

[68Ga] Ga-DOTA-FAPI-04 And [18F] FDG PET / CT For Diagnosis of Metastatic Lesions In Patients With Recurrent Papillary Thyroid Carcinoma

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Abstract

Context: PET CT imaging methods based on fibroblast activation protein inhibitors (FAPIs) have recently demonstrated promising clinical results.

Objective: We aimed to evaluate the use of ^{68}Ga -FAPI PET / CT and ^{18}F FDG PET / CT imaging techniques to detect the metastatic foci in recurrent papillary thyroid carcinoma.

Design and Patients: This is a prospective study. Patients who were diagnosed with papillary thyroid carcinoma, achieved biochemical recovery after the first operation and having recurrence for papillary thyroid carcinoma on the follow up were included in the study. [^{68}Ga] Ga-DOTA-FAPI-04 and [^{18}F] FDG PET / CT were performed for comparative purpose and detection of recurrence localization.

Results: [^{18}F] FDG PET / CT detected the metastatic foci in 21 of 29 patients (72.4%), [^{68}Ga] Ga-DOTA-FAPI-04 was able to detect the metastatic foci in 25 of 29 patients (86.2%). When the two imaging techniques were used together, the metastatic foci in 27 of the 29 patients could be detected (93.1%). Also between the [^{18}F] FDG PET / CT SUVmax values and [^{68}Ga] Ga-DOTA-FAPI-04 SUVmax values, a statistical significance was found in favor of ^{68}Ga -FAPI PET ($p = 0,002$).

Conclusion: In conclusion, ^{68}Ga -FAPI PET imaging technique can be used as an alternative method to detect the metastatic focus or foci in patients with recurrent papillary thyroid carcinoma. It can also increase the chance of metastatic focus or foci detection when used in conjunction with the ^{18}F FDG PET.

Introduction

Conventional treatment of differentiated thyroid carcinomas (DTCs) is total thyroidectomy, radioactive iodine therapy (RAI) and thyroid stimulating hormone (TSH) suppression therapy. With this highly effective treatment approach, 10-year disease-related survival is around 85% in 85% of DTCs. However, in approximately 5% of patients, the tumor may lose its differentiation and iodine uptake ability and may develop metastases.(1,2) There is no point in giving RAI treatment anymore in these patients. In patients who do not respond to tyrosine kinase inhibitors and cytotoxic chemotherapy is currently recommended in poorly differentiated thyroid cancer, but the response rate is quite low.(3) Protease molecules have recently been the subject of studies on many different subjects. There are four enzymatic members of the dipeptidyl peptidase protease molecules (DPP) protein family; fibroblast activation protein is one of them.(4,5) In studies on FAP, especially in cancer the increase in FAP expression draws attention. Therefore, it has made this protease targetable for therapeutic and diagnostic intervention. FAP expression was found to be significantly increased during wound healing, inflammation sites, atherosclerotic plaques, liver fibrosis and in 90% of epithelial carcinomas.(6) However, there is no clear information about its enzymatic role in different cancer models.(7,8,9) It has been shown that FAP, which is expressed in the cell membranes of active fibroblasts, plays a role in many enzymatic and / or non-enzymatic pathways in the extracellular matrix and is involved in many protumorigenic pathways that cause tumor progression.(4,10) PET CT imaging methods based on fibroblast activation protein inhibitors (FAPIs) have recently demonstrated promising clinical results. Studies on the use of FAPI in many different tumor screenings are included in the literature. In the literature, it has been shown that there is selective tumoral activity involvement in ^{68}Ga -FAPI PET / CT images in 28 different cancers. However, as far as we know, there is no study conducted with the FAPI PET / CT vs FDG PET / CT imaging technique on differentiated papillary thyroid carcinoma. Besides in ^{18}F FDG PET / CT, it is known that

multiple physiological involvement in various organs or ^{18}F FDG retention at different levels in infective / inflammatory events and reactive processes are observed. However, it has been stated that nonspecific involvements are almost never observed in ^{68}Ga -FAPI PET / CT.(11) Here, we tried to evaluate the ability of ^{68}Ga -FAPI PET / CT and ^{18}F FDG PET / CT imaging techniques to detect the metastatic foci in recurrent papillary thyroid carcinomas for future applications.

Materials And Methods

Patients

This prospective study was approved by the Clinical Research Ethics Committee of Gaziantep University. This study also conducted accordance with the 1964 Helsinki declaration for the ethical standards. Patients enrolled to this study from September 2020 to February 2021 ^{68}Ga -FAPI PET / CT performed after ^{18}F FDG PET / CT. Patients' inclusion criteria were ;(1) being older than 18,(2) having total thyroidectomy and papillary thyroid cancer pathology,(3) having One of the RAI-resistant thyroid carcinoma criteria according to American Thyroid Association:

- A patient with malignant or metastatic tissue (one or more lesions) detected in the first whole body scan after the first treatment, with no uptake outside the thyroid bed but not retaining I131 in the follow-up, or
- Tumor tissue that initially has iodine uptake but loses its ability to concentrate radioactive iodine on subsequent scans or treatments, or
- Patients with metastatic disease and radioactive iodine uptake in some but not all regions, or
- Patients with metastatic disease and disease progression within 1 year after treatment despite substantial radioiodine therapy.

(4) patients who were able to provide informed consent. Patients' exclusion criterias were;(1) patients with pregnancy,(2) inability or unwillingness to provide written informed consent.(3) having arthritis, chronic inflammatory condition, or cirrhosis

^{68}Ga -FAPI-04

FAPI-04 was obtained from MedChem Express LLC. The pharmaceutical grade $^{68}\text{Ge}/^{68}\text{Ga}$ generator (50 mCi) and disposable cassettes were supplied by Eckert & Ziegler Eurotope GmbH. Purification cartridge CM (Sep-Pak AccellPlus CM Plus Light Cartridge, 130 mg Sorbent per Cartridge, 37 - 55 μm , WAT023531) was well established in the cassette accessories. Other chemicals and materials were purchased from Aldrich in ultra-pure and trace metal basis grade. The HPLC analyses were performed by Modular-Lab HPLC which Eckert & Ziegler device using ACE-3 C18 150 X 3.0 mm column.

Radiolabeling Procedure

The radiolabeling process was performed by a fully-automated system without any manual interaction. $^{68}\text{Ga}^{3+}$ was eluted with 0.1 N HCl solution (8.0 ml) followed by passing through the pre-concentrated on a strong cation exchange (SCX) cartridge. The ^{68}Ga activity was recovered from the SCX cartridge by 0.9 mL eluent (5 M NaCl/HCl(0.1 M)). Reaction vial is filled by 2 ml of H_2O , 0.4 ml of sodium acetate buffer (pH is around 4.5), 0.2 ml of ethanol and 50 μg of FAPI-04. Then, ^{68}Ga -activity was transferred to the reaction vial, and it was heated to 95 $^\circ\text{C}$

for 10 min. After completion of the reaction, the reaction medium was cooled down and crude product was diluted by adding 5.0 mL of 0.9% NaCl and subsequently purified by CM cartridge. Finally, the reaction mixture was passed through a millipore filter (0.22 μm) and was injected intravenously after more than 98% radiochemical purity with 88% radiochemical yield.

The radiochemical purity was analyzed by R-HPLC and free ^{68}Ga was detected at RT = 2.2 min, whereas ^{68}Ga -FAPI-04 was detected at RT = 3.99 min. (ACE-3 C18 150 X 3.0 mm column, isocratic flow 0.6 ml/min; mobile phase: 85% H₂O (0.1 TFA) and 15% AcCN (0.1 TFA)).

^{18}F FDG

All patients fasted, except for glucose-free oral hydration, for at least 6 h before the IV injection of 370-555 MBq (10-15 mCi) of FDG. At the time of the tracer injection, blood glucose levels were checked and confirmed to be less than 150 mg/dl in all patients.

PET / CT protocol and image evaluation

All patients were examined using a PET/CT system (Discovery™ IQ; GE Healthcare) combining a dedicated, five-ring PET scanner with LightBurst technology.

PET imaging was performed 60 minutes for ^{18}F FDG and 30 minutes for ^{68}Ga -FAPI (5-6 mCi) after injection, extending from the vertex to the pelvis, with 5 bed positions of 3 min each. CT images were used for attenuation correction and fusion; no IV contrast medium was used.

The PET/CT images were carefully evaluated by one experienced nuclear medicine physician. PET, CT and fused whole-body images displayed in axial, coronal and sagittal planes were available for review. A semi quantitative analysis of tracer activity was measured as the maximal standardized value uptake (SUVmax) of ^{18}F FDG or ^{68}Ga -FAPI using the provided software (AW VolumeShare, GE Healthcare).

Images reviewed independently, and two nuclear medicine specialists reviewed the scans independently.

Nature of the metastatic lesion

After detecting uptake with imaging techniques, tyrosine kinase inhibitor was given to some of the patients and the response was followed, and for some patients, the type of tissue involved was determined by biopsy, if possible.

STATISTICAL ANALYSIS

Descriptive statistics of the data obtained from the study are given by mean and standard deviation for numerical variables, and by frequency and percentage analysis for categorical variables. ^{18}F FDG PET SUVmax and ^{68}Ga FAPI-PET SUVmax variables were evaluated with the normal distribution test Shapiro Wilk test and it was determined that they were not normally distributed ($p < 0.05$). FDG and FAPI were not normally distributed then median/IQR used. Mann-Whitney U test was used to compare these variables. Analyzes were carried out with the help of SPSS 22.0 program. A significance level of $p < 0.05$ was chosen.

Results

22 of the patients were female, only 7 were male. The disease stages of the patients were calculated according to the TNM staging before the first operation after diagnosis. The number of sessions of RAI treatment applied in the follow up and the data we consider important for the study are shown in Table 1.

Table 1 Characteristics of patients with recurrent papillary thyroid carcinoma

		Number	Percent (%)
Gender	Male	7	24,1
	Female	22	75,8
Disease stage after 1st surgery	1	5	17,2
	2	11	37,9
	3	8	27,5
	4	5	17,2
RAI treatment sessions	0	5	17,2
	1	7	24,1
	2	5	17,2
	3	7	24,1
	4	3	10,3
	5	2	6,9
Antithyroglobulin status	Negative	24	82,7
	Positive	5	17,2
RAI screening status before Fdg or fapi screening for metastasis	Negative	12	41,3
	Positive	17	58,6
FDG-PET results for metastasis	Negative	8	27,5
	Positive	21	72,4
Ga 68-FAPI results for metastasis	Negative	4	13,7
	Positive	25	86,2
Pathological variant of papillary thyroid carcinoma	Classic	16	55,1
	Tall cell	6	20,6
	Follicular	3	10,3
	Poor differentiated	4	13,7

The comparison of TSH-stimulated maximum thyroglobulin levels before RAI scanning and the highest SUVmax values among metastatic lesions in ¹⁸FDG PET and ⁶⁸Ga-FAPI PET imaging are shown in Table 2. Between the

¹⁸F PET SUVmax values and ⁶⁸Ga FAPI PET SUVmax values, a statistical significance was found in favor of ⁶⁸Ga-FAPI PET.

Table 2 Characteristics of patients with recurrent papillary thyroid carcinoma

	Mean ± Standard Deviation	P value
Age	45,83(±16,39)	
Thyroglobulin level (IU/mL) on the follow up when TSH > 30 mIU/L	1552,92 (±5691,87)	
FDG/PET CT SUV-Max	3,70(±3,41)	p = 0,002*
FAPI/PET CT SUV-Max	7,50(±6,61)	

*Shows the statistical significance result between ¹⁸F PET / CT SUVmax and ⁶⁸Ga-FAPI PET / CT SUVmax.

While ¹⁸F PET / CT detected the metastatic foci in 21 of 29 patients, ⁶⁸Ga FAPI PET / CT was able to detect the metastatic foci in 25 of 29 patients. When the two imaging techniques were used together, the metastatic foci in 27 of the 29 patients could be detected. The characteristics of the patients with ¹⁸F or ⁶⁸Ga-FAPI detected metastatic foci are shown in table 3. While the metastatic focus detection percentage of ¹⁸F was 72.4% among all patients, the detection percentage of ⁶⁸Ga-FAPI PET was 86.2%. When both techniques were used together, the percentage of metastatic focus detection was 93.1%. Image examples of metastatic lesions with both techniques given in figure 1.

Table 3 Metastatic focus detection rates of imaging techniques according to disease characteristics

	FDG Positive	FDG Negative	Positivity Ratio FDG	Positivity Ratio for FAPi	Ga68 FAPi Positive	Ga68 FAPi Negative	Total detection rate for FDG	Total detection rate for FAPi
TG Level								
2-10	3	1	75,0%	50,0%	2	2		
11-300	9	5	64,3%	85,7%	12	2		
> 300	9	2	81,8%	100%	11	0		
Anti-thyroglobulin antibody status								
Positive	5	0	100%	80,0%	4	1		
Negative	16	8	66,6%	87,5%	21	3		
Total body screening status after active radioiodine treatment							72,4%	86,2%
Positive	12	5	70,5%	94,1%	16	1		
Negative	9	3	75,0%	75,0%	9	3		
Pathological feature								
Classic	11	5	68,7%	87,5%	14	2		
Tall cell	3	3	50,0%	66,6%	4	2		
Follicular	3	0	100%	100%	3	0		
Poor differentiated	4	0	100%	100%	4	0		

Metastatic focus detection rates of imaging techniques according to disease characteristics are given in Table 3 in detail.

Discussion

The frequency of thyroid cancer in the world is gradually increasing with the development of imaging techniques. Thyroid cancer is now detected much earlier and in much smaller sizes. (12) It is expected to be the 2nd or 3rd most common cancer in the 2020s. (13) It is important for clinicians to constantly question the availability of new imaging techniques for the ideal follow-up of their patients. Because the number of patients who are difficult to manage has increasing along with the increasing patient volume. In addition, traditional techniques are not sufficient for all patients. The use of ¹⁸FDG PET / CT in papillary thyroid cancer is generally prioritized in patients with post-operative, high serum thyroglobulin levels and negative radioactive iodine (RAI) whole body scans. Preoperative use of ¹⁸FDG PET / CT is still controversial. (14,15) But the patient group in our study is a high-risk patient group with recurrence despite post-operative radioactive iodine treatments. Therefore, it seems reasonable to use ¹⁸FDG PET / CT in such patients where conventional techniques are insufficient. However, despite the use of ¹⁸FDG PET / CT, there are still patients whose metastatic focus or foci cannot be detected hence who cannot be cured. In such cases, a new imaging technique that could help the clinicians will come to the fore to guide the management. Therefore, in our study, we focused on the use of ⁶⁸Ga-FAPi in patients with recurrent thyroid cancer.

In a multi-center study conducted by Kratochwil, the use of ^{68}Ga -FAPI-PET / CT in 28 different cancer types was examined. Six of the 80 patients in this study were patients with differentiated thyroid cancer, and the histological analysis or subgroups in the study were unknown. Although low SUVmax values were detected, imaging with ^{68}Ga -FAPI PET / CT was detected in differentiated thyroid cancer. The fact that our patient group is advanced recurrent patients may explain our higher SUVmax values in ^{68}Ga -FAPI imaging. Also, cancer-associated fibroblasts and extracellular fibrosis may increase in desmoplastic tumors, leaving the original tumor cells in the minority. (4)

According to the pathological classification of papillary thyroid carcinoma; The classic variant and follicular variant that exist in our patient groups are well differentiated, while the tall cell variant is moderately differentiated. (16) With all these recurrence and tumor differentiation features, it was shown in our study that ^{68}Ga -FAPI was not inferior to ^{18}F FDG for papillary thyroid cancer patients with recurrence who's previously treated with radioactive iodine. Moreover, ^{68}Ga -FAPI PET can be used as complementary method with ^{18}F FDG to detect metastatic foci.

As the tumor progresses and the tumor cells lose their ability to uptake iodide, the cancer becomes resistant to radioactive iodine therapy and causes negative iodine uptake screening. (17) In addition, as thyroid cancers become more aggressive, they cause a decrease in sodium iodide symporters and overexpression of the GLUT1 transporter and become radioactive iodine refractory. (18) Hereby more radioactive iodine utilization is expected in differentiated tumors, while radioactive iodine uptake decreases in poorly differentiated tumors. In our study, detection rate of metastatic foci with ^{18}F FDG PET was 70.5% in patients with positive radioactive iodine screening, while detection rate of ^{68}Ga -FAPI PET was 94.1%. Considering that the ^{18}F FDG PET gives more successful results in less differentiated thyroid carcinomas in contrast to well differentiated ones, such a limitation of ^{68}Ga -FAPI PET has not been observed. However, studies with specific patient groups with larger numbers of patients are needed.

We observed that in both imaging techniques, as the TG value increases, the rate of metastasis detection has increased also. ^{68}Ga -FAPI PET imaging was able to detect all metastasis foci when TG > 300 (in all 11 patients). It has been suggested that low TG in patients with recurrent thyroid carcinoma may be associated with tumor dedifferentiation. (19) Regarding this, at lower TG levels (Thyroglobulin antibody negative), ^{18}F FDG PET detected the foci in 3 of 4 patients and ^{68}Ga -FAPI PET was able to detect the foci in 2 of 4 patients.

There are studies showing that ^{68}Ga -FAPI PET imaging is more specific in tumoral conditions because of the low levels of expression of fibroblast activated protein in the body, but high expression of cancer-associated fibroblasts in the presence of tumor cells. (20,21,22) According to the results of our study, using both imaging techniques together seems more useful in detecting the metastatic focus or foci in difficult cases such as recurrent papillary thyroid cancer.

The limitations of our study are as follows; the low number of patients in the study because the study was conducted with a specific disease group and histopathological confirmation of metastases could not be made consequent to their anatomical localization.

In conclusion, ^{68}Ga -FAPI PET imaging technique can be used as an alternative method to detect the metastatic focus or foci in patients with recurrent papillary thyroid carcinoma. It can also increase the chance of metastatic focus or foci detection when used in conjunction with the ^{18}F FDG PET.

Declarations

Compliance with Ethical Standards:

Funding: There is no funding in this study study w

Conflict of Interest: We declare there is no conflict of interest between the authors

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent: Informed consent was obtained from all individual participants included in the study.

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Figures

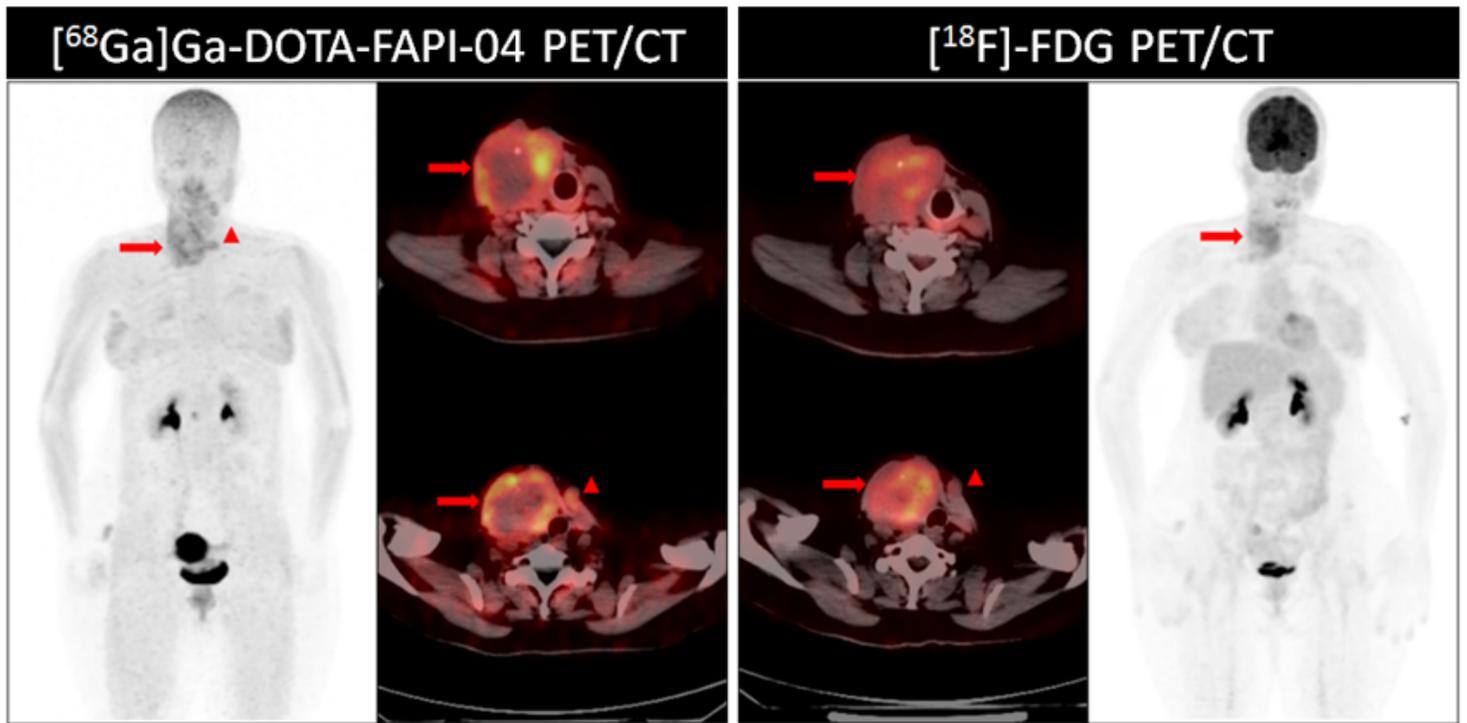


Figure 1

31 years old female with papillary thyroid carcinoma (Encapsulated follicular variant) who has undergone total thyroidectomy and presented with recurrent disease. Both modalities showed significant activity uptake in conglomerated lymph nodes (arrow) observed in right anterolateral side of the neck (68Ga-FAPI PET / CT SUVmax: 7.5; 18FDG PET / CT SUVmax: 7.4). However, more discrete activity distribution was noted in 68Ga-FAPI PET / CT imaging. Moreover, the lymph node located in left anterolateral side of the neck (arrow head) showed markedly elevated 68Ga-FAPI uptake (SUVmax: 3.7), whereas showed no significant 18FDG retention.