ncbi.nlm.nih.gov/clinvar/ in order to estimate splice site effects, protein damage or clinical signification. An online web-server HOPE was used to analyze the effects of point mutations on protein hydrophobicity, chemical and physical properties, spatial structure and function http://www.cmbi.ru.nl/hope/ (Venselaar et al., 2010). Moreover, effects of synonymous mutations on messenger ribonucleic acid (mRNA) folding were predicted using Mfold web server http://www.bioinfo.rpi.edu/applications/mfold (Zuker, 2003).

The full mRNA sequence of BRAF (reference and mutated) and the nucleotide sequence surrounding the synonymous variation were analyzed.

Statistical Analysis

Differences in categorical variables, such as gender, age, tumour size, and stages, between patients with and without BRAF mutations were evaluated for significance with chi-squared test. Molecular data was analysed by SPSS version 20.0 statistical software. P value < 0.05 was considered to indicate a statistically significant difference.

Results

Sample features

The study enrolled 44 patients, 36 female (81.8%) and 8 male (18.2%). The patients were aged between 18 and 90 years with a mean age of 46.8 years at the time of surgery. The specimens included 26 (59.1%) PTC, 9 (20.4%) follicular-variant PTC, 4 (9.1%) FTC, 3 (6.8%) MTC, one ATC and one poorly differentiated carcinoma

Table 1. Clinicopathological Characteristics of the Studied Patients (n=44)

	N	%
Gender		
Female	36	81.8
Male	8	18.2
Age years		
≤45 years	22	50
>45 years	22	50
Type of Thyroid Cancer		
Papillary carcinoma	26	59.1
Papillary carcinoma follicular variant	9	20.4
Follicular carcinoma	4	9.1
Medullary carcinoma	3	6.8
Anaplastic carcinoma	1	2.3
Poorly differentiated carcinoma	1	2.3
Size, cm		
≤2	14	31.8
>2	30	68.2
Surgery		
Left lobe	10	22.7
Right lobe	14	31.8
Total thyroid	20	45.5
Clinical Stage		
I	28	63.6
II	5	11.4
III	10	22.7
IV	1	2.3
Lymph node involvement		
N0	37	84.1
N1	7	15.9

(PDC). The tumours were found in the right thyroid lobe in 14 cases and in both lobes in 20 cases with spread to lymph nodes found in 7 cases (15,9%). The tumour sizes varied from 1 to 10 cm with a mean of 4.05 cm. According to the TNM classification of head and neck tumours 2016, 28 patients (63.6%) were in stage I and only one (2.3%) in stage IV. Table 1 summarizes clinicopathological data of the study series.

Mutational BRAF analysis

The BRAF exon 15 mutations were detected in 19 (43.2%) of the thyroid cancer cases. No mutation was detected in normal thyroid gland, adenoma or thyroid Hashimoto disease. The V600E was the most frequent one found in 15/44 (34.1%) cases. We also detected 6 other variants in 7 patients (15.9%) (Figure 1), among them 3 had a double mutation. The S616F and the W619R mutations found in one patient each and the T599S found in two patients, were all reported as pathogenic by prediction tools (Table 2).

Three mutations were associated with V600E, the L584I, the D587Y and the synonymous L597L; Only the D587Y was not reported previously. All except one of these mutations were in patients with PTC (Table 3).

The BRAF V600E mutations were found in (36.4%) of patients under 45 years, in 33.3% of women and in 42.9% of right thyroid. They were significantly associated only with type PTC (p= 0,014). The non V600E mutations were found in 7/44 (15.9%) of patients. 71.4% of them in patients over 45 years, 100% in woman and 85.7% in papillary carcinoma. There was no association otherwise between V600E nor for the different variations in BRAF gene and clinicopathological characteristics or histological risk factors (Table 4).

Effect of the mutations on the protein structure and function

This analysis evaluates the effect of the mutation on the following features: Contacts made by the mutated residue, structural domains in which the residue is located, modifications on this residue and known mutations for this residue (Figure 2). The V600E (c.1799T>A) gives a protein a bigger size and changes its charge to negative which enhances the protein activity 500-fold and

Table 2. In silico Analysis of BRAF Variations

	Variant type	Sequence variation (Nucleotide)	Mutation Taster	UMD Predictor	Provean	Sift
L584I	SNV	c.1750 C>A	Disease causing: amino acid sequence changed, protein features (might be) affected, splice site changes	Probably Pathogenic	Neutral	Damaging
D587Y	SNV	c.1759 G>T	Disease causing: amino acid sequence changed, protein features (might be) affected	Pathogenic	Deleterious	Damaging
L597L	Synonymous SNV	c.1791 A>T	Disease causing: amino acid sequence changed, protein features (might be) affected	Polymorphism	Neutral	Tolerated
T599S	Synonymous SNV	c.1795A>T	Disease causing: amino acid sequence changed, protein features (might be) affected	Probably Pathogenic	Neutral	Tolerated
S616F	Synonymous SNV	c.1847 C>T	Disease causing: amino acid sequence changed, protein features (might be) affected	Pathogenic	Deleterious	Damaging
W619R	Synonymous SNV	c.1855T>A	Disease causing: amino acid sequence changed, protein features (might be) affected , splice site changes	Pathogenic	Deleterious	Damaging

SNV, Single nucleotide variant; Mutation taster: http://www.mutationtaster.org/ ; UMD predictor, A mutation pathogenicity prediction system: http://umd-predictor.eu/analysis.php; PROVEAN (Effect Analyzer): http://provean.jcvi.org/protein_batch_submit.php?species=human; SIFT Sorting Intolerant From Tolerant: http://sift.bii.a-star.edu.sg/

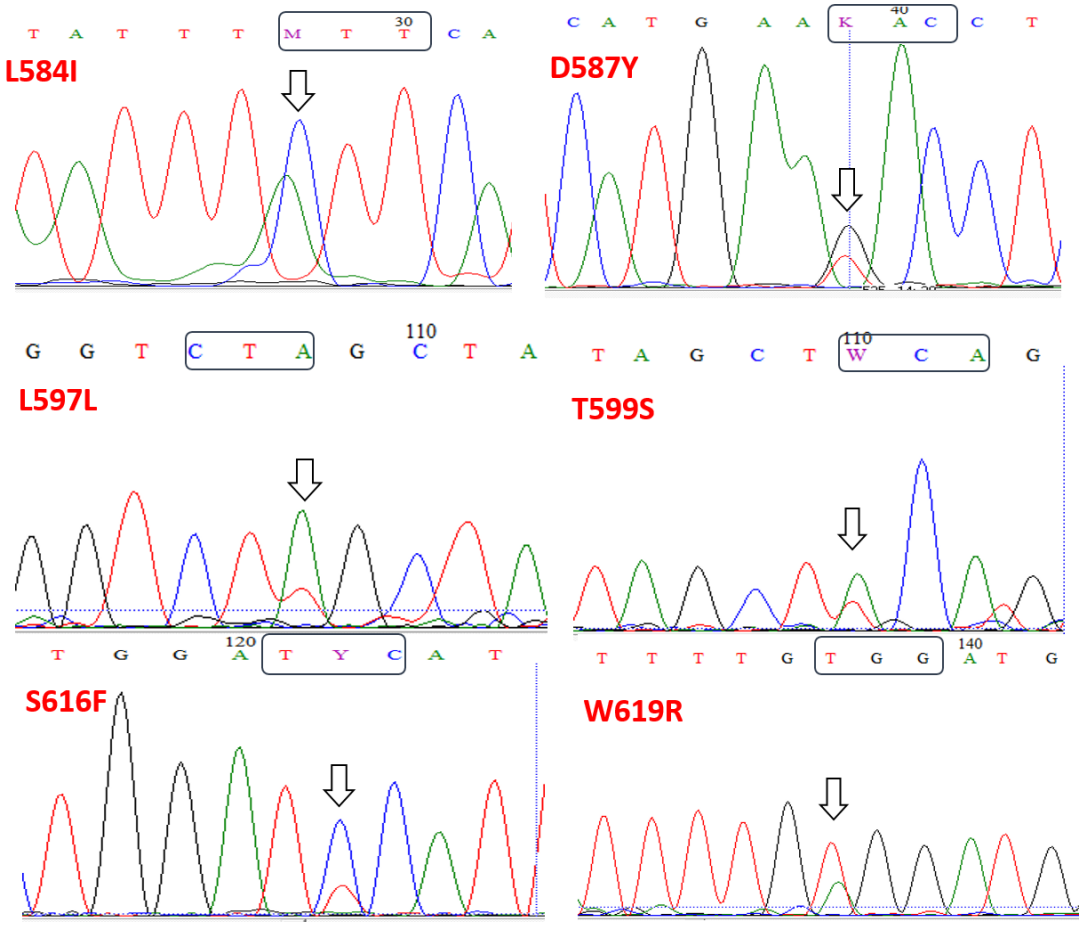

Figure 1. Sequencing Chromatograms at the Nucleotide Position of Interest Representing the Six Novel Variants of BRAF Mutations.

stimulates constitutive MEK–ERK signaling in cells. It could disturb the interaction between the protein and other molecules and thereby affect signal transfer.

The D587Y (c.1750 C>A) was found in one patient aged 35 years with papillary carcinoma. The wild-type residue forms a salt bridge with Lysine at position 552. The mutation of an Aspartic Acid into a Tyrosine at position 587 will disturb the ionic interaction made by the original, wild-type residue. The residue is located on the surface of the protein. Mutation of this residue can disturb interactions with other molecules or other parts of the protein, which might indicate that the mutation is possibly damaging to the protein.

The T599S (c.1795 A>T), will give a smaller residue than the wild type and hens disturb the hydrogen bond with a Glutamic Acid at position 501. The mutated residue is located in a domain that is important for the activity of the protein and in contact with residues in another domain. It is possible that this interaction is important for the correct function of the protein. The mutation can affect this interaction and as such affect protein function.

For the S616F (c.1847 C>T) mutation, the mutant residue is bigger and more hydrophobic than the wild-type residue. The wild-type residue forms a hydrogen bond with: Leucine at position 618. The differences between wild-type and mutant residue make that the new residue is not in the correct position to make the same hydrogen bond as the original wild-type residue did. In both the

Table 3. Clinicopathological Characteristics of Mutations other than V600E

Mutation	N	Histological Type	Gender		Age years		Size		Surgery			Clinical Stage			
			F	M	≤45	>45	≤2	>2	L	R	T	I	II	III	IV
L584I	1*	Papillary	+		+		+			+		+			
D587Y	1*	Papillary	+		+			+	+			+			
L597L	1*	Papillary FV	+			+		+			+	+			
T599S	1	Papillary	+			+	+			+		+			
	1	Anaplastic	+			+		+			+				+
S616F	1	Papillary	+			+		+			+			+	
W619R	1	Papillary FV	+			+	+		+			+			

*Patients with V600E mutation

Table 4. Association between BRAF Mutations and Clinicopathological Characteristics

Variables	BRAF V600E mutations			All BRAF mutations*		
	Positive (%) (n=15)	Negative (%) (n=29)	P value	Positive (%) (n=19)	Negative (%) (n=25)	P value
Gender						
Female	12 (80)	24 (82.8)	0.562	16 (84.2)	20 (80)	0.519
Male	3 (20)	5 (17.2)		3 (15.8)	5 (20)	
Age years						
≤45 years	8 (53.3)	14 (48.3)	0.5	8 (42.1)	14 (56)	0.272
>45 years	7 (46.7)	15 (51.7)		11 (57.9)	11 (44)	
Type of thyroid cancer						
Papillary carcinoma	12 (80)	14 (48.3)	0.274	14 (73.7)	12 (48)	0.126
Papillary carcinoma	3 (20)	6 (20.7)		4 (21.1)	5 (20)	
Follicular variant						
Follicular carcinoma	-	4 (13.8)		-	4 (16)	
Medullary carcinoma	-	3 (10.3)		-	3 (12)	
Anaplastic carcinoma	-	1 (3.4)		1 (5.2)	-	
Poorly differentiated	-	1 (3.4)		-	1 (4)	
Type of thyroid cancer						
Papillary carcinoma	15 (100)	20 (69)	0.014	18 (94.7)	17 (68)	0.032
Others types of carcinoma	-	9 (31)		1 (5.2)	8 (32)	
Size, cm						
≤2	6 (40)	8 (27.6)	0.307	8 (42.1)	6 (24)	0.171
>2	9 (60)	21 (72.4)		11 (57.9)	19 (76)	
Surgery						
Left lobe	2 (13.3)	8 (27.6)	0.504	3 (15.8)	7 (28)	0.606
Right lobe	6 (40)	8 (27.6)		7 (36.8)	7 (28)	
Total thyroid	7 (46.7)	13 (44.8)		9 (47.4)	11 (44)	
Clinical Stage						
I	11 (73.3)	17 (58.6)	0.612	13 (68.4)	15 (60)	0.539
II	2 (13.3)	3 (10.3)		2 (10.5)	3 (12)	
III	2 (13.3)	8 (27.6)		3 (15.8)	7 (28)	
IV	-	1 (3.4)		1 (5.3)	-	
Lymph node involvement						
N0	12 (80)	25 (86.2)	0.448	16 (84.2)	21 (84)	0.657
N1	3 (20)	4 (13.8)		3 (15.8)	4 (16)	

*V600E and non V600E mutations

PDB-le and in the PISA-assembly, this residue was found to be involved in a multimer contact. The PISA-database contains protein assemblies that are highly likely to be biologically relevant. This is a strong indication that the residue is indeed in contact with other proteins.

The L584I (c.1750 C>A), mutated residue is located in a domain that is important for the activity of the protein and in contact with another domain that is known to be involved in binding. The interaction between these domains could be disturbed by the mutation, which might affect the signal transduction between the domains. The mutated residue could affect the splice site.

The W619R (c.1855T>A) mutant residue is smaller than the wild-type residue with a positive charge and lesser hydrophobicity. The wild-type residue forms a hydrogen bond with Glutamic Acid at position 648. The differences in size and hydrophobicity between wild-type and mutant residue will affect hydrogen bond formation. The synonymous mutation L597L (c1791A >T) change the splice site and might affect the protein features.

Discussion

Thyroid cancer is characterized by the presence of different genetic mutations including BRAF gene (Younis, 2017). To our knowledge, this is the first study to examine BRAF mutations in thyroid cancer of Libyan patients.

BRAF is highly expressed in hematopoietic cells, head and neck tumours, melanoma and thyroid follicular cells, and over 90% of BRAF mutations are BRAF V600E (Fagin et al., 2008). Many studies have been done to investigate the clinicopathological characteristics, and

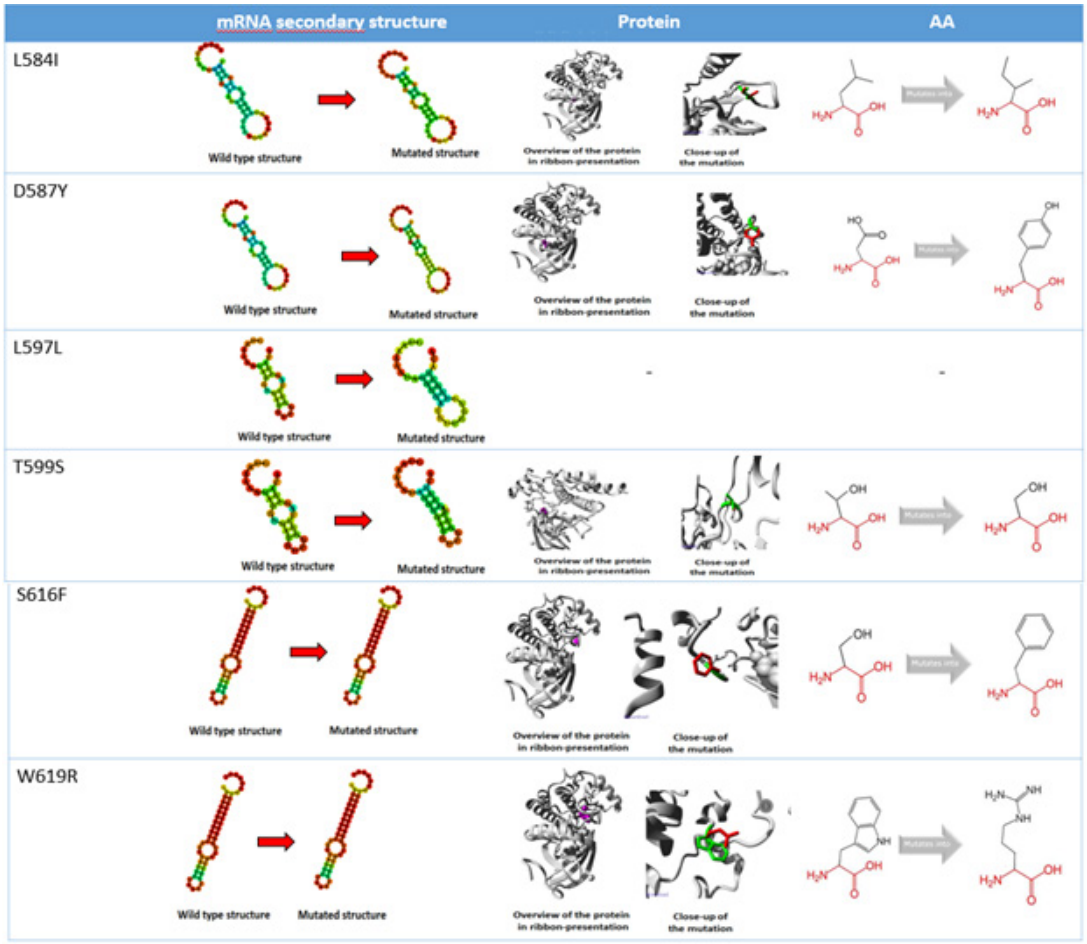

Figure 2. The Effect of Different Variations on mRNA Secondary Structure, 3D BRAF Protein and Amino-Acid.

the potential utility of BRAF V600E mutation on the diagnostic, prognostic and therapeutic aspects of TC (Adeniran et al, 2006; Al Salam et al, 2020). Unlike the highly consistent results obtained from in vitro studies, the current clinical data shows controversial results regarding BRAF V600E mutation prevalence and its role as a genetic prognostic marker of TC (Tang et al., 2010). In this study, we found this mutation in 34.1% of TC. This prevalence was almost similar to those reported in other Mediterranean regions and several European and Asian countries but lower than those reported in eastern Asian countries and north American ones. Su-jin kim (2012) in a study on 547 patients reported a prevalence of BRAF V600E mutation in 69.7% of cases and in a study conducted to compare patients with thyroid cancer in Japan and Vietnam, it was found that the prevalence of BRAF V600E mutation in PTC was rather high but similar in both countries; 82.1% in Japanese and 83.0% in Vietnamese patients (Vuong et al., 2016). Several factors could explain the discrepancy in the prevalence of BRAF mutation like the types of thyroid cancer studied, the sensitivity of the technique or the genetic variability. Many reports have shown that thyroid cancers are very frequent in countries with high radiation levels which could lead to high level of BRAF mutations. However, comparison between radiation exposed groups and non-exposed groups didn't show difference in mutation levels of BRAF in TC; instead, it showed high RET-PTC rearrangement (Dinets et al., 2012; Hamatani et al., 2008; Handkiewicz-Junak et al., 2016).

BRAF V600E mutation was higher in men 37.5% than in women 33.3% but without significant association. Previous studies have reported a higher incidence of BRAF mutations in females compared to males, but no one was able to establish the relationship between sex and BRAF mutation (Elisei et al., 2008; Gouveia et al., 2013; Kim et al., 2012; Yip et al., 2009)

There are many reports that showed the relationship between age and BRAF mutation rate. In our study, the mutation was higher in young patients (<45 years) (53.3%) than in people aged over 45 years (46.7%) (p=0.750). Similar results were reported by Al salem et al., (2020) and Geng et al., (2017) who showed higher frequency in paediatric age group. Other studies, however reported higher prevalence in patients over 55 years (Pessoa-Pereira et al., 2019; Xing et al., 2015; Xing et al., 2005). These differences in age pattern could be related to patient sample.

We found that the BRAF V600E was more frequent in stage I of thyroid cancer (73.3%), however, this association with tumour stage was not statistically significant. This is consistent with the study conducted in Brazil, which showed the presence of the BRAF mutation in stage I and II at 65.3%, while in stages III and IV at 61% (Andrade et al., 2013). These results could indicate that BRAF mutation is involved in the pathogenesis of TC indeed it

is not associated with poor prognosis because all patients under 45 years are classified as stage I.

Controversial results were reported concerning the BRAF mutation as prognostic factor. Many reports showed correlation between BRAF mutation and poor prognostic factors (Henke et al., 2015; Liu et al., 2014; Tufano et al., 2012) lymph node invasion (Frasca et al., 2008; Kebebew et al., 2007; Oler et al., 2009; Yip et al., 2009) tumour size (Adeniran et al., 2006; Xing et al., 2005) or male gender. Other reports however didn't find any association (Romei et al., 2021; Wang et al., 2016; Zhang et al., 2016). In our study, BRAF mutation was more frequent in small tumours, in young patients and in early stages of the disease. We didn't find any association with poor prognostic factors. Similar results were reported by Christopher Gouveia et al., (2013). A new study of whole exome made by the cancer genome atlas research network have found that the index RAS/RAF mutations is more precise than BRAF mutation for evaluating prognosis in TC (Cancer Genome Atlas Research Network, 2014).

Currently BRAF mutation is used also as a diagnostic marker on thyroid cytology to classify thyroid nodules as benign or suspect. Many studies have demonstrated the utility of BRAF V600E mutation for the therapeutic decision after thyroid FNA (Chen et al., 2022).

In the present study, BRAF V600E mutation was found in 46.2% of PTC and in 33.3% of follicular-variant PTC but none in other types of TC. These levels are in part different from those reported previously. In fact, the prevalence of BRAF V600E in PTCs varies from 29% to 83%, and is almost ~60% in classic PTC, ~77% in tall-cell variant, and ~25% in PTC-derived ATC, but it is rare (0–12%) in follicular-variant PTC and is not found in FTC (Fymat, 2017). The results of a study in Italy showed that the BRAF gene mutation was present in the PTC and type ATC and was not found in other types (Fernandez et al., 2013). Al salem (2020) also showed higher prevalence in classic PTC than follicular variant of PTC and papillary microcarcinoma.

In the current study we found 7 patients with mutations other than BRAF V600E. They were detected in 15.9% of cases; among them 6.8% had mutation associated with V600E. This high level of non-V600E mutations was not reported previously in thyroid cancer nor in other malignancies. Reports concerning mutations other than BRAF V600E are scarce. They estimate their prevalence at 2-3% of BRAF mutations in PTC, and they are mainly located near codon 600. A literature review indicates that some of our non V600E mutations have been described in other tumours like malignant melanoma, adenocarcinoma of the pancreas and the colon) (Kirschner et al., 2005; Schonleben et al., 2008). This indicates that these mutations may have carcinogenic potential, which may be the cause of PTC. All these mutations were found in women with PTC except for the T599S (c.1795A>T) found in two patients, one of them was a 55-year woman with an advanced stage of anaplastic carcinoma, the second T599S mutation was found in a 47-year women with papillary thyroid carcinoma. This mutation was reported previously in a patient with melanoma (Wheler et al., 2015) but there is no data concerning its pathogenicity.

The BRAF W619R (c.1855T>A) was reported once previously in a patient with melanoma (Kirschner et al, 2005). It is described in COSMIC as pathogenic (0, 99). In our study it was found in a woman aged 76 years with a 2 cm papillary thyroid carcinoma. The S616F (c.1847 C>T) however was previously found in pancreas carcinoma and cutaneous melanomas (Schonleben et al., 2008; Schonleben et al., 2008; Schulten et al., 2012; Si et al., 2012); it was classified as probably damaging causing alteration of the cell adhesion and impacting MAPK pathway. It was found in a women aged 64 years with a 5 cm papillary thyroid carcinoma.

The L584I (c.1750C>A) was reported in COSMIC as pathogenic (score 0.98) and it was reported previously in the colon adenocarcinoma (Kong et al., 2017). This mutation was detected in a women aged 43 years with papillary thyroid carcinoma. The L597L (c.1791A>T) mutation was found in a 47-year women with papillary thyroid carcinoma.

The L597L (c.1791 A>T) mutation was found in a 47-year women with papillart thyroide carcinoma. This mutation was reported previously in one over 83 Basal cell carcinomas of the skin (Stamatelli et al., 2011) and it was considered as pathogenic in COSMIC (score 0.95)

One not previously reported mutation; the D587Y (c.1759 G>T) was associated to V600E mutation in young women (35 year) with early-stage papillary carcinoma. In silico analysis shows that this mutation is deleterious for the protein function and probably damaging according to COSMIC.

After reviewing the published data, more than 50 BRAF mutations in lung; skin; colon and pancreatic cancer have been discovered to date. Considering the frequency of theses mutations and their effect on the protein, we think that they should be assessed in TC.

In conclusion, BRAF mutation in the thyroid cancer of Libyan patients is frequent but have some particularities. It is not associated with clinicopathological characteristics and almost 30% of them are non-V600E. Theses mutations are rare, but they affect the protein function leading to its activation. These data should be validated on larger series to assess the real impact of these mutations on the carcinogenesis of PTC in Libyan patients.

Author Contribution Statement

Study conception and design: HTG, HT, IBA, OA. Data acquisition: HTG, MA, HT, IBA. Analysis and interpretation of data: HTG, HT, IBA. Bioinformatic analysis: NBJ, IBA. Technical experiment: HT, IBA. Full manuscript redaction: HTG, HT, IBA. Involvement in the drafting of the manuscript: SB, OA Critical revision of the article: HTG, IBA and OA. Submission procedure: HTG and IBA. All authors read and approved the final manuscript.

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Ethics approval

The ethical approval for the study was provided by the ethical committee of Musrata Cancer Centre.

Conflict of Interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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