Papillary thyroid carcinoma with squamous differentiation: A report of two cases and a brief review of the literature

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Abstract. Papillary thyroid carcinoma (PTC) is the most common type of thyroid cancer with a good prognosis. However, in rare cases, it can behave aggressively and exhibit unusual features such as squamous differentiation, where squamous cells appear alongside typical PTC cells. PTC with squamous differentiation (PTC-SD) is extremely rare and is thus not well understood. The present study reports two cases of PTC-SD. The first case was that of a 28-year-old female patient who presented with a neck mass and hoarseness, and another that of a 49-year-old male patient who presented with an incidental finding of a thyroid lesion during a neck ultrasound for a dental issue. Upon investigations, both patients had suspicious thyroid nodules. A total thyroidectomy was performed for both cases. A histopathological examination confirmed PTC-SD. Levothyroxine was prescribed for both patients, and radioactive iodine ablation was scheduled for the first case. Squamous epithelium in the thyroid is rare and can result from various causes, including primary thyroid squamous cell carcinoma, anaplastic transformation, metastasis, or PTC-SD. Although the available data are limited, they indicate that PTC-SD may be associated with a poorer prognosis compared to conventional PTC. Herein, in a review of 4 cases reported in the literature, it was found that 1 patient succumbed and another experienced recurrence. Despite its rarity, recognizing this variant is crucial for an accurate diagnosis and appropriate treatment planning. Patients with PTC-SD require careful monitoring, with the majority undergoing surgical resection. Though rare, clinicians should recognize that PTC can undergo squamous differentiation.

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Introduction

Papillary thyroid carcinoma (PTC) is the most commonly occurring type of thyroid cancer, accounting for >80% of all thyroid malignancies. Typically, PTC is associated with a favorable prognosis and a slow progression. Although uncommon, PTC can exhibit an aggressive behavior, such as invading the vascular system and extending into the major vessels (1). PTC occurs two- to four-fold more frequently in women than in men. It can exhibit a wide range of gross morphologic appearances, and the tumors may arise in any region of the thyroid gland (2). In a normal thyroid gland, the squamous epithelium is absent; therefore, the presence of squamous epithelial cells is considered unusual. Squamous differentiation in a thyroid tumor denotes a well-differentiated carcinoma, such as PTC, that exhibits areas of squamous differentiation without any anaplastic or poorly differentiated features (3). This may occur, for instance, when well-differentiated thyroid carcinomas such as PTC, particularly the tall cell variant, undergo further differentiation (4). While PTC is frequently associated with focal or extensive squamous metaplasia in ~20-40% of cases, the occurrence of true squamous differentiation within PTC is exceedingly rare (3). These rare variants of thyroid malignancy tend to exhibit an aggressive clinical behavior, and their prognostic outcomes remain poorly understood (5). The present study reports two cases of PTC with squamous differentiation (PTC-SD). In accordance with the CaReL guidelines, a brief literature review was also included in the present study, and all references have been checked to ensure no content was cited from blacklisted journals (6,7). The literature search was conducted on Google Scholar using the keyword 'allintitle: squamous AND thyroid AND carcinoma AND papillary'.

Case report

Case one

Patient information. On December 1, 2024, a 28-year-old female patient presented to Smart Health Tower (Sulaymaniyah, Iraq) with a neck mass and hoarseness. An analysis of her medical history did not reveal any notable findings, apart from a prior cesarean section. She had no family history of thyroid disease or malignancy.

Clinical findings. A physical examination revealed a firm, non-tender grade I goiter without any other abnormalities.

Diagnostic approach. An ultrasonography of the thyroid revealed scattered microcalcifications in the lower third of the right lobe. Additionally, a solid hypoechoic nodule with an irregular surface, measuring 16x14x12 mm, was observed in the middle to lower third of the left lobe (data not shown). It contained both micro- and macrocalcifications, increased perinodular and intranodular vascularity, and was classified as TR5. Multiple small lymph nodes (LNs) were observed around the gland, predominantly on the left side. The largest, measuring 10x7 mm, was located below the lower pole of the left lobe and appeared highly suspicious. Furthermore, a few left-sided cervical LNs were noted, with the largest measuring 6x3 mm in the left group III. A suspicious LN, measuring 5.6x3.7 mm, was identified in the left group IV, located behind the distal left jugular vein. The patient subsequently underwent fine needle aspiration cytology, which yielded a Bethesda II result (data not shown).

Therapeutic intervention. A total thyroidectomy with left lateral and central neck dissection was performed through a collar incision. Both recurrent laryngeal nerves and parathyroid glands were carefully preserved. Hemostasis was achieved, and the wound was closed in layers with a left-sided drain. A histopathological examination of the surgical specimen was performed by the hospital laboratory. This was performed on 5-μm-thick paraffin-embedded sections. The sections were fixed in 10% neutral buffered formalin at room temperature for 24 h and stained with hematoxylin and eosin (Bio Optica Co.) for 1-2 min at room temperature. The sections were then observed under a light microscope (Leica Microsystems GmbH). The histopathological examination revealed papillary formations lined by thyroid follicular cells that had elongated, crowded, and cleared nuclei with crowding, grooves and pseudoinclusions. Separated by areas of fibrosis, parts of the tumor were composed of larger cells with an abundant eosinophilic cytoplasm and squamous differentiation. These features were indicative of PTC-SD (Fig. 1). Out of the 34 LNs examined, six were involved by the papillary component of the tumor, four of which were from the central compartment, and two from the lateral compartment. Immunohistochemistry (IHC) was performed as follows: The paraffin blocks were cut into 4-6-μm-thick sections and transferred onto charged glass slides. Subsequently, they were placed in an oven at 60˚C overnight. Antigen retrieval was performed using the Dako PT Link (Agilent Technologies, Inc.) by boiling the sections at 100˚C for 5 to 10 min. A solution of pH 6.0 or pH 9.0 was used for the target antibody. The slides were then subjected to a 15-min wash with a 20 ml buffer solution (0.05 mol/l Tris/HCl, 0.15 mol/l NaCl, 0.05% Tween-20, pH 7.6) at room temperature. To facilitate the process, the slides were welled using the Dako Pen (Agilent Technologies, Inc.). Furthermore, endogenous peroxidase was blocked using 3% hydrogen peroxide. Subsequently, the primary antibodies [CK5/6 (1:100, mouse monoclonal, clone D5/16 B4, cat. no. M7237), p40 (1:100, mouse monoclonal, clone BC28, cat. no. M7317) and TTF-1 (1:100, mouse monoclonal, clone 8G7G3/1, cat. no. M3575) all form Dako; Agilent Technologies, Inc.] were applied at room temperature and left for 80 min. The secondary antibody, which was horseradish peroxidase-conjugated (1:200, cat. no. P0447, Dako; Agilent Technologies, Inc.) was then applied, along with the chromogen (diaminobenzidine) (MilliporeSigma), both at room temperature for 15 min. To achieve counterstaining, hematoxylin Gill II (Thermo Fisher Scientific, Inc.) was applied at room temperature for a duration of 30 sec. The slides were dried and coverslips were applied. IHC revealed the positive staining of the squamous areas for CK5/6 and p40, with negative staining for TTF-1, whereas the conventional papillary areas showed the opposite staining patterns (Fig. 2).

Follow-up and outcome. The post-operative period was uneventful, and levothyroxine (100 mg daily) was prescribed. Subsequently, the patient was referred for radioactive iodine ablation. Following the ablation, thyroglobulin levels decreased to <0.04, indicating a favorable response. The patient condition was in a good at 3-months of follow up.

Case two

Patient information. A 49-year-old male patient was being treated for a dental issue when, during an unrelated neck ultrasound, a suspicious thyroid lesion was incidentally discovered. The patient was referred to Smart Health Tower on October 22, 2024. The analysis of the past medical and family history of the patient yielded negative results for any thyroid disease.

Clinical findings. Upon a physical examination of the cervical region, there was no palpable mass.

Diagnostic approach. The patient was euthyroid, with the following laboratory results: Thyroid-stimulating hormone level of 1.58 mIU/l (normal range, 0.4-4.0 mIU/l) and free FT4 level of 18.0 pmol/l (normal range, 12.0-22.0 pmol/l). An ultrasonography of the neck revealed a well-defined hypoechoic nodule in the mid-upper third of the right thyroid lobe (14x10x8 mm) classified as TR3, and a smaller nodule in the lower third of the left lobe (6x5x5 mm), classified as TR1 (image was not archived). Both thyroid lobes were normal in size, with a homogeneous echotexture and normal vascularity. No marked cervical pathological lymphadenopathy was detected, and the submandibular and parotid glands appeared normal with no focal lesions. Due to the right mid-upper lobe nodule, further evaluation included serum calcitonin, which was normal (8.99 pg/ml), and ultrasound-guided fine-needle aspiration of the TR3 nodule, which yielded a Bethesda VI result, highly suspicious for malignancy.

Therapeutic intervention. Pre-operatively, the patient underwent an evaluation, which revealed normal bilateral vocal cord function and normal serum calcium levels (9.16 mg/dl). Thyroglobulin was measured at 33.2 ng/ml for baseline assessment. The patient underwent a total thyroidectomy. A histopathological examination specimen was performed by the hospital laboratory. This was performed on 5-μm-thick

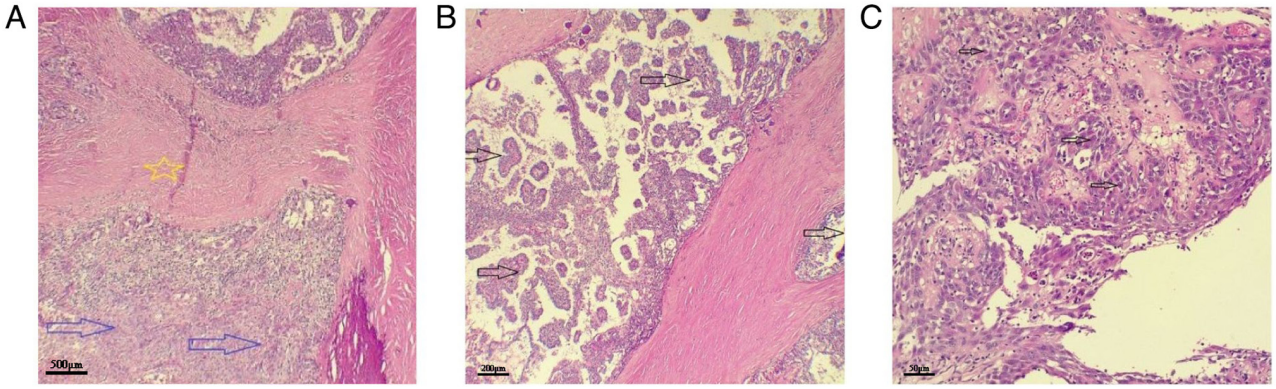

Figure 1. (A) Histopathology results of case one illustrating well-formed papillary structures in the upper part of the image with an area below it illustrating squamous cell differentiation (blue arrows). The two areas are separate by a thick fibrous stroma (yellow star) (hematoxylin and eosin staining; magnification, x4). (B) Higher power view of the classical subtype of the papillary thyroid carcinoma, which is composed of complex papillary structures covered by follicular epithelial cells (black arrows) (hematoxylin and eosin staining; magnification, x10). (C) Higher power view of the squamous differentiation area, illustrating dyskeratotic squamous cells (black arrows) without nuclear features of papillary thyroid carcinoma in this area (hematoxylin and eosin staining; magnification, x40).

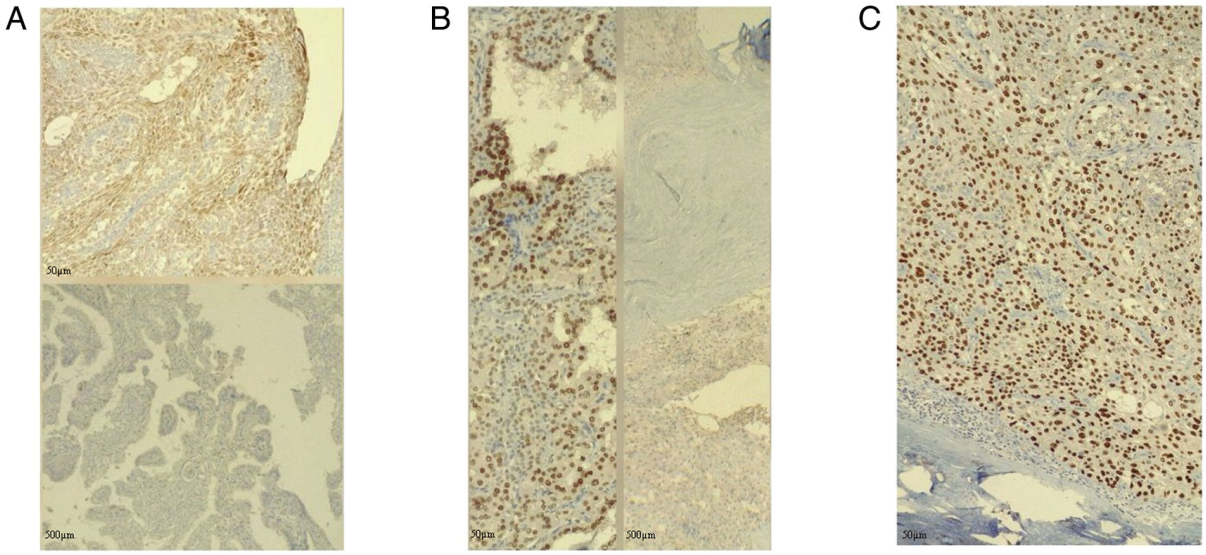

Figure 2. Immunohistochemistry results of case one. (A) CK5/6 immune staining illustrating a cytoplasmic staining pattern in the upper image (squamous cell area) and negative in the lower image (papillary thyroid carcinoma area). (B) TTF1 immune staining, illustrating nuclear positivity on the left side (papillary thyroid carcinoma area) and negative on the right side (squamous area). (C) P40 staining illustrating a diffuse, strong nuclear staining pattern in the squamous area.

paraffin-embedded sections. The sections were fixed in 10% neutral buffered formalin at room temperature for 24 h and stained with hematoxylin and eosin (Bio Optica Co.) for 1-2 min at room temperature. The sections were then observed under a light microscope (Leica Microsystems GmbH). The histopathological examination revealed a unifocal, unencapsulated PTC-SD (Fig. 3) in the right lobe, measuring 1.2 cm, comprising areas of conventional PTC, as well as areas of squamous differentiation without anaplastic dedifferentiation or high-grade features (increased mitotic activity or necrosis). There was no evidence of lympho-vascular invasion, perineural invasion, or extrathyroidal extension. The background parenchyma showed thyroid follicular nodule disease. A total of five LNs were isolated, and all were tumor-free.

Follow-up and outcome. Post-operatively, the patient remained stable with no complications and began receiving levothyroxine. Serum calcium levels remained within the normal range (9.45 mg/dl), and thyroglobulin decreased to 0.338 ng/ml. The patient was scheduled for further follow-up. At 3-months of follow up, the condition of the patient was stable.

Discussion

The presence of squamous epithelium in the thyroid can result from various causes, including primary thyroidal squamous cell carcinoma (SCC), benign squamous metaplasia in conditions such as nodular goiter or lymphocytic thyroiditis, SCC arising through dedifferentiation in recurrent thyroid cancers often with anaplastic features, thymus-like differentiation in a cancer, metastasis from a squamous carcinoma elsewhere, or a typical PTC undergoing squamous differentiation at initial

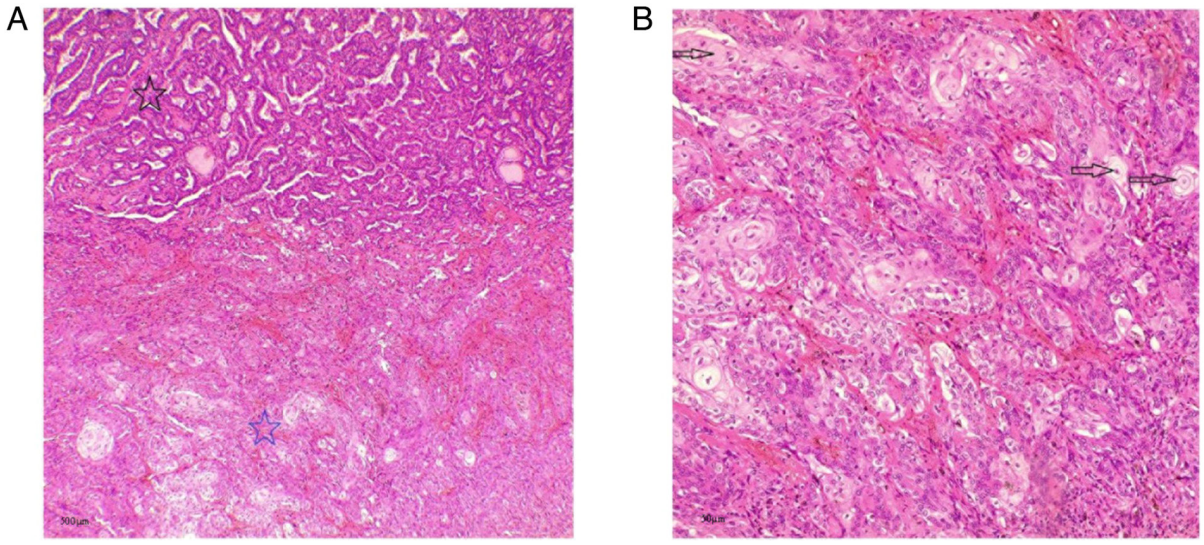

Figure 3. (A) Histopathology results of case two illustrating complex papillary structures in the upper part of the image that are covered by follicular epithelial cells (black star) with an area of squamous differentiation in the lower part of the image (blue star) (hematoxylin and eosin staining; magnification, x4). (B) Higher power view of the same area in the above image, illustrating squamous differentiation with a clear eosinophilic cytoplasm that contains intracellular eosinophilic keratinous material accumulation (black arrows) (hematoxylin and eosin staining; magnification, x40).

diagnosis (3). Anaplastic carcinomas may sometimes exhibit focal squamous differentiation on histological examination. However, they are typically non-keratinizing and poorly differentiated, displaying marked pleomorphism along with classic anaplastic features, such as spindle cell and giant cell components, unlike PTC-SD (8,9).

Squamous differentiation in PTC is considered to develop either through the metaplastic reprogramming of thyroid follicular cells or via a multistep dedifferentiation of the original tumor clone. Chronic inflammation may promote metaplasia, as observed in thyroid cancers and Hashimoto's thyroiditis, which often co-express the squamous transcription factor p63 (10). Similarly, Ding *et al* (11) reported cases in which Hashimoto's foci adjacent to PTC exhibited squamous metaplastic changes. Other studies support a clonal progression toward a squamous phenotype. Thewjitcharoen *et al* (12) described a metastatic case exhibiting intermixed classical PTC and poorly differentiated squamous cells sharing thyroid markers (PAX8 and CK19), while only the squamous component was CK5/6-positive, indicating a common origin. Handra-Luca (13) likewise observed PTC metastases, in which p63-positive papillary epithelium gave rise to CK5/6-positive squamous cells, suggesting the activation of a latent squamous differentiation program. On a molecular level, the progression from PTC to squamous morphology parallels anaplastic transformation, with tumors typically harboring early MAPK pathway mutations (BRAF or RAS) alongside additional alterations, such as TP53 and TERT promoter mutations and CDKN2A loss (11). Advanced primary cancers from nearby components, such as the larynx, base of the tongue, upper esophagus, or pharynx, can directly extend into the thyroid gland. Although the thyroid has an extensive blood supply, metastases to the thyroid are uncommon, representing only 2-3% of all cancerous thyroid tumors, with SCC comprising only a small portion of these cases (8). Due to their varied clinical and biological characteristics, it is crucial to distinguish PTC-SD from other conditions that involve squamous epithelium in a thyroid specimen (3). Even though PTC generally has a favorable prognosis, with an >90% survival rate at 20 years, several variables can negatively impact the outlook. These include being >45 years of age at diagnosis, incomplete surgical removal, large tumors, an advanced stage, the presence of metastasis, specific histological variants such as columnar, tall cell and diffuse sclerosing types, as well as evidence of local tissue invasion (3). Squamous cells of the thyroid are documented in connection with the tall cell variant of PTC. This variant is generally larger than the classic form, tends to arise in older individuals, and is associated with a more aggressive clinical behavior. The observed link between the tall cell variant and squamous differentiation implies a potential histopathological relationship in the progression of thyroid cancer (9). However, the tall cell variant PTC was not present in the cases described herein.

In the present study, four cases of PTC-SD were reviewed in the literature (3,9,13,14). Although the sample size in these studies was small, the findings suggested a poorer outcome than conventional PTC, with 1 patient succumbing to the disease and another developing a recurrent lesion in the left clavicle (Table I). IHC examinations were performed in all the cases to determine origin of the tumor, which assisted in the differential diagnosis. Beninato *et al* (5) reviewed patients with PTC-SD identified either during lymph node dissection for recurrent disease or at initial total thyroidectomy from a single-center cancer registry (1995-2015) and identified only 10 cases. A total of 4 patients had PTC-SD metastases detected during surgery for recurrence, while 6 patients exhibited PTC-SD in primary tumor specimens at an initial thyroidectomy. Despite the small sample size, their findings suggested that this variant represents an aggressive form of thyroid carcinoma (5). Similarly, Ito *et al* (4) identified only 10 patients with PTC containing squamous components among 5,749 cases evaluated between 2006 and 2010, accounting for

Table I. Review of four cases of papillary thyroid carcinoma with squamous differentiation identified in the literature.

First author, year of publication	Age, years	Sex	Clinical finding	Imaging	Histopathological and Immunohistochemical examinations	Fine needle aspiration	Molecular examinations	Treatment	Postoperative treatment	Outcome	(Refs.)
Sheta, 2023	30	M	Left-sided neck swelling	US: Thyroidal homogeneous echo-texture, solitary thyroid nodule	HPE: PTC and malignant squamous epithelial cells, psammoma bodies. IHC: +ve CK5/6 and Tg	Suspicious for PTC and staged as Bethesda V	Not mentioned	Total thyroidectomy, axillo-bilateral breast approach	Not mentioned	Stable condition	(3)
Dennis, 2018	65	F	Fluctuant left-sided neck mass	None	IHC: +ve p63, CK5/6 and BRAF V600E, -ve TTF-1 and Tg. HPE: Admixed PTC and squamous cells	Brightly eosinophilic cells with squamoid features	Not mentioned	Surgical resection	Chemoradiation (carboplatin and taxol), and radioactive iodine ablation	Left clavicular lesion 2 months later	(9)
Grove, 2018	68	M	Neck mass, history of PTC	Unknown	IHC: +ve CK5/6, P63, and PAX8	Keratinizing squamous cells, papillary follicular cells, amongst malignant squamous cells	Not mentioned	Total laryngectomy	Not mentioned	Deceased	(14)
Handra-Luca, 2018	21	F	Neck mass	None	HPE: multifocal thyroiditis and a neck thymus parathyroid unit. IHC: +ve P63, TTF1 Tg, and B-Raf in thyroid PTC, the same plus Ck5/6 in LN squamous differentiation	Not mentioned	Not mentioned	Total thyroidectomy with neck lymph node dissection	Radioactive iodide & substitutive thyroid hormones	Stable condition	(13)

US, ultrasonography; CT, computed tomography; PET, positron emission tomography; HPE, histopathological examination; IHC, immunohistochemistry; PTC, papillary thyroid carcinoma; CK5/6, cytokeratin 5/6; TTF-1, thyroid transcription factor 1; Tg, thyroglobulin; PAX8, paired-box gene 8; LN, lymph node; -ve, negative; +ve, positive; M, male; F, female.

0.2% and confirming its rarity. The atypical behavior of PTC is frequently observed in elderly patients who demonstrate rapid disease progression. Accordingly, cases of PTC with squamous differentiation are also commonly reported in older individuals (3). In the study by Beninato *et al* (5), at least half of the PTC-SD cases demonstrated multifocal tumors, extrathyroidal extension and lymph node metastases with extranodal spread. Moreover, all patients exhibited tumor involvement at the inked surgical margins (5).

IHC analysis with markers specific for thyroidal and squamous differentiation aids diagnosis by demonstrating the characteristic biphasic pattern (3). Handra-Luca (13) reported a case of a 21-year-old patient with a neck mass who underwent total thyroidectomy with LN dissection. The thyroid tumor was identified as bilateral PTC. IHC analysis revealed focal p63 expression, diffuse TTF-1 positivity, and the presence of thyroglobulin and B-Raf. Notably, squamous differentiation was detected in 15 of the 19 metastatic lymph nodes, with positive staining for CK5/6, p63, TTF-1, thyroglobulin and B-Raf, indicating squamous differentiation arising from PTC metastases (13). In the present study, in the first reported case, IHC revealed positive staining of the squamous areas for CK5/6 and p40, with negative staining for TTF-1.

The morphological features of PTC-SD and its potential occurrence at metastatic sites can make distinguishing between squamous metaplasia and true squamous differentiation difficult when based solely on histology. The detection of BRAF mutations can serve as a valuable tool in excluding metastatic SCC (13). Negative molecular prognostic indicators include TERT promoter mutations and the coexistence of multiple concurrent mutations, while the prognostic significance of the BRAFV600E mutation remains controversial (3). Although the incidence of well-differentiated thyroid cancer is increasing and overall survival remains high, supporting more conservative surgical and adjuvant approaches, certain subtypes are associated with a poorer prognosis and require timely identification and aggressive management. Patients with PTC-SD should undergo careful, lifelong monitoring and, in the absence of a specific treatment protocol, should be managed according to established evidence-based guidelines for high-risk thyroid cancers (5). The majority of reported cases in the literature underwent surgical resection, often followed by post-operative radioactive iodine ablation (5,9). The main limitations of the present case report include the lack of molecular testing due to the high cost for patients and the inability to retrieve preoperative ultrasound image data, cell counts, and nuclear characteristics. A minor limitation is the lack of an IHC control sample.

In conclusion, clinicians should consider the possibility of squamous differentiation in PTC, despite its rarity. Hence, recognizing this variant is crucial for accurate diagnosis and appropriate treatment planning.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

FHK and AMS were major contributors to the conception of the study, as well as to the literature search for related studies. HAA, AAQ and RMA contributed to the clinical management of the patients, assisted in data acquisition and interpretation, and participated in the literature review and manuscript preparation. HAY and TOS contributed to the conception and design of the study, in the literature review, in the critical revision of the manuscript, and in the processing of the table. IJH and SHH assisted in the diagnosis and management of the patients and participated in manuscript review. AMA was the pathologist who performed the diagnoses. FHK and AMS confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Written informed consent was obtained from the patients for participation in the present study.

Patient consent for publication

Written informed consent was obtained from the patients for the publication of the present case report and any accompanying images.

Competing interests

The authors declare that they have no competing interests.

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