# Relation between *BRAF* V600E mutation and $^{18}$F-FDG avidity in radioiodine refractory differentiated thyroid carcinoma

**Mai Hong Son, Nguyen Thi Nhung, Hoang Quoc Truong, Ngo Thi Minh Hanh, Le Ngoc Ha**

***108 Military Central Hospital***

## Summary

*Objective*: The *BRAF* V600E mutation is one of the prognostic factors in thyroid carcinoma related to GLUT-1 expression which increases FDG uptake. In this study, we investigated the relationship between *BRAF* V600E mutation, clinicopathologic factors and $^{18}$F-fluoro-2-deoxyglucose ($^{18}$F-FDG) avidity in radioiodine-refractory differentiated thyroid carcinoma (DTC). *Subject and method:* Total 46 radioiodine-refractory DTC patients who underwent *BRAF* V600E analysis from the biopsy and $^{18}$F-FDG positron emission tomography/computed tomography from 2011 to 2018. Semi-quantitative analysis of highest hypermetabolic lesion was accessed by automated polygonal regions of interest (ROIs) drawing on attenuation-corrected PET images the Workstation AW4.7 (GE). The relationship between *BRAF* mutation, clinicopathologic factors, and $^{18}$F-FDG avidity was investigated. *Result:* Patients with the *BRAF* V600E mutation present higher $^{18}$F-FDG uptake (median of SUVmax: 7.11) than those without mutation (median of SUVmax: 4.91) but the difference is not statistic significance (p=0.236). The tumor size (p=0.006) and distant metastases (0.03) were significantly associated with $^{18}$F-FDG uptake in univariate analysis. Aggressive histopathologic type of DTC was only the factor (p = 0.01) related to FDG uptake significantly (p=0.01) in both univariate and multivariate analysis. *Conclusion:* The aggressive and classic type of DTC were the factors significantly related to $^{18}$F-FDG avidity in both univariate and multivariate analysis. The effect *BRAF* V600E of mutation on glucose metabolism in radio iodine refractory patients needs further study in larger groups of patients.

*Keywords:* $^{18}$F-fluorodeoxyglucose, positron-emission tomography, *BRAF* V600E mutation, histopathologic type, radioiodine refractory.

## 1. Background

Thyroid carcinoma is one of the most popular endocrine cancer worldwide. Among common thyroid cancer types, differentiated thyroid cancer (DTC) has taken into account of 90% [1]. However, there is 5% of DTC patients developed more aggressive disease with radioiodine-refractory therapy, often becoming the cause of mortality tumor recurrence and distant metastases [2]. These patients were predicted to have poorer prognosis and absence of an absolute effective treatment among surgery, radiation therapy, chemotherapy and target therapy. As a result, risk stratification and prognostic evaluation should be focused by nuclear physicians to define the appropriate treatment modality for these patients.

**Correspondence to:** Mai Hong Son - Department of Nuclear Medicine, 108 Military Central Hospital

**Email:** alex.hong.son@gmail.com

$^{18}$F fluorodeoxyglucose (FDG) positron emission tomography/computed tomography (PET/CT) is a noninvasive diagnostic modality benefit for localize residual, recurrent disease and particularly when iodine avid has been lost [3], [4]. *BRAF* V600E mutation have been revealed in variety of cancers including thyroid cancer with radioiodine - refractory therapy. As a prognostic factor, *BRAF* V600E mutation and FDG uptake on PET/CT could provide additional information for risk stratification and prognosis in group of DTC patients. Recent studies showed the relationship between *BRAF* V600E mutation and FDG uptake in papillary DTC patient [5]. The *BRAF* V600E mutation was related to GLUT-1 expression which increases FDG uptake [6]. However, the role of $^{18}$F-FDG PET/CT and *BRAF* mutation in risk stratification and prognosis in group of radioiodine - refractory therapy patients has not been studied well. Based on the results of previous studies, our hypothesis was that the *BRAF* mutation may increase the $^{18}$F-FDG uptake in radioiodine - refractory patients. The aim of this study was to investigate the relationship between *BRAF* mutation status and $^{18}$F-FDG avidity in radioiodine - refractory patients.

## 2. Subject and method

Approving from the thyroid cancer tumor board of 108 Military Central Hospital from 2015 - 2018, 46 consecutive patients with diagnose of radioiodine - refractory differentiated thyroid carcinoma were recruited retrospectively. Diagnosis of radioiodine refractory DTC was based on American Thyroid Associate (ATA) 2015 including one of criteria [7]. Total of 46 radioiodine refractory patients underwent whole body $^{18}$F - FDG PET/CT for restaging and risk stratification before selection of treatment strategies. Recurrent and or metastatic lesions on $^{18}$F-FDG PET/CT was biopsied and analized *BRAF* V600E mutation.

PET/CT examination was performed, using GE Discovery Lightspeed scan (STE) and Discovery 710, according to the European Association of Nuclear Medicine (EANM) guidelines, version 1.0 [7]. For patient preparation, the serum glucose level was checked to exclude hyperglycemia. Afterwards, the patients were allowed to rest in the waiting room before intravenous injection of 2.5MBq/kg body weight (±10%) of $^{18}$F-FDG. Contrast enhancement CT was used with 100ml of the contrast material was used with a scan delay of 30s and an injection rate of 3ml/s from the skull base to the mid-thigh were performed 60 min after $^{18}$F-FDG injection. The parameters of CT scan were as follows: 120kVp, 100mA, helical thickness of 3.75mm and 0.5s/rotation. PET images were reconstructed using an iterative algorithm with attenuation correction with CT.

Semi-quantitative analysis of highest hypermetabolic lesion were accessed by automated polygonal regions of interest (ROIs) drawing on attenuation-corrected PET images the Workstation (version 4.7, GE Healthcare). For lesions suspected malignant on CT images but do not show the visual uptake $^{18}$F-FDG on PET, the manual ROIs were drawn on contrast enhance CT and cloned to PET images to record the metabolic uptake. In cases of multiple malignant lesions, an ROI was drawn on the hottest lesions on PET. Maximum standardized uptake value (SUVmax) was calculated with the injected dose and patient's body weight.

*BRAF* V600E mutation was analyzed in formalin-fixed paraffin-embedded tissues by using two methods including immunohistochemistry and real-time PCR in Department of Pathology, 108 Military Central Hospital.

The statistic software Statistical Package for the Social Sciences (SPSS) 20.0 and GraphPad Prism (version 8.0, GraphPad software) have been used to analyze the data. The multivariate analysis was analyzed by logistic regression. All p-values were calculated by t-test, 2 tailed Mann-Whitney U test

and Fisher extact test and p less than 0.05 was considered statistically significant.

## 3. Result

### *3.1. Patient characteristics*

**Table 1. Clinicopathologic characteristics of radioiodine - refractory patients**

| Clinicopathologic characteristics of the patients | number (n = 46) | percent (%) |
|---|---|---|
| Age (mean ± SD) | 48.2 ± 15.4 | |
| ≤ 45 | 23 | 50 |
| > 45 | 23 | 50 |
| Gender | | |
| Male | 7 | 15.2 |
| Female | 39 | 84.8 |
| Accumulative dose | | |
| < 600mCi | 41 | 89.1 |
| ≥ 600mCi | 5 | 10.9 |
| The time of I-131 treatment | 2.96 ± 1.39 (minimum: 2, maximum: 9) | |
| Serum Tg level (ng/ml, mean ± SD ) | 285.1 ± 188.74 (minimum: 16.3; maximum: 1000) | |
| Recurrent/metastatic location | | |
| Thyroid bed | 12 | 26.1 |
| Cervical lymph node | 42 | 91.3 |
| Distant metastases | 9 | 19.6 |
| Histopathological type | | |
| Classical type | 28 | 60.9 |
| Aggressive type | 18 | 39.1 |
| *BRAF* V600E mutation | | |
| Positive | 38 | 82.6 |
| Negative | 8 | 17.4 |
| SUV (mean ± SD, median/min/max) | 9.46 ± 7.35; 6.5/1.98/36.2 | |

The ratio of patient under forty five years old and patients older than forty five years old were the same. The percentage of female patients contributed the major part of overall (84.8%). Among 46 patients in this study, the *BRAF* V600E mutation was prensent in 38 (82.6%) patients. In the histopathologic variants, the proportion of classical type was 60.9% higher than those of aggressive type (39.1%). Localization of recurrent and or metastatic lesions were seen mostly on cervical lymph node in

42/46 patients (91.3%) while the other lesions on thyroid bed and other organs were only in 12/46 patients (26.1%) and 9/46 (19.6%) respectively (Table 1).

### 3.2. Relationship between SUVmax, BRAF V600E mutation and histopathologic types


A B

**Figure 1.** a. Distribution of SUVmax for *BRAF* V600E mutation negative and positive.
b. Distribution of SUVmax for histopathological type of thyroid carcinoma

The $^{18}$F-FDG uptake of iodine refractory lesions were analyzed semi-quantitatively using SUVmax, the median SUVmax of the *BRAF* V600E mutation-positive tumors was not significantly higher than that of the *BRAF* mutation negative tumors (7.1 vs. 4.91, p=0.23, Mann-Whitney U test, Figure 1a). In stark contrast, the median SUVmax of the aggressive type of histopathology was significantly higher than median of the classic type (13.24 vs. 5.285, p=0.003, Mann-Whitney U test, Figure 1b).

There was no significant difference of $^{18}$F-FDG uptake between subgroup of classic type with *BRAF* V600E positive and *BRAF* V600E negative. However, we observed a significant difference of median of SUVmax when comparing between aggressive type thyroid carcinoma with and without *BRAF* V600E mutation lesions (13.2 vs. 5.6, p=0.03, Mann-Whitney U test, Figure 2).

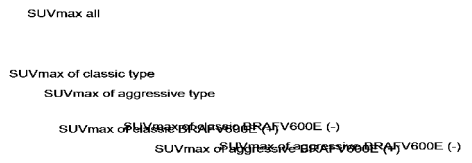


**Figure 2.** Distribution of SUVmax for RAI refactory, histopathological type of thyroid carcinoma, all *BRAF* V600E positive and negative lesions.

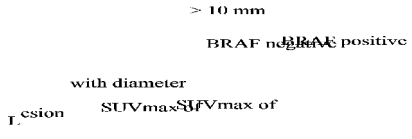


**Figure 3.** Comparing the SUVmax of tumors (> 10mm) between *BRAF* V600E negative and positive

There was no signigicant difference of SUVmax of tumor with diameter > 10mm between *BRAF*

V600E negative and positive group. The median of SUVmax in group of *BRAF* V600E negative was lower than that of *BRAF* V600E positive (7.5 vs 9.410, p=0.5145, Mann-Whitney U test, Figure 3).

Table 2. The relationship between BRAF V600E mutation and histopathological type of thyroid carcinoma

| Histopathological type *BRAF* V600E | | Differentiated thyroid carcinoma (DTC) | | | | p |
|---|---|---|---|---|---|---|
| | | Classic type | | Aggressive type* | | |
| | | n | % | n | % | |
| *BRAF* | Positive | 23 | 82.1 | 15 | 83.3 | 0.622 |
| | Negative | 5 | 17.9 | 3 | 16.7 | |
| ***Total*** | | ***28*** | ***100*** | ***18*** | ***100*** | |

In group of DTC classic type and aggressive type, the proportion of *BRAF* V600E mutation was 23/28 (82.1%) and 15/18 (83.3%) respectively. As a result, there was no significant difference between *BRAF* V600E mutation and histopathological type of DTC (p=0.622, Fisher extact test, Table 2).

**Table 3. Unvariate and multiple variate analysis logistic regression analysis of the factors related to SUVmax (n = 46)**

| Factors | n | SUVmax (mean ± SD) | Univariate and p value | Multivariate and p value |
|---|---|---|---|---|
| Age | | | | |
| ≤ 45 | 23 | 7.0 ± 1.5 | 0.026* | 0.117 |
| > 45 | 23 | 11.9 ± 1.5 | | |
| Gender | | | | |
| Male | 39 | 8.4 ± 7.06 | 0.03* | 0.043* |
| Female | 7 | 15.0 ± 8.08 | | |
| Histopathological type | | | | |
| Classic type | 28 | 6.6 ± 1.26 | 0.01* | 0.01* |
| Aggressive type | 18 | 13.9 ± 9.43 | | |
| Mutation | | | | |
| *BRAF* V600E (+) | 38 | 6.81 ± 4.15 | 0.279 | 0.244 |
| *BRAF* V600E (-) | 8 | 10.01 ± 7.99 | | |
| Tg (ng/ml) | | | | |
| ≤ 40 | 3 | 8.8 ± 6.3 | 0.876 | 0.473 |
| > 40 | 43 | 9.5 ± 7.66 | | |
| Lesion's size | | | | |
| 5 - 10mm | 27 | 6.94 ± 4.02 | 0.006** | 0.091 |
| > 10mm | 19 | 13.03 ± 9.77 | | |
| Recurrence/ metastases | | | | |
| Local recurrence | 12 | 14.6 ± 10.06 | 0.05 | 0.048* |
| No local recurrence | 34 | 7.6 ± 5.55 | | |
| Cervical lympho node | 37 | 8.5 ± 6.51 | 0.097 | 0.214 |
| No cervical lympho node | 9 | 13.2 ± 10.43 | | |
| Distant metastases | 5 | 14.3 ± 8.51 | 0.03* | 0.142 |

| | | |
|---|---|---|
| No distant metastases | 41 | 8.3 ± 7.53 |

To analyze the relationship between clinicopathologic variables and SUV max in RAI refractory patient, the univariate and multivariate analysis were performed. Univariate analysis revealed the significant relation between SUVmax and age, lesion's size, distant metastases ($p<0.05$). Local recurrence is only the factor affected significantly to $^{18}$F-FDG uptake on PET in multivariate analysis. On the other hands, the gender and histopathologic type of thyroid carcinoma were independent factors associated with FDG uptake in RAI refractory patient in both multivariate and univariate analysis ($p<0.05$). In contrary, *BRAF* V600E mutation was not significantly associated with SUVmax ($p=0.279$. 0.244 in univariate and multivariate analysis).

## 4. Discussion

Our study indicated that the *BRAF* V600E mutation and other factors such as size, Tg, cervical, distant metastases were not related to SUVmax in comparison of median and univariate, multivariate analysis. The small number of patients in group of negative mutation in comparison with mutation positive (8/48 vs 38/48) might affect to the statistic analysis in comparison of mean, median and univariate, multivariate analysis. On the other hand, histopathologic types including classic type and aggressive type of thyroid carcinoma played significant role in the avidity of $^{18}$F-FDG.

The recent studies reported that $^{18}$F-FDG uptake is significantly higher for tumors with *BRAF* V600E mutations than for tumors that are negative *BRAF* V600E [8], [9], [10]. *BRAF* mutation inhibited the expression of sodium iodide symporter (NIS) which was playing an important role in iodine uptake [11], [12]. FDG-PET imaging was based on metabolic glucose in cancer cells which was related to it glucose transporters GLUT-1, GLUT-3, and GLUT-4 expressions. The hypothesis that *BRAF* mutation activated the MAPK pathway and stimulating GLUT-1, GLUT-3, and GLUT-4 expressions and increased FDG-uptake. In a meta-analysis study, there were 1144 patients including 843 patients with *BRAF* V600E positive and 301 with *BRAF* V600E negative showed that the *BRAF* V600E mutation had significantly greater chance of having $^{18}$F-FDG avid lesions.

Most of studies were investigated on primary papillary thyroid cancer patient otherwise the patients in our study were iodine refractory. Lee et al concluded that *BRAF* mutation was associated with over GLUT expression in both primary papillary and metastatic thyroid cancer. However, that study depicted the correlation between *BRAF* mutation and FDG uptake on cervical nodal metastases. Another study reported the inverse relationship between RAI and FDG uptake in patients with metastatic lesions of differentiated thyroid cancer. To our knowledge, the relationship between *BRAF* mutation and FDG avidity in radio - iodine refractory patients with metastatic lesions are limited. In our study, local recurrence in radioiodine refractory patients was the factor affected significantly to $^{18}$F-FDG uptake on PET in multivariate analysis and distant metastases was the factor related to glucose metabolism in univariate analysis.

In both univariate and multivariate analysis, our study found that 18-FDG uptake on PET/CT were significantly affected by the histopathologic types including classic type and aggressive type of thyroid carcinoma. The largest group of patient contributed for iodine refractory and PET-positive supposed to be poor differentiated thyroid carcinoma and most of them presented with stage III or IV disease.

A flip-flop phenomenon was observed between radioiodine and FDG uptake in differentiated thyroid cancer cells [13]. A study highlighted the fact that aggressive thyroid cancer cells upregulated glucose transporter (GLUT) and reduced expression of sodium-iodide symporter (NIS) while the classic thyroid cancer cells maintaining the iodine avidity. This phenomenon proposed that FDG-PET/CT as efficient diagnostic tools in aggressive subtypes of thyroid cancer due to high FDG avidity. In those studies, the sensitivity of FDG-PET/CT was 100% in staging and postoperative staging. In aggressive thyroid tumor including poor differentiated thyroid carcinoma and anaplastic thyroid carcinoma presented chromosomal alteration, RAS, BRAF, TP53,

and b-catenin mutations. These genetic pathways could affect to the different FDG avidity pattern in different histopathologic subtypes of thyroid cancer.

In this study, there was no significant difference of $^{18}$F-FDG uptake between subgroup of classic type with *BRAF* V600E positive and *BRAF* V600E negative. There is the possibility that a further study with larger sample size need to be performed to investigate the pattern of FDG uptake between subtypes of thyroid cancer including classic type and aggressive type.

## 5. Conclusion

Among the clinicopathologic factors, these data suggest that aggressive histopathologic type of thyroid carcinoma was significantly more $^{18}$FDG-avid than classic type. There was no relationship between *BRAF* V600E mutation and $^{18}$FDG uptake in tumor cell.

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