# Braf Mutation In Differentiated Thyroid Cancer And Thyroid Nodules

Esmaeil Faraji[1*], Amir Bahrami[2], Morteza Jabbarpoor Bonyadi[3], Morteza Gojazadeh[4], Nikou Fotouhi[5], Akbar Aliasgarzadeh[6], Jaafar Shadi[1], Omid Mashrabi[7]

1- Fellow of Endocrinology and Metabolism, Internal Medicine Department, Faculty of Medicine, Tabriz University of Medical Sciences, Iran.
2- Professor of Endocrinology and Metabolism, Internal Medicine Department, Faculty of Medicine, Tabriz University of Medical Sciences, Iran.
3- Associate Professor, Molecular Genetics, Faculty of Medicine, Tabriz University of Medical Sciences, Iran.
4- PhD Physiologist, statistician, Faculty of Medicine, Tabriz University of Medical Sciences, Iran.
5-M.Sc of Molecular genetic, Faculty of Natural Science, University of Tabriz, Iran
6- Associate Professor of Endocrinology and Metabolism, Internal Medicine Department, Faculty of Medicine, Tabriz University of Medical Sciences, Iran.
7- Resident of Internal Medicine, Internal Medicine Department, Faculty of Medicine, Tabriz University of Medical Sciences, Iran.
*_Corresponding author: Esmaeil Faraji (smlfrj@yahoo.com)

**Abstract: Introduction:** Thyroid cancer is the most common cancer of the endocrine system. It`s incidence increases with age. Genetic alterations such as BRAF mutations may play a role in thyroid cancer pathogenesis. These mutations occur almost always in papillary thyroid cancer and have been the focus of interest in recent years. **Patients and Methods:** In a cross sectional study, 55 patients including 40 patients with benign thyroid nodule and 15 patients with malignant thyroid nodule were enrolled into two groups A and B respectively. Group A was assigned to clinical follow up and group B underwent total thyroidectomy. Both groups were tested for BRAF mutation. The data was compared. **Results:** The mean age of the patients was 40.15 (18-81) years. BRAF mutation was present in 4 (26.7%) of the group B pts and none of the group A which was statistically significant (p=0.004). There was an association between BRAF mutation and extra thyroidal extension and local aggressiveness of the tumor. No correlation was found between BRAF and age of the patients (r=0.16, p=0.22). NO association was found between BRAF and stage of the thyroid cancer (p=0.17). **Conclusion:** There is an association between BRAF mutation and papillary thyroid cancer and tumor local aggressiveness. However, at this time BRAF mutation is not a robust tool for the management of thyroid cancers and more studies are needed to establish its role as a diagnostic factor.

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## 1. Introduction

Thyroid cancer is the most common cancer of the endocrine system. Its incidence increases with age and reaches a plateau after age 50. It's F: M ratio is 2:1 but has poorer prognosis in males. Childhood radiation exposure is an important risk factor (Davies and Welch, 2006). Palpable thyroid nodules are common in the adult population with an estimated prevalence in the United States of 4–7%, resulting in 10–18 million affected individuals. The frequency of thyroid nodules detectable by ultrasonography is even higher and may exceed 50% in patients over 65 yrs old (Saavedra, 2007). The vast majorities of thyroid nodules are benign and can be managed conservatively, whereas approximately 5–15% of nodules examined by ultrasound and fine-needle aspiration (FNA) cytology are malignant (Burgess and Tucker, 2006). A challenge facing the physician is to distinguish between benign nodules and malignant tumors to ensure that each patient receives timely and appropriate treatment, while minimizing the risk of unnecessary intervention (Nikiforov and Nikiforova, 2011). FNA biopsy and cytologic examination of collected cells is the most accurate and widely used diagnostic tool at this time. It provides a definitive diagnosis of a malignant or benign nodule in most cases. However, a conclusive diagnosis cannot be obtained by use of FNA cytology for about 25% of all nodules in which case the clinical management of patients with these nodules faces difficulty. New diagnostic approaches for such nodules are in need (Nikiforov and Nikiforova, 2011).

Knowledge of genetic alterations occurring in thyroid cancer has rapidly expanded in the past decade. This improved knowledge has provided new insights into thyroid cancer etiology and has offered novel diagnostic tools and prognostic markers that

enable improved and personalized management of patients with thyroid nodules (Nikiforov and Nikiforova, 2011). The association of T1799A BRAF kinase and thyroid cancer has been noticed in recent years. This mutation occurs almost always in papillary thyroid cancer (Nikiforov and Nikiforova, 2011). Many studies have shown the association of this mutation with clinico-pathologic factors effective in progression, recurrence and treatment failure in papillary thyroid cancer. BRAF is a serine-threonine kinase that is translocated to the cell membrane after being bound and activated by RAS, which results in the phosphorylation and activation of MAPK kinase and other downstream targets of the MAPK signaling pathway (Jin, 2006). In other words, BRAF mutation activates a kinase which stimulates MAP protein kinase activated by MAPK mitogen and this shows that activation of MAPK cascade is necessary for tumor genesis (Kim, 2006).

In thyroid cancer BRAF is activated as a result of many mutations. The most common mutation is in locus 1799 which causes a substitution of T to A and as a result Valin to Glutamate (V600E) in the resultant protein. This leads to aberrant gene expression and tumor genesis. So this mutation can be used in the diagnosis of malignant from a benign nodule and prognosis of aggressiveness, recurrence and risk of metastasis in thyroid cancer (Lee, 2006). Many studies have been conducted across the world with different results which were occasionally contradictory. Inasmuch as we know there has been no such a study in our region, so regarding the importance of improving diagnostic methods in thyroid nodules, we conducted a study of BRAF mutation in differentiated thyroid cancers for the first time in our country.

## 2. Material and Methods

**Patients and Methods:** In a cross sectional study, 55 patients (40 with benign, 15 with malignant thyroid nodule) were studied. They were enrolled into two groups of A and B respectively. The study was done from March 2012 to March 2013 in Endocrinology Clinic of Imam Reza Hospital, Tabriz University of Medical Sciences, IRAN.

Patients with thyroid nodule underwent FNA biopsy of the nodule, after history and physical examination. FNA was done by a senior endocrinology fellow verified by attending professors. All patients were provided complete information about the study. Two samples were prepared, one for cytology, another for genetic analysis of BRAF mutation. Cytology specimens were studied by qualified pathologists. 100% of preparations was adequate for diagnosis. Specimens for BRAF, preserved in a special nucleic acid preservative solution (PBS), were submitted to a qualified genetic specialist in a standard genetic lab. All samples provided sufficient material for DNA extraction. The geneticist did not know the results of patients` cytology. Patients with benign cytology were followed clinically and those with malignant or suspicious cytology underwent total thyroidectomy by high volume thyroid surgeons. Surgeons` reports of extension of the tumor and the results of BRAF study and the results of post -operative pathology and pre operative cytology of all patients were compared.

**Nuclear acid isolation and detection of point mutation:**

All aspirations provided sufficient material for DNA extraction. Nuclear acid in the sample was extracted using a standard kit. The specimen was tested for BRAF V600E mutation using primers, PCR amplification and DNA sequencing in a qualified genetic lab.

**Statistical analysis**

The data were analyzed using SPSS16. Independent-samples T test was used for quantitative variables and chi-square for qualitative variables and Fisher`s Exact Test in both. The associations were significant if $p \leq 0.05$.

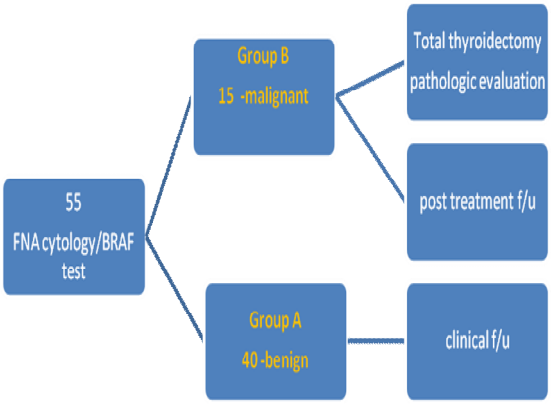


**Fig.1: schematic representation of study design**

## 3. Results

The mean age of the patients was 40.15(18-81) years. The mean age of group A was 41.3±13.8 (18-80) years and that of group B was 39±18.3 (22-81) years which was not statistically significant (p=0.61). 12 patients were male (21.8%) and 43 patients were female (78.2%) of whom 7(17.5%) males and 33(82.5%) females were in group A and 5(33.3%) males and 10(66.7%) females were in group B. The distribution difference was not statistically significant (p=0.18). 3 patients (7.5%) in group A and 1 pt.

(6.7%) in group B had a history of non thyroid cancer of whom 2 patients had cutaneous and one patient had breast cancer in group A. The patient in group B had germ cell tumor of the testis. This was not statistically significant (0.7). History of systemic disease was present in 7 (17.5%) in group A [4 patients had hypertension, 2 patients had T2DM, one patient had pemphigus vulgaris) and in 3 patients (20%) of group B [one pt. had hypertension and one pt. had migraine headache] which was not statistically significant (p=0.55).

The results of FNA cytology, post –op pathology and BRAF test of benign and malignant nodules are shown in table 1 and 2 respectively.

**Table1: Results of cytology and BRAF test in benign nodules**

| No | Cytology |
|---|---|
| 12 | Adenomatoid nodule |
| 5 | Colloid nodule |
| 5 | Follicular adenoma |
| 14 | Nodular goiter |
| 2 | Hashimoto thyroiditis |
| 2 | Lymphocytic thyroiditis |

**Table 2: Results of cytology, BRAF and post-op pathology in malignant nodules**

| No | Cytology | Post–op pathology | BRAF status |
|---|---|---|---|
| 5 | Follicular neoplasm | 3 adenomas | All neg. |
| | | 2 PTCs | |
| 4 | Suspicious for malignancy | 1 adenoma | 1 pos. 3 neg. |
| | | 3 PTCs | |
| 5 | Papillary thyroid cancer | All 5 PTC | 3 pos. 2 neg. |
| 1 | Atypia of unknown significance | NA | Neg. |

BRAF mutation was negative in all patients of group A (100%). In group B it was positive in 4 patients (26.7%) and negative in 11 patients (73.3%). The difference in BRAF status between the two groups was significant (p=0.004). There was no association between BRAF status and gender (p=0.16). There was no correlation between BRAF status and age (p=0.22, R=0.16).There was no association between BRAF and higher stages of the disease. All 5 patients (group B) who had PTC on FNA cytology had extra thyroidal extension and invasion of the tumor. Three of these patients had BRAF mutation which shows an association between BRAF and local invasiveness.


**Fig.2 MAPK pathway [adapted from Xing] (Xing, 2007)**

## 4. Discussions

Many studies have shown a correlation between BRAF mutation and papillary thyroid cancer, it's aggressiveness, extension and recurrence. BRAF is a serine tyrosine kinase which is translocated into the plasma membrane of the cells and after activation by RAS, activates phosphorylation reactions and their downstream signals (Jin, 2006). In other words BRAF activates a kinases and MAP-MAPK cascade. It seems that MAP cascade activation is necessary for tumorgenesis (Kim, 2006). The most common mechanism of activation is a point mutation that involves a thymine is substituted with adenine at nucleotide position 1799(T1799A), which results in a valine-to-glutamate replacement at residue 600 (Val600Glu). This BRAF mutation constitutes 98–99% of all BRAF mutations found in thyroid cancer (Nikiforov and Nikiforova, 2011).

Many studies, some with contradictory results, have been conducted on the association of BRAF mutations and differentiated thyroid cancers across the world(Stanojevic, 2011; Puxeddu and Moretti, 2007; Lim, 2013; Czarniecka, 2010; Kim, 2012). In our study 4 patients (26.7%) with papillary thyroid cancer had a mutation. In contrast, no mutation was found in any patient with benign nodules. In a Japanese study, an association of BRAF mutation with advanced disease stages of PTC was observed by Namba et al. In this multicenter study on 207 patients ,BRAF mutation was present in 24.6% of all patients.,28.8% of papillary cancers ,2 of 6 undifferentiated carcinomas, one of follicular adenomas and none of the follicular cancers(Namba, 2003).

In a large series of PTC cases consisting of mainly American patients, a significant association of BRAF mutation with extra thyroidal invasion and advanced disease stages III and IV was reported by Nikiforova et al. In this study on 320 patients BRAF mutation was positive in 38% of papillary cancers, 13 % of poorly differentiated thyroid cancers, and 10% of undifferentiated thyroid cancers and none of the follicular thyroid cancers (Nikiforova, 2003). Xing et

al in 2005 did a similar study on 219 patients from multiple centers. BRAF mutation was present in 49% of papillary cancers. There was an association between BRAF mutation and extrathyroidal extension, lymphatic metastasis and more advances stages of the disease which was significant statistically (Xing, 2005).

Kim et al in 2004 conducted a similar study demonstrating an association of BRAF and lymphatic or extrathyroidal extension of thyroid cancer (Kim, 2004). In our study we studied the association of BRAF mutation with age and gender.

Neither age nor gender had a significant association with BRAF status. There was an association between BRAF mutation and extrathroidal extension and local aggressiveness of the tumor. Although there have been many studies supporting the role of BRAF mutation as a contributing risk factor in thyroid cancer, there also were other studies with inconsistent results (Fugazzola, 2004; Li, 2012; Pelttari, 2012; Ahn, 2012; Liu, 2005; Kim, 2005). This may be due to ethnic differences, diversity of medical records, often different reports by pathologist or surgeons and differences in BRAF expression in various subtypes of tumors not considered by reporting pathologists. So what does BRAF status means in the management of patients with papillary thyroid carcinoma? Sarne, in an editorial, poses the question and after a good review of recent studies concludes that there is not as yet a clear picture of the place of BRAF status in the management of patients with thyroid cancer (Sarne, 2012).

Results of our study show an association between BRAF mutation and papillary thyroid cancer and extrathyroidal extension and local aggressiveness of the tumor. It doesn't help determine malignancy in follicular neoplasms, an issue we are so much in need of. So we conclude that, at this time, there are no robust data in support of implementation of BRAF testing in the management and clinical decision making in thyroid cancers except in a little subset of patients.

Disclosure: No conflict of interest relevant to this article is reported hereby.

**Corresponding Author:**
Dr. Esmaeil Faraji:
Internal medicine Department, Faculty of Medicine, Tabriz University of Medical Sciences, Iran.
E-mail: smlfrj@yahoo.com

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