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The Role of BRAF Mutation (V600E) in Papillary Thyroid Carcinoma (PTC)

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Abstract

Introduction. Thyroid cancer is usually associated with genetic alterations. Papillary thyroid carcinoma (PTC) was found to be accompanied by point mutation occurs in a part of exon 15 in specific gene called BRAF which is a part of pathway that responsible for cell main functions such as division. The role of BRAF gene point mutation (V600E) in the occurrence of PTC was investigated in this study.

Study designs and subjects: 70 patients previously diagnosed with PTC in addition to 35 controls were recruited in this control-based study. Patients were grouped according to their ages and gender. Blood samples were collected for DNA extraction. PCR was run to detect V600E point mutation in BRAF gene.

Results: PTC incidence was found to be higher in patients aged from 40-49 years. The percentage decreased as ages increased. Females had higher PTC frequency than males, 66% to 34%, respectively. The V600E point mutation was detected in 35 patients out of 70 (50%).

Conclusion. Genetic studies have to be a part of thyroid cancer investigations. The study confirmed that PTC is usually associated with molecular defect. Other studies such as thyroid profile tests (TSH, T3 or T4) have to be done along with genetic studies for more confirmation and more understanding of this molecular defect.

Key words*: PTC, BRAF, V600E and point mutation.*

Introduction

Thyroid cancer is considered one of the most prevalent endocrine tumor with higher incidence rates (three-fold) in women than in men [(1)]. In 2008, thyroid cancer was the sixth most diagnosed cancer in women [(1,2)]. The frequency of thyroid cancer has increased in the recent decades due to the improvement in the diagnostic procedures and increased use of therapeutic irradiation but it still constitutes about 1% of all human malignant tumors [(3)]. Thyroid tumors are derived primarily (90%) from thyroid follicular cells while the remainder is arising from calcitonin-producing cells or other thyroid cells [(4)]. Papillary thyroid carcinoma (PTC) comprises about 90% of all thyroid cancers with incidence ages ranges from 20-50 years [(4)]. Somatic mutations in BRAF gene; which is a part of signaling pathway known as RAS/MAPK pathway that responsible for controlling cell main functions such as proliferation, differentiation, migration and apoptosis, has been associated with many types of cancer including thyroid cancers [(5)]. PTC is usually related to molecular defect resulted from an activating point mutation in BRAF gene [(2)]. This point mutation is a result of thymine to adenine transversion at position 1799 (T1977A) in exon 15, leading to a replacement of valine amino acid with glutamic acid at residue 600 (V600E) which terminating with constitutive activation of BRAF kinase [(2)]. The active kinase is believed to be produced constitutively in the presence of mutation due to the disruption in the hydrophobic interaction between the activation loop and residues in the ATP binding sites that allow the kinase to be in an inactive conformation [(6)]. The mutation leads to more

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interaction to be generated rendering the kinase into a catalytically competent structure [6]. Mutant BRAF correspondingly exhibits higher level of kinase activity [6]. The prevalence of BRAF mutation was reported by some studies in 29-83% of PTC cases as the most common detected oncogene [7-10]. Poor prognosis and high aggressiveness are both related with PTCs with BRAF mutation [11]. The activating point mutation in BRAF gene leads to overexpression of BRAF protein which was found in some studies to be significantly higher than that in other thyroid disorders such as multinodular goiter (MNG) [2]. It is also higher in PTC with lymph nodes metastasis (LNM) than that without metastasis [2].

Keeping into mind the increased occurrence of PTC nowadays, this study was aimed to point out the frequency of BRAF point mutation (V600E) in PTC cases. The frequency of PTC among specific age groups or gender was studied as well.

Material and Method

Patients and controls

A total of 70 histologically confirmed patients with thyroid cancer admitted to Al-Sadder Teaching Hospital in Basra province /oncology unit where they prospectively considered. Patients with other types of cancer, patients undergone radiotherapy, or chemotherapy in the last three weeks; and patients with other active medical conditions such as (benign thyroid tumor, heart failure, hepatic disorder, renal failure, uncontrolled diabetes and infections) were excluded. Additional 35 persons with benign thyroid tumors were enrolled as a control group. The Ethics Committee at our institute has approved the study. The stages of PTC were classified according to the TNM staging system and related information were taken from saved patients records [12].

Sample collection/ storage

70 blood samples were collected from all patients in EDTA tubes for DNA extraction. PCR was run to determine the presences of BRAF (T1799A) mutation. Genomic DNA was extracted from EDTA anticoagulant peripheral blood leukocytes using Accupower® Genomic DNA extraction kit (Bioneer. Korea), and then stored at -20 C till the day of use.

Polymerase chain reaction

Primers used for amplification of the BRAF gene and BRAF mutant genes were designed using Primer Premier 7.0 (Bioneer. Korea). The upstream and downstream primers; respectively, were: 5'-TCATAATGCTTGCTCTGATAGGA-3', and 5'-GGCCAAAAATTTAATCAGTGGA-3'. The amplified fragment was 224 bp in length. The reaction mix was done in 25 µl volumes including 5µl of template DNA, 12.5 µl of GoTaq®Promega Green Master Mix 2X, 2µl of primers (foreword and reverse) and 3.5µl of nuclease Free water (Applied PCR system, USA).

PCR conditions were as following: initial denaturation at 94°C for 5 min, followed by denaturation at 94°C for 30s, annealing at 60°C for 1 min and 1 min of extension at 72°C, with a final extension of 7 min at 72°C. The fragments were visualized by electrophoresis on a 1% agarose gel stained with 5% ethidium bromide. The gel was then analyzed under UV light using Cleaver Gel Documentation System (Cleaver Scientific Ltd, UK) for the presence or absence of an allele specific band.

Statistical Analysis

Graf pad prism 5 computer software was used to statically analyze the results. The strength of association between 2 categorical variables, such as the presence of certain grade, stage, sex and disease status, also the differences of genotype between thyroid cancer patients and control groups, the odds ratio (OR) and 95% confidence intervals (CIs) were assessed by a special $\chi 2$ formula. The difference was considered significant, if $p < 0.05$.

Results

1-Demography study

The present study showed that maximum cases; where V600E point mutation presented, were observed in the age group (40-49 yrs) that recorded 47%, ages range between (50-59 yrs) recorded 27%, followed by 16% in (60-69 yrs) and finally 10% (70-79 yrs), Figure 1-A. The results also showed that the prevalence of mutation was higher in females ((23) 65.72%)) than in males ((12) 34.28%)), Figure 1-B.

2-Disterbution of patients according to Staging and Grading

By returning to the saved patients' records and as shown in table 2, the patients were classified according

to the size of tumor (T), the spread of cancer to lymph nodes (+ve for spreading and –ve for not) as well as if there is a metastasis (M1) or not (M0) were all clarified (Table 1). A significant difference (P≤0.05) was seen among different stages.

The results also showed that 14 cases (20%) were classified as grade I, while 25 cases (33.7%) and 31 cases (44.3%) were classified as grade II and III, respectively. The difference among different grades was significant (P ≤0.05).

Results of DNA sequencing

Part of exon 15 of BRAF gene was amplified using specific primers which ensures yielding a product where the length of the wild-type BRAF is 97bp while the mutant BRAF is 224bp. The results of electrophoresis of the PCR reaction products are shown in Figure 2. Nine samples were included, eight samples with PTC and one with benign thyroid tumor. The product of the eight samples were between 200 and 300 bp, while the wild-type showed a product of about 97bp. In total, BRAF V600E mutation was detected in 35 out of 70 cases (50%). No BRAF V600E mutations were detected in 35 benign thyroid cancers controls.

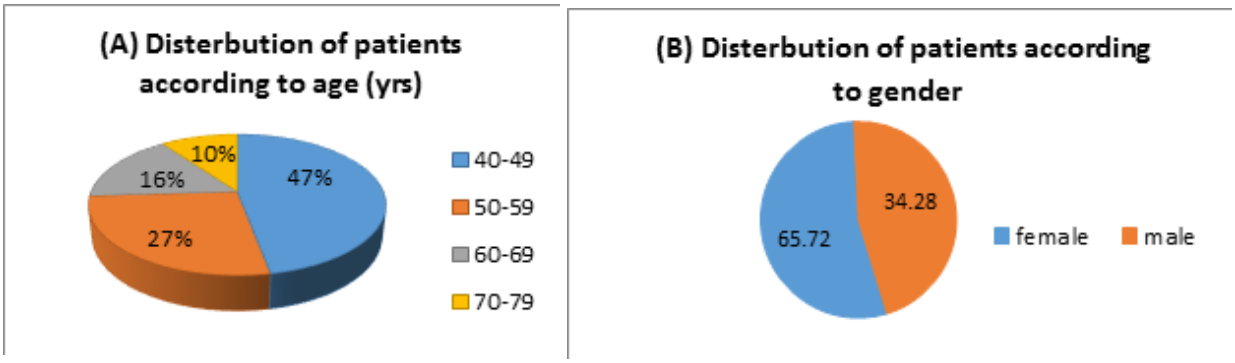

Figure 1: distribution of patients according to age (A) and gender (B)

Table 1: TNM classification and grading in thyroid cancer patients

Gategory	thyroid cancer No (%)
Tumor stage	
T 1-2	32 (45.7 %)
T 3-4	38 (54.3 %)
Lymph node	
N +ve	40 (57.2 %)
N -ve	30 (42.8 %)
Metastatic	
M0	24 (34.3%)
M1	46 (65.7%)
Grade	
1	14 (20 %)
11	25 (35.7 %)
111	31 (44.3 %)

X2 = 16.060 , df = 3, P value≤0.01)

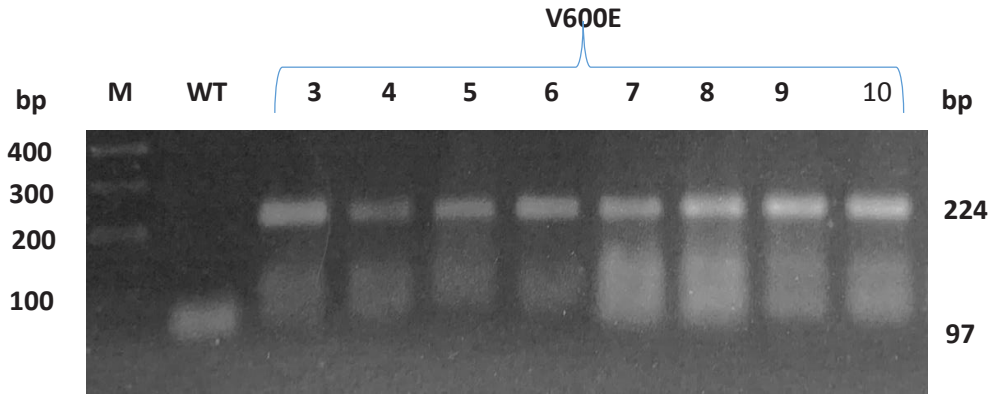

Figure 2: 1% agarose gel electrophoresis of Nine DNA samples. The 1st lane represents the marker DNA. The 2nd lane represents the wild-type BRAF of 97bp product. Lanes (3-10) represent samples from 8 patients with PTC, all showing a product of 224bp.

Discussion

Papillary thyroid cancer (PTC) is considered the most frequent malignancy that is usually associated with somatic mutations in oncogene BRAF gene [13]. Because of its importance in the risk prognosis and management of PTC, this gene has attracted the attention of researchers through the world in the recent years. Our study's aim was to point out the association between the inherited BRAF variants and PTC based on a case–control study. The study showed that 35 out of 70 patients with PTC were diagnosed with V600E point mutation with a majority observed in the age group (40-49 years). The chance of having the mutation decreases gradually after the age of 50. The results also showed that gender could be a determination factor of V600E occurrence where 23 females (65.72%) were diagnosed with PTC, comparing to 12 males (34.28%). V600E has been described by many studies as the most common mutation in the BRAF which occurs in about 29%-83% of PTC cases, where classical PTC and worse patients' outcomes can be associated. (13) Importantly, research found that BRAF could play an important role in the tumor development and evolution in addition to its main role in the tumergenesis [14]. Kim et al. reported that metastasis in the lymph nodes of PTC could be predicted from the BRAF V600E mutation in the primary lesion, and could be more valuable than other PTC- related factors such as age, clinical stage, and tumor size [15].

Another study confirmed that BRAF gene alterations are not restricted to thyroid cancers, but they also could be seen in various cancers like malignant melanoma (27–70%), colorectal cancer (5–22%), and serous ovarian cancer (<30%) [2]. The progression and aggressiveness in the carcinogenesis of thyroid tissue can be indicated by the BRAF mutation and over-expression. Many other studies have focused on the association between Thyroid stimulating hormone (TSH) and PTC. A study conducted by Huang et al. found that serum TSH level below normal ranges associated with high risk of PTC among women in contrast to the risk of PTC in men where high TSH level was associated [16]. Boelaert et al. found high aggressiveness of malignancy in patients with solitary nodules and elevated TSH levels [17]. It is highly recommended that in patients at stage III or IV, the TSH level must be targeted at the undetectable level until further investigations on BRAF mutation become available [18].

In conclusion, in this study we tried to point out the role of BRAF point mutation (V600E) in PTC patients especially in the ages above 40 years. Patients aged 40 to 49 years were at higher risk of having V600E mutation. As observed by many other studies, our study found that V600E is highly presented in females. The mutation was found in 50% of our study's patients which reflect the role of this mutation in the occurrence of PTC. However, the study had many limitations such as the accessibility to only 70 patients which is considered a small sample size, and the lack of other tests results such as TSH or other thyroid hormones which could have drawn a conclusion on the relationship between any of these hormones and PTC.

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