Comparison of ^{68}Ga-FAPI-04 and ^{18}F-FDG PET/CT for diagnosis of metastatic lesions in patients with recurrent papillary thyroid carcinoma

Zeynel Abidin Sayiner[1] MD, Umut Elboğa[2] MD, Ertan Sahin[2] MD, Sadettin Ozturk[1] MD, Yusuf Burak Cayirli[2] MD, Yusuf Zeki Celen[2] MD, Ersin Akarsu[1] MD, Ilkay Dogan[3] MD, Benan Kilbas[4] MD, Kurtulus Eryilmaz[4] MD, Davut Cakici[4] MD

1. Gaziantep University, Department of Endocrinology and Metabolism, Turkey
2. Gaziantep University, Department of Nuclear Medicine, Turkey
3. Gaziantep University, Department of Biostatistics, Turkey
4. Moltek Health Services Production & Marketing Inc., Department of R&D, Kocaeli, Turkey

Keywords: ^{68}Ga-FAPI-04 - Thyroid cancer - ^{18}F-FDG PET/CT - Diagnosis thyroid carcinoma

Corresponding author:
Sadettin Ozturk MD,
Gaziantep University, Department of Endocrinology and Metabolism, Turkey
Tel.: +090 507 543 43 18
sadettinozturk27@hotmail.com

Received:
18 February 2023
Accepted revised:
12 April 2023

Abstract

Objective: We aimed to evaluate the gallium-68-labeled fibroblast-activation protein inhibitor (^{68}Ga-FAPI) positron emission tomography/computed tomography (PET/CT) in localizing papillary thyroid carcinoma (PTC) foci in patients with biochemical relapse. Papillary thyroid carcinoma has achieved biochemical recovery after appropriate treatment and had biochemical relapse in the last follow-up were included in this retrospective study. Gallium-68-FAPI and fluorine-18-fluorodeoxyglucose (^{18}F-FDG) PET/CT were performed to detect recurrence foci. **Subjects and Methods:** Biochemically relapsed patients who underwent total thyroidectomy and were diagnosed with pathologically differentiated thyroid cancer were included in our study. Gallium-68-FAPI and ^{18}F-FDG PET/CT imaging methods were used to determine the focus of metastasis or recurrence in all patients. **Results:** Among 29 patients enrolled to the study, pathological subgroups were papillary (n=26) and poorly differentiated (n=3) PTC. Anti-thyroglobulin (TG) antibody positivity were noted in 5 of the patients, while all 29 of them were TG positive and had been consist of three groups as follows: 2-10ng/mL (n=4), 11-300ng/mL (n=14), 301ng/mL and above (n=11). Recurrence was detected in 72.4% (n=21) and 86% (n=25) of the patients via ^{18}F-FDG and ^{68}Ga-FAPI, respectively. Accuracy of detection noted as 100% (5/5), 75% (3/4), and 92.9% (13/14) in groups with the anti-TG antibody positivity, TG levels of 2-10ng/mL and 11-300 ng/mL, respectively, when the two imaging modalities were utilized together. Furthermore, accuracy of ^{68}Ga-FAPI was 100% (11/11) in the group with TG levels of 301ng/mL and above, whereas accuracy of ^{18}F-FDG was 81.8% (9/11). Lastly, median maximum standardized uptake value (SUVmax) of recurrent lesions detected by the ^{68}Ga-FAPI (median SUVmax: 6.0) were statistically higher than the ones detected by the ^{18}F-FDG (median SUVmax: 3.7) (P=0.002). **Conclusion:** In recurrent PTC especially in case of higher TG levels, ^{68}Ga-FAPI can be used in patients with inconclusive ^{18}F-FDG findings.

Hell J Nucl Med 2023; 26(1): 41-46 *Published online: 28 April 2023*

Introduction

Conventional treatments for differentiated thyroid carcinomas (DTC) include complete thyroidectomy, radioactive iodine (RAI), and suppression of thyroid stimulating hormone (TSH). With such highly effective treatments, the 10-year disease-related survival rate for DTC is approximately 98%. In 5% of individuals, however, the tumor may lose its differentiation, iodine uptake capabilities or become metastatic [1, 2]. These patients would be RAI-resistant and represent the vast majority of recurring cases. In poorly differentiated thyroid carcinoma, tyrosine kinase inhibitors and cytotoxic chemotherapy are now recommended, but response rates are quite low [3].

Recently, a member of the dipeptidyl peptidase protease IV (DPP-IV) family, fibroblast activation protein (FAP), has been the subject of studies, especially on its relatively specific expression in many cancers [4, 5]. Also, FAP expression was found to be increased in some benign conditions such as wound healing, chronic inflammation atherosclerosis and liver fibrosis. Recent studies have revealed the increased expression level of FAP, particularly in epithelial carcinomas. Significant FAP expression in different malignancies makes it a potential diagnostic and therapeutic target [6-10]. Fibroblast activation protein has been shown to be expressed in the cell membranes of active fibroblasts and to play various roles in many enzymatic and/or non-enzymatic extracellular matrix pathways; thus, it participates in many cancer pathways that cause tumor progression [4, 11]. Recently, positron emission tomography/computed tomography (PET/CT) imaging based on gallium-68 (^{68}Ga)-labeled FAP inhibitors (FAPI) has shown encouraging diagnostic effects. Gallium-68-FAPI PET/CT has demonstrated selective tumoral activity in twenty-eight distinct malignancies, including thyroid carcinoma [12]. In this study, we evaluated the ability of ^{68}Ga-FAPI PET/CT and fluorine-18-fluorodeoxyglucose (^{18}F-FDG) PET/CT imaging modalities to detect metastatic foci in recurrent papillary thyroid carcinomas for potential future applications.

Subjects and Methods

Patients

This retrospective study was approved by the Clinical Research Ethics Committee of Gaziantep University. This study was also conducted in accordance with the 1964 Helsinki Declaration for ethical standards. The ethics approval number is 2021 or 261. Patients enrolled in this study from September 2020 to February 2021, and ^{68}Ga-FAPI PET/CT was performed after ^{18}F-FDG PET/CT. Patients' inclusion criteria were: (1) being older than 18, (2) having total thyroidectomy and papillary thyroid cancer, (3) having biochemical recurrence detected by elevated thyroglobulin (TG), and (4) patients who were able to provide informed consent. Patients' exclusion criteria 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

The DOTA-FAPI-04 precursor was supplied by MedChem Express LLC. The pharmaceutical-grade germanium-68 (^{68}Ge)/^{68}Ga generator (50mCi) and disposable cassettes were obtained from Eckert & Ziegler Eurotope GmbH. CM cartridge (Sep-Pak AccellPlus CM Plus Light Cartridge, 130mg Sorbent per Cartridge, 37-55µm, WAT023531) was utilized in the cassette system. Other chemicals and materials were manufactured from Aldrich in ultra-pure and trace metal basis grade. The HPLC analyses were performed by Shimadzu LC20A and Eckert & Ziegler HPLC Scan devices using an ACE-3 C18 150x3.0mm column.

Radiolabeling Procedure

The radiolabeling process was performed by a fully automated ML-Eazy system. The $^{68}GaCl_3$ was eluted with 0.1M HCl solution (8.0mL) followed by passing through the preconditioned on a strong cation exchange (SCX) cartridge. The ^{68}Ga activity was purely picked up from the SCX cartridge by the 0.9mL eluent (5M NaCl/HCl (0.1M)). The reaction vial is prepared with sodium acetate buffer (pH is around 4.5), 0.2mL of ethanol and 50g of DOTA-FAPI-04. Afterwards, ^{68}Ga-activity was automatically transferred to the reaction vial and the reaction was conducted at 95°C for 10 min. After completion of the reaction, the reaction medium was cooled to room temperature and ^{68}Ga-DOTA-FAPI-04 was diluted by adding 5.0mL of 0.9% NaCl and subsequently purified using a CM cartridge. Finally, the product was passed through a sterile filter (0.22m) with 98% radiochemical purity and 88% radiochemical yield. The radiochemical purity was well analyzed by R-HPLC, and free ^{68}Ga was recorded at RT=2.15min, whereas ^{68}Ga-DOTA-FAPI-04 was recorded at RT=3.96min. (ACE-3, C18, 150x3.0mm column, isocratic flow 0.6mL/min; mobile phase: 0.1 TFA) H2O: AcCN (85:15).

^{18}F-FDG

All patients were fasted, except for glucose-free oral hydration, for at least 6h before the IV injection of 370-555MBq (10-15mCi) of ^{18}F-FDG. At the time of the tracer injection, blood glucose levels were checked and confirmed to be less than 150mg/dL in all patients.

PET/CT protocol and image evaluation

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

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

The PET/CT images were carefully evaluated by three independent, experienced nuclear medicine physicians. Positron emission tomography, 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 Volume Share, GE Healthcare).

Nature of the metastatic lesion

After detecting uptake with imaging techniques, the type of tissue involved was determined by biopsy as the gold standard. Both imaging techniques were applied to the patients at the same time.

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. Fluorine-18-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 ($P<0.05$). Fluorine-18-FDG and FAPI were not normally distributed then median/IQR used. A Mann-Whitney U test was used to compare these variables. Analyses were conducted with the help of the SPSS 22.0 program. A significance level of $P<0.05$ was chosen.

Results

Twenty-two of the patients were female and the other 7 were male. The disease stage of the patients was determined according to the TNM staging preoperatively. The number of cycles of RAI treatment administered in the follow up and the descriptive analyses of the study are shown in Table 1.

The comparison of TSH-stimulated maximum TG levels before RAI scanning and the highest SUVmax values among metastatic lesions in ^{18}F-FDG and ^{68}Ga-FAPI PET/CT imaging are shown in Table 2. A statistical significance was found between the SUVmax values of ^{18}F-FDG PET/CT and ^{68}Ga FAPI PET/CT, in favor of ^{68}Ga-FAPI.

While ^{18}F-FDG PET/CT revealed metastatic foci in 21 of 29 patients, ^{68}Ga-FAPI PET/CT detected metastatic foci in 25 of 29 patients (Table 3). When the two imaging modalities were

combined, metastatic foci could be detected in 27 of 29 individuals. Table 3 shows the characteristics of patients with ^{18}F-FDG or ^{68}Ga-FAPI-detected metastatic foci. In comparison to ^{18}F-FDG, which detected metastatic foci in 72.4% of patients, ^{68}Ga-FAPI PET/CT detected metastatic foci in 86.2% of patients. When both approaches were performed together, 93.1% of metastatic foci were detected. Figure 1 shows images of metastatic lesions addressed with both modalities. The suspected metastatic nature of the tumor was later verified by biopsy. Table 4 shows the detection rates of metastatic foci by imaging modalities according to disease features.

Table 1. *Characteristics of patients with recurrent papillary thyroid carcinoma.*

Overall n=29		
Age (years); Mean (SD)	45.83(±16.39)	
Stimulated TG levels (IU/mL); (Mean (SD)	1552.92 (±5691.87)	
Sex; n (%)	Female	7 (24.1)
	Male	22 (75.9)
Stage after 1st Surgery; n (%)	I	5 (17.2)
	II	11 (37.9)
	III	8 (27.6)
	IV	5 (17.2)
RAI treatment cures (#); n (%)	1	7 (24.1)
	2	6 (20.7)
	3	9 (31.0)
	4	5 (17.2)
	5	2 (6.9)
Antithyroglobulin antibody; n (%)	Positive	24 (82.8)
	Negative	5 (17.2)
Whole body ^{131}I imaging; n (%)	Positive	12 (41.4)
	Negative	17 (58.6)
Pathological Variant; n (%)	Classical	16 (55.2)
	Tall cell	6 (20.7)
	Follicular	3 (10.3)
	Poorly differentiated	4 (13.8)

Table 2. *Characteristics of patients with recurrent papillary thyroid carcinoma.*

Modality	**Mean SUVmax (SD)**	**P value**
^{18}F-FDG PET/CT	3.70 (±3.41)	0.002
^{68}Ga-FAPI PET/CT	7.50 (±6.61)	

Table 3. *Detection rates of both imaging.*

Modality		n (%)
^{18}F-FDG PET/CT	Positive	21 (72.4)
	Negative	8 (27.5)
^{68}Ga-FAPI PET/CT	Positive	25 (86.2)
	Negative	4 (13.8)

Table 4. *Metastatic focus detection rates of imaging techniques according to disease characteristics.*

		^{18}F-FDG (+) (n)	^{18}F-FDG (-) (n)	Positivity rate (%)	^{68}Ga-FAPI (+) (n)	^{68}Ga-FAPI (-) (n)	Positivity rate (%)
TG Levels (IU/mL)	2-10	3	1	75.0	2	2	50.0
	11-300	9	5	64.3	12	2	85.7
	>300	9	2	81.8	11	0	100
Anti-TG Antibody	Positive	5	0	100	4	1	80.0
	Negative	16	8	66.6	21	3	87.5
Whole body ^{131}I imaging	Positive	12	5	70.5	16	1	94.1
	Negative	9	3	75.0	9	3	75.0
Pathological Variant	Classical	11	5	68.7	14	2	87.5
	Tall cell	3	3	50.0	4	2	66.6
	Follicular	3	0	100	3	0	100
	Poorly Diff.	4	0	100	4	0	100

Discussion

With the improvement of imaging techniques, the reported prevalence of thyroid cancer is increasing significantly. The detection of thyroid cancer is now considerably earlier and at a much lower size [13]. The thyroid carcinoma is predicted to be the second or third most prevalent malignancy in the 2030s [14]. Increasing patient volume is accompanied by an increase in the percentage of patients who are difficult to manage; therefore, traditional procedures are insufficient for all patients. For appropriate patient follow-up, it is vital for physicians to continually evaluate the availability of novel imaging techniques. Patients with post-operatively high blood thyroglobulin levels and negative radioactive iodine (RAI) whole body scans are given preference for ^{18}F-FDG PET/CT use in papillary thyroid cancer. The use of ^{18}F-FDG PET/CT prior to surgery is still controversial [15, 16]. The patient population in our study, however, is a high-risk patient population with recurrence despite radioactive iodine therapy following surgery. Therefore, it seems reasonable to utilize ^{18}F-FDG PET/CT in patients for whom standard procedures are inadequate. However, despite the use of ^{18}F-FDG PET/CT, there are still patients whose metastatic focus or foci cannot be detected and who therefore cannot be treated. In such situations, a new imaging technology that could aid clinicians will emerge to guide treatment. Therefore, we focused on the utilization of ^{68}Ga-FAPI in patients with recurrent thyroid carcinoma. In a multicenter study conducted by Kratochwil, the utilization of ^{68}Ga-FAPI-PET/CT was evaluated

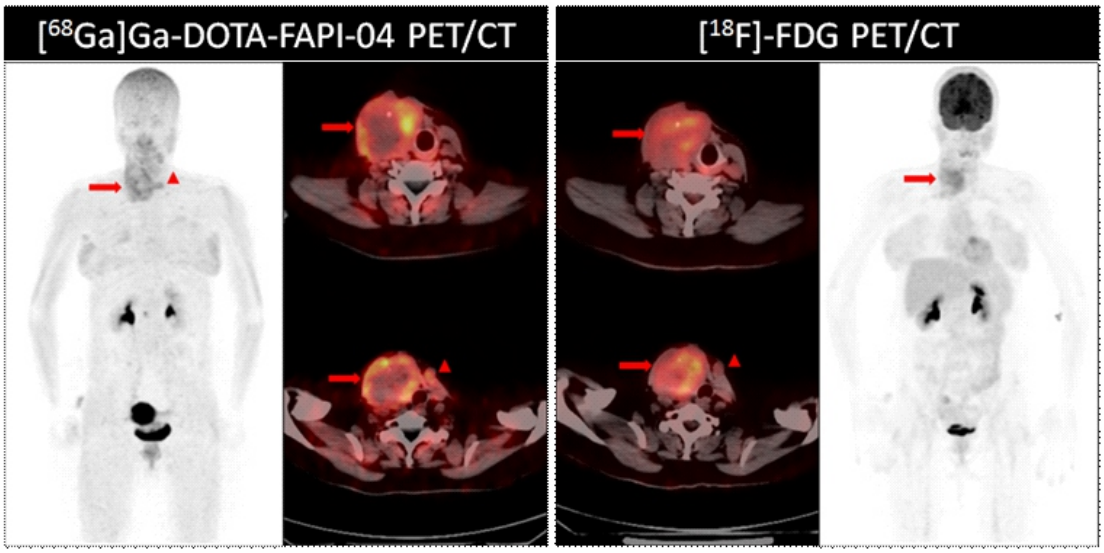

Figure 1. A-31-year-old woman with papillary thyroid cancer (Encapsulated follicular variant) who has had a total thyroidectomy and now has recurrent disease. Significant activity uptake was noted in conglomerated lymph nodes (arrow) on the right anterolateral side of the neck with both modalities (^{68}Ga-FAPI PET/CT SUVmax: 7.4; ^{18}F-FDG PET/CT SUVmax: 7.4). In contrast, ^{68}Ga-FAPI PET/CT imaging revealed a more distinct distribution of activity. In addition, the lymph node positioned on the left anterolateral side of the neck (arrowhead) demonstrated significantly enhanced ^{68}Ga-FAPI uptake (SUVmax: 3.7) but no substantial ^{18}F-FDG retention.

in 28 different cancer types.

Despite the discovery of low SUVmax values, ^{68}Ga-FAPI PET/CT imaging was beneficial in identifying thyroid cancer foci. Increased ^{68}Ga-FAPI PET/CT SUVmax results in our study may be related to the advanced recurrent nature of our patient cohort. Possible pathophysiological explanation for this outcome is that cancer-associated fibroblasts promote extracellular fibrosis, leaving the original tumor cells in a minority [4].

According to the pathological categorization of papillary thyroid cancer, the classic variant and follicular variant found in our patient populations are well differentiated, but the tall cell variant is moderately differentiated [17]. With all of these recurrence and differentiation characteristics, our study demonstrated that ^{68}Ga-FAPI was not inferior to ^{18}F-FDG for patients with recurrent papillary thyroid carcinoma who were previously treated with radioiodine. Additionally, ^{68}Ga-FAPI PET can be utilized in conjunction with ^{18}F-FDG to detect more metastatic foci.

As the tumor progresses and the tumor cells lose their ability to use iodide, the cancer becomes resistant to radioactive iodine therapy and causes negative iodine uptake imaging [18]. In addition, as thyroid cancers become more aggressive, sodium iodide symporters decrease and over-expression of the GLUT1 transporters is seen; hence, they become radioactive iodine-refractory [19]. More radioactive iodine utilization is expected in differentiated tumors, while radioactive iodine uptake decreases in poorly differentiated tumors. In our study, the detection rate of metastatic foci with ^{18}F-FDG PET/CT was 70.5% in patients with positive radioactive iodine imaging, while the detection rate with ^{68}Ga-FAPI PET/CT was 94.1%.

We noticed that as the TG value increased in both imaging modalities, the rate of metastatic identification also increased. When TG exceeded 300, ^{68}Ga-FAPI PET/CT imaging was able to detect all metastatic foci (in all 11 patients). Low TG levels in patients with recurrent thyroid cancer have been linked to dedifferentiation of the tumor [20]. At lower TG levels (thyroglobulin antibody negative), ^{18}F-FDG PET/CT detected the foci in three out of four patients, whereas ^{68}Ga-FAPI PET/CT detected the foci in two out of four patients.

There are studies showing that ^{68}Ga-FAPI PET/CT imaging is more accurate in tumoral conditions with more desmoplastic reactions, such as pancreatic cancer, cholangiocarcinoma, hepatocellular carcinoma, sarcoma, oesophageal cancer and gastric cancer [21-23]. According to the results of our study, using both imaging techniques together seems more useful in detecting one or multiple metastatic foci, in difficult cases such as recurrent papillary thyroid cancer, which is not known as a desmoplastic tumor.

The limitations of our study are as follows: the small number of patients in the study because the study was conducted with a specific disease group and the inability to obtain histopathological confirmation of all metastases independent of their anatomical localization.

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

Institutional Review Board Statement

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Gaziantep University (protocol code 2021/261;05.08.2021).

The authors declare that they have no conflicts of interest.

Bibliography

1. MarottaV, Ramundo V, Camera L et al. Sorafenib in advanced iodine-refractory differentiated thyroid cancer: efficacy, safety, and exploratory analysis of role of serum thyroglobulin and FDG-PET. *Clin Endocrinol (Oxf)* 2013; 78(5): 760-7.

2. Cabanillas M. E, Waguespack S. G, Bronstein Y et al. Treatment with tyrosine kinase inhibitors for patients with differentiated thyroid cancer: The M. D. Anderson experience. *J Clin Endocrinol Metab* 2010; 95(6): 2588-95.
3. Chrisoulidou A, Mandanas S, Margaritidou E et al. Treatment compliance and severe adverse events limit the use of tyrosine kinase inhibitors in refractory thyroid cancer. *Onco Targets Ther* 2015; 8: 2435-42.
4. Hamson E. J, Keane F.M, Tholen S et al. Understanding fibroblast activation protein (FAP): substrates, activities, expression and targeting for cancer therapy. *Proteomics Clin Appl* 2014; 8(5-6): 454-63.
5. Gorrell M.D,ParkJ. E, in: N.L. Rawlings, G. Salvesen (Eds.), Handbook of Proteolytic Enzymes, 3rd edition, Elsevier, San Diego 2013, pp. 3395-401.
6. Aertgeerts K, Levin I, Shi L et al. Structural and kinetic analysis of the substrate specificity of human fibroblast activation protein Alpha. *J Biol Chem* 2005; 280: 19441-4.
7. Bauer S, Jendro M. C, Wadle A et al. Fibroblast activation protein is expressed by rheumatoid myofibroblast-like synoviocytes. *Arthritis Res Ther* 2006; 8: R171.
8. Wang X. M, Yu D.M.T, McCaughan G.W et al. Fibroblast activation protein increases apoptosis, cell adhesion and migration by the LX-2 human stellate cell line. *Hepatology* 2005: 42: 935-45.
9. Brokopp C.E, Schoenauer R, Richards P et al. Fibroblast activation protein is induced by inflammation and degrades type I collagen in thin-cap fibroatheromata. *Eur Heart J* 2011: 32: 2713-22.
10. Ballal S, YadavM.P, Moon E.S et al. Novel Fibroblast Activation Protein Inhibitor-Based targeted Theranostics for Radioiodine Refractory differentiated Thyroid Cancer Patients: A Pilot Study. *Thyroid* 2022; 32(1): 65-77.
11. Siveke J.T. Fibroblast-activating protein: targeting the roots of the tumor microenvironment. *J Nucl Med* 2018; 59: 1412-4.
12. Kratochwil C, Flechsig P, Lindner T et al. ^{68}Ga-FAPI PET/CT: Tracer Uptake in 28 Different Kinds of Cancer. *J Nucl Med* 2019; 60(6): 801-5.
13. Davies L, Welch H.G. Current thyroid cancer trends in the United States. *JAMA Otolaryngol Head Neck Surg* 2014; 140(4): 317-22.
14. Brito J.P, Morris J.C, Montori V.M. Thyroid cancer: zealous imaging has increased detection and treatment of low risk tumours. *BMJ* 2013; 347: f4706.
15. Piccardo A, Puntoni M, Bertagna F et al. ^{18}F-FDG uptake as a prognostic variable in primary differentiated thyroid cancer incidentally detected by PET/CT: a multicentre study. *Eur J Nucl Med Mol Imaging* 2014; 41: 1482-91.
16. Kim S.K, So Y, ChungH.W et al. Analysis of predictability of ^{18}F-fluorodeoxyglucose-PET/CT in the recurrence of papillary thyroid carcinoma. *Cancer Med* 2016; 5: 2756-62.
17. Thompson L. D. Ninety-four cases of encapsulated follicular variant of papillary thyroid carcinoma: A name change to Noninvasive Follicular Thyroid Neoplasm with Papillary-like Nuclear Features would help prevent over treatment. *Mod Pathol* 2016; 29(7): 698-707.
18. Antonelli A, Fallahi P, Ferrari S.M et al. Dedifferentiated thyroid cancer: A therapeutic challenge. *Biomed Pharmacother* 2008; 62: 559-63.
19. Hanna G.J, Busaidy N.L, Chau N.G et al. Genomic correlates of response to everolimus in aggressive radioiodine-refractory thyroid cancer: a phase II study. *Clin Canc Res* 2018; 24: 1546-53.
20. Lin J. D. Thyroglobulin and human thyroid cancer. *Clin Chim Acta* 2008; 388(1-2):15-21.
21. Taveira M. Comparison of ^{68}Ga-FAPI versus ^{18}F-FDG PET/CT for Initial Cancer Staging. *Radiol Imaging Cancer* 2021; 3(2): e219007.
22. Chen H, Pang Y, Wu J et al. Comparison of ^{68}Ga-DOTA-FAPI-04 and ^{18}F-FDG PET/CT for the diagnosis of primary and metastatic lesions in patients with various types of cancer. *Eur J Nucl Med Mol Imaging* 2020; 47: 1820-32.
23. Ou L, Wu J, Yang F, Zhang C. Comparison of ^{68}Ga-FAPI and ^{18}F-FDG PET/CT in metastasis of thyroid papillary carcinoma. *Hell J Nucl Med* 2021; 24(1): 100-1.