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Gestational trophoblastic neoplasia of intermediate trophoblasts: Epithelioid trophoblastic tumor and placental site trophoblastic tumor, a study of morphologic, immunohistochemical, and next generation sequencing

İntermediate trofoblastların gestasyonel trofoblastik neoplazisi: Epitelioid trofoblastik tümör ve plasental site trofoblastik tümörlerin morfolojik, immünohistokimyasal ve yeni nesil dizileme çalışması

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Abstract

Objective: Gestational trophoblastic tumors are very rare neoplasms. We determined the distinctive morphological, immunohistochemical, and clinical features of placental site trophoblastic tumors (PSTT) and epithelioid trophoblastic tumors (ETT) in our cohort.

Materials and Methods: Nine cases of PSTT and four cases of ETT were retrieved from the archives. Histomorphologic, immunohistochemical, and clinical features were noted. A molecular study was performed on one PSTT and one ETT case using next-generation sequencing.

Results: While the nodular pattern, geographic necrosis, and extracellular eosinophilic globules were peculiar to ETTs, vessel wall affinity, marked pleomorphism, intranuclear pseudoinclusion, spindle tumor cell, and vacuolar degeneration were more specific for PSTTs in our series. An immunohistochemical panel of p63, hPL, and CD146 were helpful for the exact typing of the tumor. p63 positivity supports the ETT and diffuse staining of hPL and CD146 supports the PSTT diagnosis. Three of the patients with metastatic disease (lung and brain metastasis) except one have a high mitotic count (12 and 8) and a long interval between (8 and 10 years) antecedent pregnancy and diagnosis. While KIT and TP53 mutations were observed only in PSTT, amino acid changes in KDR, APC, and SMAD4 genes were detected both in the ETT and PSTT cases.

Conclusion: In the prediction of metastasis, the long intervals between antecedent pregnancy and diagnosis, deep myometrial invasion, mitotic count, and Ki67 proliferation index were involved rather than other histomorphological parameters, but none of the parameters is an absolute predictor of the metastasis.

Keywords: Epithelioid trophoblastic tumor, gestational trophoblastic neoplasia, immunohistochemistry, placental site trophoblastic tumor, prognosis

PRECIS: In this cohort, we evaluate the distinctive morphological, immunohistochemical, and clinical features of PSTT and ETT which are quite rare gestational trophoblastic neoplasms.

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Öz

Amaç: Bu çalışmanın amacı nadir görülen gestasyonel trofoblastik neoplaziler olan Plasental Site Trofoblastik Tümör (PSTT) ve Epiteloid trofoblastik tümör (ETT) için ayırt edici klinik, morfolojik, immünohistokimyasal ve moleküler genetik özellikleri belirlemektir.

Gereç ve Yöntemler: Hacettepe Üniversitesi'nde 2001-2020 yılları arasında tanı alan 9 PSTT ve 4 ETT olgusu incelenmiş olup karakteristik histomorfolojik, immünhistokimyasal ve klinik özellikler kaydedilmiştir. Bir PSTT ve bir ETT olgusuna uygulanmış olan yeni nesil dizileme çalışmasının verileri değerlendirilmiştir.

Bulgular: Morfolojik olarak nodüler patern, coğrafi nekroz ve ekstraselüler eozinofilik globüller ETT'ye özgü iken; damar duvarı afinitesi, belirgin pleomorfizm, intranükleer psödoinklüzyonlar, iğsi tümör hücreleri ve vakuolar dejenerasyon PSTT'ye daha spesifiktir. İmmünohistokimyasal olarak p63 pozitifliği ETT'yi, hPL ve CD146'nın yaygın boyaması PSTT'yi desteklemektedir. p63, hPL ve CD146'dan oluşan bir panelin kullanılmasının tümörün tiplendirilmesi için oldukça yardımcı olabileceği görülmüştür. KIT ve TP53 mutasyonları sadece PSTT'de gözlenirken, hem ETT hem de PSTT olgusunda KDR, APC ve SMAD4 genlerinde amino asit değişiklikleri tespit edilmiştir. İzleminde metastatik hastalığı olan üç hastanın biri hariç diğerlerinde yüksek mitoz sayısı (12 ve 8) ve bununla korele olarak yüksek Ki67 proliferasyon indeksi (%28 ve %30), derin myometrial invazyon ve önceki gebelik ile tanı arasında uzun bir zaman aralığı (8 ve 10 yıl) olduğu kaydedilmiştir.

Sonuç: ETT ve PSTT'lerde kötü prognoz ve metastaz tahmininde, histomorfolojik parametrelerden ziyade, önceki gebelik ile tanı arasında uzun bir zaman aralığı olması, derin myometriyal invazyon, mitotik aktivite ve Ki67 proliferasyon indeksi önem taşımaktadır; ancak hiçbir parametre metastaz için mutlak bir belirleyici değildir.

Anahtar Kelimeler: Epiteloid trofoblastik tümör, gestasyonel trofoblastik neoplazi, immünohistokimya, plasental site trofoblastik tümör, prognoz

Introduction

The World Health Organization classification of gestational trophoblastic disease (GTD) includes complete and partial hydatidiform mole, invasive hydatidiform mole, choriocarcinoma (CC), placental site trophoblastic tumor (PSTT), epithelioid trophoblastic tumor (ETT), exaggerated placental site, and placental site nodule. Neoplastic forms of GTDs are called gestational trophoblastic neoplasms (GTNs) [1]. This group includes CC, PSTT, and ETT[2]. Among them, PSTT and ETT arising from intermediate trophoblasts (IT) are much rarer and a limited number of reported cases are present in the literature, most of them are case reports and their exact incidence is unknown[3-6].

PSTT is formed by the neoplastic transformation of the implantation site IT, whereas ETT occurs by the neoplastic transformation of chorionic type IT. Although the histopathological examination shows that they have similar morphology with the type of IT they originate, these morphological findings may not always be recognizable in curettage specimens, which contain a limited amount of tumor samples. Accurate recognition of these tumors, especially differentiating them from choriocarcinoma and the benign mimics of GTN, is critical for the patient to receive the appropriate treatment. Although most of the PSTT and ETT behave benignly, in some series, approximately 15-25% of patients had recurrence and metastasis, and half of them died from tumor, but prognostic morphologic and immunohistochemical parameters predictive of these tumors could not be established[7,8]. Because of having difficulty making an accurate diagnosis for these rare tumors, in most countries, GTDs are diagnosed and treated in certain centers by gynecologic pathologists and oncologists who are particularly interested in GTDs[9].

In our study, we determined the distinctive morphological, immunohistochemical, and clinical features of PSTT and ETT in our cohort. Furthermore, we assessed common mutations in GTNs arising from ITs by using next-generation sequencing (NGS) in two cases.

Materials and Methods

The Hacettepe University Non-interventional Research Ethics Committee approved this study (the Ethics board approval number is 2022/12-13). All patients consented to the research and publications.

Patient selection: Nine PSTT and four ETT cases were retrieved from the archives of the department of pathology, Hacettepe University diagnosed between 2001 and 2020. Seven of 13 cases were sent to our department for a second opinion. Therefore, we have limited clinical information about these patients. Nevertheless, some patients' survival information was learned by telephone recall.

Histomorphological evaluation: Only archive materials were used and no new study was performed. All hematoxylin-eosin (H&E) and immunohistochemically stained slides were reviewed. While three of the nine PSTT cases have both curettage and hysterectomy samples, four PSTT cases have only hysterectomy samples and two PSTT cases have only curettage samples. Three hysterectomy specimens and one curettage specimen of four ETT patients were reevaluated.

H&E-stained tumor slides were examined for the architecture of the tumor (discohesive cell layers, cohesive islands, nests, and single cells), characteristic invasion patterns (myometrial invasion pattern, vessel wall infiltration), ratio, and characteristics of necrosis (geographic, fibrinoid), presence of extracellular deposits (fibrinoid substance, eosinophilic globules), presence of peri-/intra-tumor lymphocyte infiltration, nuclear features (pleomorphism, nucleoli, hyperchromasia, multinucleation, bizarre nucleus, intranuclear pseudoinclusion), cytoplasmic features (round, spindle-like, polygonal cells, vacuolar degeneration, hemosiderin pigment), the mitotic activity [average mitoses that were counted in 10 consecutive high power fields (HPF)].

Immunohistochemical evaluation: Immunohistochemical stains that were performed at the time of diagnosis (CK7, inhibin, hPL, CD146, p63, beta-hCG, Ki-67) were re-examined. No additional immunohistochemical study was performed because of the absence of paraffin blocks. Cytoplasmic or membranous staining of the tumor cells was accepted to be positive for CK7, inhibin, hPL, CD146, and beta-hCG, and nuclear staining was considered positive for p63 and Ki67. The percentage of stained tumor cells was recorded by counting at least 500 cells in the sections of hysterectomy, and at least 100 cells in curettage. Additionally, the heterogeneity of staining (when the percentage of positive staining cells between the highest and least stained tumor areas differed by less than 20% it was referred to homogenous staining; whereas the difference was >20%, referred heterogeneous staining) was also evaluated.

Molecular study: A molecular study was performed on one PSTT and one ETT case. DNA was extracted from FFPE tissue samples using a QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. DNA concentration was measured using a Qubit 3.0 fluorometer and Qubit® dsDNA HS Assay kit (Thermo Fisher Scientific, Waltham, MA, USA). Ten nanograms of DNA were used for preparing amplicon libraries using IonAmpliSeq TM Library kit 2.0 (Thermo Fisher Scientific). To amplify the template, Ion AmpliSeq Cancer Hotspot Panel v2 (Life Technologies, California, USA) was used. The panel consisted of a primer pool of 207 amplicons covering 2800 hotspot mutations in 50 genes. The amplified libraries were purified using Agencourt AMPure XP beads (Beckman Coulter Genomics, High Wycombe, UK). Library concentrations were measured using Qubit® dsDNA HS Assay kit and Qubit® 3.0 Fluorometer (Thermo Fisher Scientific). The libraries were stored at -20 °C until the sequencing step was performed. The purified libraries were diluted to 100 pM and then amplified on Ion SphereTM particles (Life Technologies). The templates were prepared and enriched using the Ion OneTouch TM 2 System (Life Technologies), an automated emulsion polymerase chain reaction system. Sequencing was performed on an Ion Personal Genome Machine System (PGM TM; Life Technologies) using Ion 318 TM chips and Ion PGM TM Sequencing Hi-Q view kit v2.

Results

Clinical and laboratory findings: Clinical and laboratory findings are summarized in Table 1. The mean age of PSTT patients was 29 while it was 37 for ETT patients. All except one PSTT patient had a previous normal or molar pregnancy or abortus history. Clinical symptoms were vaginal bleeding, vaginal discharge, amenorrhea, and inguinal pain. Eleven of the 13 patients underwent hysterectomy. We don't have further clinical information about the remaining 2 patients.

Macroscopic and histomorphologic findings: Six of the 11 patients' hysterectomy specimens were examined at our center. Available macroscopic findings and mitotic counts are shown in Table 2.

Histologically, PSTTs are mostly hemorrhagic, nodular, exophytic, and/or invade the uterus wall. Nodularity was not obvious in the curettage specimens. In all PSTT cases but one, mostly discohesive, and sometimes both discohesive and cohesive cells formed cell layers/sheets. Accompanying this pattern, individual scattered cells, nests, and cell cords/trabeculae were seen. All but one case revealed smooth muscle infiltration of tumor and vascular wall involvement (Figure 1a). There was geographic type necrosis in up to 75% of the tumor. Focal or diffuse fibrinoid material accumulation and peri-intra-tumor lymphocyte infiltration were present. Spindle cells were seen in 3 cases.

Table 1. Immunohistochemical findings, number of positive staining cases, ratio, heterogeneity[a], and intensity of staining

	Positive cases (n/n)		Positive tumor cell ratio (%)		Heterogeneity of staining[a]		Staining intensity (weak-strong)	
	PSTT	ETT	PSTT	ETT	PSTT	ETT	PSTT	ETT
CK7	4/4	4/4	100	100	Homogenous	Homogenous	Strong	Strong
Inhibin	6/6	3/3	1-100	5-80	Homogenous/ heterogeneous	Homogenous/ heterogeneous	Weak/strong	Strong
hPL	8/8	4/4	60-100	1-20	Homogeneous except one	Heterogeneous	Strong	Strong
βHCG	6/7	1/4	5-30	20	Homogenous/ heterogeneous	Heterogeneous	Strong	Strong
P63	0/7	3/3	-	20-75	Negative	Homogenous/ heterogeneous	-	Strong
CD146	5/5	2/2	50-100	25-30	Homogeneous except one	Heterogeneous	Strong	Strong
Ki-67	8/8	4/4	1-30	5-80	Homogeneous except two	Homogenous	Strong	Strong

[a]: If the percentage of positive staining cells between the highest and least stained tumor areas differed less than 20% it was referred "homogenous staining"; if the difference was >20%, referred "heterogeneous staining"

Table 2. Clinical and morphological prognostic parameters, follow-up times, and metastasis status (disease-free survival: DFS, loss to follow up: LTFU)

	Diagnosis	Age	Previous pregnancy	Time since antecedent pregnancy (months)	Clinical symptoms	Beta-HCG (mIU/mL)	Tumor size (cm)	Myometrial invasion	FIGO stage	Mitotic count (10 HPF)	ki67 index	Necrosis	Follow-up time	Metastasis	Survival status
1	PSTT	26	-	-	Vaginal bleeding	-	-	-	-	2*	10%	no	7 years	no	DFS
2	PSTT	27	Abortion	3 months	Vaginal bleeding	87	2.5	-	-	2	15-20%	no	-	-	LTFU
3	PSTT	23	Molar pregnancy	12 months	Vaginal bleeding	330	5	-	-	3*	10%	yes	6 years	lung (in 4 month)	DFS
4	PSTT	35	Healthy pregnancy	30 months	Amenorrhea	17	0.5	Superficial	I	1 in curettage and hysterectomy	10%	no	-	-	LTFU
5	PSTT	33	Molar pregnancy	120 months	Vaginal discharge	78	2.4	Deep	I	3 in curettage/8 in hysterectomy	30%	no	2 years	Lung and brain (in 2 years)	LTFU
6	PSTT	34	Healthy pregnancy	12 months	Vaginal bleeding	210	5.5	Superficial	I	0 in curettage/1-2 in hysterectomy	1%	yes	-	-	LTFU
7	PSTT	30	Healthy pregnancy	36 months	Vaginal bleeding	4	3.5	Deep	I	0-1	<5%	no	10 years	no	DFS
8	PSTT	24	Healthy pregnancy	6 months	Vaginal bleeding	27	-	-	-	1	-	yes	-	-	LTFU
9	PSTT	32	Molar pregnancy	18 months	Vaginal bleeding and inguinal pain	13	3	Superficial	I	1	15%	yes	2 years	no	DFS
10	ETT	41	Healthy pregnancy	48 months	Vaginal bleeding	-	1	Superficial	I	1	10%	no	9 years	no	DFS
11	ETT	44	Healthy pregnancy	96 months	Vaginal bleeding	-	5	Deep		12	28%	no	8 years	Lung (in 2 years)	DFS
12	ETT	40	Abortion	36 months	Inguinal pain	-	4	Deep		2	15%	yes	-	-	LTFU
13	ETT	24	Molar and healthy pregnancy	12 months	Vaginal bleeding	151	-	-		10*	80%	yes	3 year	no	DFS

*In these cases, mitotic count was evaluated on the curettage specimens

Whether focal or diffuse, significant nuclear pleomorphism (Figure 1b) was seen in all PSTT cases. In three cases, there was a vesicular nucleus and prominent nucleoli. In most cases, nuclear hyperchromasia, bi/multinucleation, bizarre nucleus, and intranuclear pseudoinclusion were observed (Figure 1b, c). Cytoplasms were mostly both clear and eosinophilic with fine granulation. Prominent cell membranes and vacuolar degeneration (Figure 1e), intracytoplasmic hemosiderin pigment, and signet ring-like cells in 2 cases were seen.

In most ETT cases, cohesive cell layers and some cases accompanying single scattered cells and nests were seen. In hysterectomy specimens, nodular, (Figure 2a) expansive myometrial invasion patterns were noted. The vascular wall involvement was not observed. Geographic necrosis (Figure 2b) was seen in all cases and ranged between 10-60% of the tumor. Eosinophilic globules (Figure 2c) and intra-peritumoral lymphocyte infiltration and hyaline-band-like material surrounding the cell groups were noticed. Spindle cells were not observed. There were varying degrees of moderate to severe nuclear pleomorphism and multinucleated giant cells, vesicular nucleus, and prominent nucleoli. Intranuclear pseudo-inclusion was seen only in the case that had focal pleomorphism. Clear and eosinophilic cells with finely granular cytoplasm were observed together in all cases. Koilocytosis-like sub-membranous cytoplasmic condensation and intracytoplasmic hemosiderin pigments were observed. Vacuolar degeneration was not observed.

Immunohistochemical findings: Immunohistochemical findings of the tumors are summarized in Table 1. CD146 and hPL are usually positive in PSTT and showed homogenous staining (Figure 1f-g). In contrast, these markers showed heterogeneous staining in ETT (Figure 2d-e). All ETTs were positive with p63 (Figure 2f; in contrast, all PSTTs were negative with p63 (Figure 1h).

Follow-up findings and treatment: Follow-up time and metastasis status are shown in Table 2. Five of the PSTT patients' follow-up information was available. Only one of them got a medical treatment that an EMA/CO (etoposide, methotrexate, actinomycin D, cyclophosphamide, vincristine/oncovine) regimen. Two of the patients had metastatic disease. The first patient (case 3) didn't receive any treatment after diagnosis, had a lung metastasis, and underwent a lung wedge resection in another hospital. The second patient (case 5) had both lung and brain metastases and was treated with EMA/CO regimen and cranial radiotherapy.

Three ETT patients' follow-up information was available. A patient (case 11) had lung metastasis two years after the diagnosis. A metastasectomy procedure was performed without adjuvant treatment. A patient (case 13) was treated with EMA/CO regimen.

Molecular analysis: We applied NGS for one of the ETT and PSTT cases, which were both operated and followed up at our institution without recurrence or metastasis. KIT, KDR, APC, TP53, and SMAD4 somatic variants were identified. While KIT and TP53 mutations were observed only in PSTT, amino acid changes in KDR, APC, and SMAD4 genes were detected both in the ETT and PSTT cases. Furthermore, ETT showed missense mutations in PIK3CA, RB1, and SMARCHB1.

Discussion

PSTT and ETT are GTNs that usually occur years after a normal or molar gestation, and both arise from IT trophoblasts; implantation site, and chorionic type respectively. Although their characteristic morphologic features are defined in textbooks, they fall short of enabling even gynecologic pathologists to recognize and differentiate these tumors, particularly in curettage specimens, due to their low frequency and difficulty in detecting their characteristics in a limited

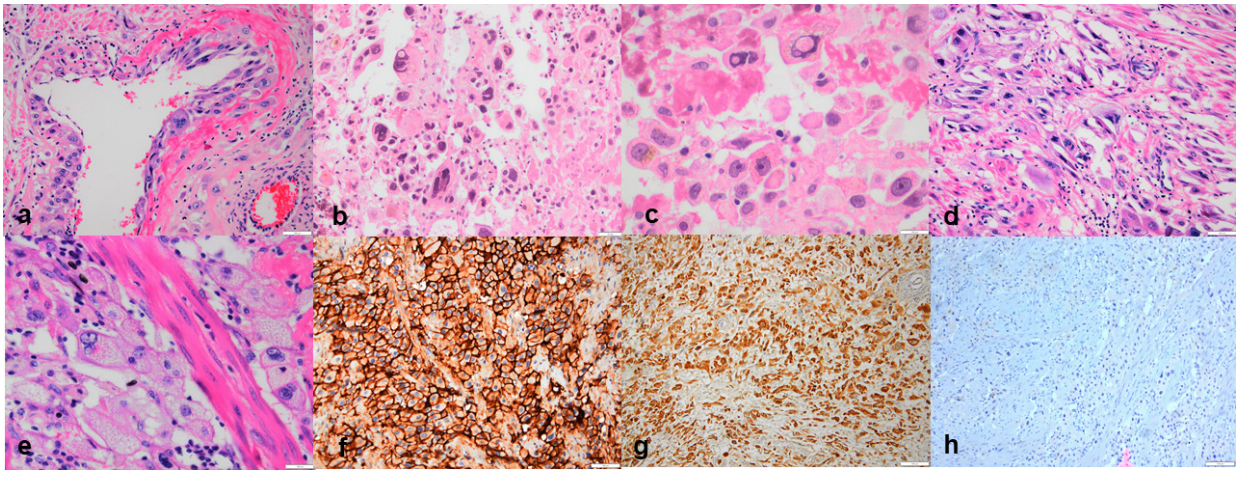

Figure 1. In addition to classical histological features in PSTT, a) vessel wall affinity (H&E 200x), b) marked pleomorphism (H&E 100x) and c) intranuclear pseudo inclusion (H&E 200x), d) spindle tumor cell (H&E 200x), e) vacuolar degeneration of tumor cell (H&E 200x) are observed as specific findings for PSTT in our cases. Immunohistochemically f) CD146 (100x) and g) HPL (100x) showed homogenous strong positivity h) while p63 (100x) was negative

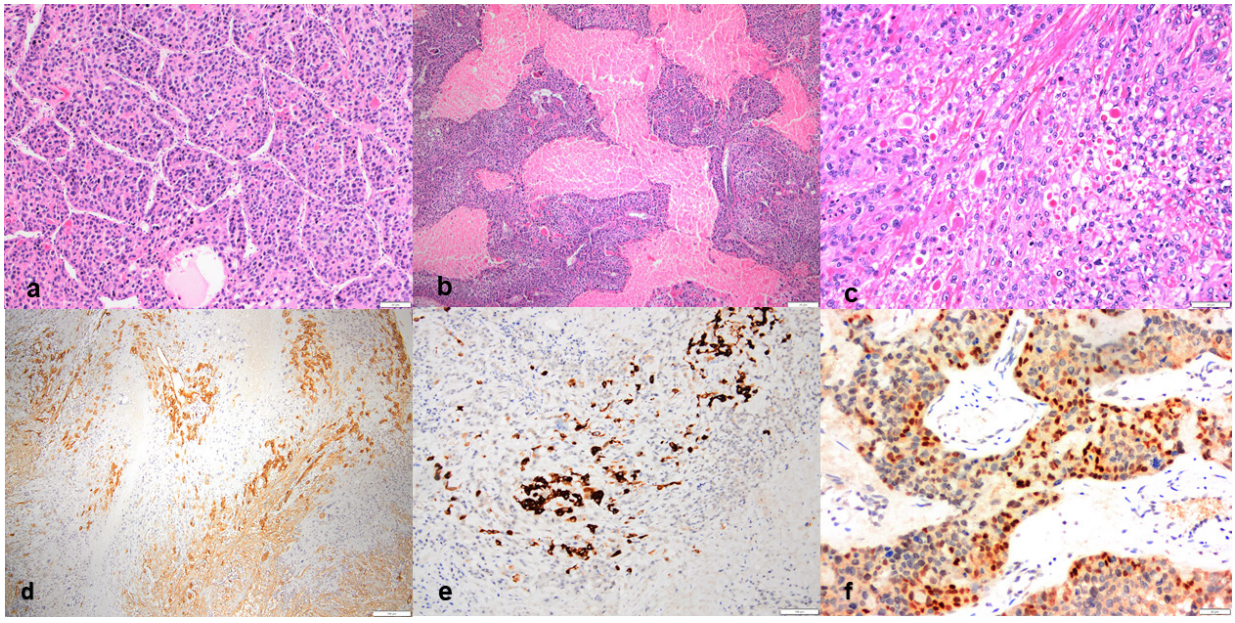

Figure 2. In our series a) nodular pattern (H&E 200x), b) geographic necrosis (H&E 100x) and c) extracellular eosinophilic globules (H&E 200x) were peculiar in ETT cases. Immunohistochemically d) CD146 (40x) and e) HPL (100x) showed heterogeneous strong positivity f) while p63 (200x) was positive (200x).

tissue fragment. Accurate recognition of these tumors is critical for the patient to receive appropriate treatment. Hysterectomy is recommended for ETT and PSTT because of their relative resistance to chemotherapy, which is used just for metastatic patients or patients with adverse prognostic factors[7,8,10]. Histomorphologic features of PSTTs typically consist of sheets of cells and ETTs exhibit nests and cords[11,12]; in our cohort the most commonly encountered architectural pattern was sheets/layers of cells in both tumor groups. The cells-forming these sheets were often discohesive in PSTTs, and cohesive in ETTs. However, in both groups' sheets of cohesive and discohesive cells were coexisting. The nested pattern also appeared in both the groups.

Differentiating PSTT and ETT in biopsy specimens is also hard, especially for non-gynecologic pathologists. It is stated that one of the main distinctive features is the growth pattern of the tumor[11,13,14]. In PSTTs, tumor cells infiltrate the myometrium in the form of single cells, cords, or nests in the periphery of the tumor, whereas ETTs are nodular and generally well-circumscribed tumors, but they may have focal infiltrative areas[13]. We have a similar observation in hysterectomy specimens. However, in curettage specimens, it is not usually possible to distinguish whether the tumor has a predominantly infiltrative or an expansive border.

It is already known that PSTT shows vessel wall affinity[13]. Similar to normal implantation sites, tumor cells migrate through vessel walls and replace them while maintaining the vascular architecture[14]. In our cohort, we also demonstrated vessel wall affinity as a specific feature of PSTT. The frequent presence of such findings, even in curettage specimens, can be considered as a diagnostic for PSTT. However, while deciding on a truly transformed vessel, it should be well distinguished from perivascular tumor infiltration that may also be seen in ETT[11,14].

In this study, necrosis was a common finding with varying degrees in both groups. One of the remarkable findings in our series was a demonstration of geographic necrosis in ETTs, in addition to fibrinoid necrosis seen in both tumor groups. Tumor involvement of the vessel wall leads to fibrinoid necrosis and extracellular fibrinoid or hyaline material accumulation, which is accepted as the characteristic feature of ETT[11,14,15]. This morphologic appearance was present in both groups of ETT and PSTT in our series; therefore, it should not be considered a feature directly in favor of ETT. In our study, we also noticed the presence of extracellular hyaline globular bodies in ETTs, and this finding has not been previously reported in the literature so far.

It has been reported that generally, a monomorphic cell population is dominant in both tumor groups[11,14]. Also, there are publications indicating that nuclear atypia is modest in ETTs, whereas there are varying degrees of pleomorphism in PSTTs[12,16]. In our cohort, cells with marked nuclear pleomorphism were seen in the cases of PSTT, but in only one of the ETT cases. Therefore, nuclear pleomorphism favors PSTT when accompanying other specific morphologic features.

The presence of intranuclear pseudo-inclusion has not been considered a conspicuous finding in these tumors heretofore. However, it was a striking feature in all cases of the PSTT in our study. In ETTs, it was seen only in very few cells where marked nuclear pleomorphism was observed. In addition to that finding, bizarre nuclei, bi/multinucleation, cytoplasmic vacuolization, and spindle cells, which are thought to be secondary to the

degeneration of tumor cells, were often determined in PSTTs in our series.

When we evaluated the immunohistochemical findings, there was no contradictory result with the previously reported data[17-19]. CK7 demonstrated the tumor architecture better than inhibin. While hPL and CD146 were highly positive in PSTTs, p63 was immunoreactive in most of the ETT cells. However, it is noteworthy to be aware of the misleading immunostaining patterns to evaluate the immunohistochemical studies correctly, particularly in curettage specimens. Immunomarkers weren't positive in 100% of cells, and heterogeneous patterns can be seen. Thus, to agree on the positivity of the tumor, a threshold should be determined. According to our data, in ETT, there may be low heterogeneous staining with hPL and CD146 (heterogeneous staining patterns up to 20% with hPL and up to 30% with CD146 should be considered against PSTT). p63 is a quite reliable immunomarker for the diagnosis of ETT because of it's negative in PSTTs. In PSTTs when 60% or more tumor cells are positive with hPL and 75% or more with CD146, you may establish the diagnosis reliably.

The mean age was 29 for PSTT, and 37 for ETT similar to the literature. As antecedent pregnancy history in PSTT patients was mostly term and molar pregnancies may be present[16], also in our cohort, the patients had either molar or normal pregnancies before the diagnosis.

Metastasis rates reported for GTN are 25-30% and the most common metastasis sites are the lungs and brain[20]. The prognostic factors for PSTT and ETT are quite similar. In the literature, age >40 years, >48 months since previous pregnancy, FIGO stage, the presence of metastases, necrosis, increased mitotic count (>2.5/mm^2 or >5/10 HPF), deep myometrial invasion, and increased beta-HCG levels have been reported as possible poor prognostic factors[12,16,20,21].

In our series, 2 of the 5 PSTT patients with follow-up information had metastatic disease. The first patient (case 3) had no prominent worse prognostic features but had lung metastasis. The second patient (case 5) had a history of molar pregnancy 10 years before the diagnosis, had a deep myometrial invasion, with a mitotic count of 8/10 HPF in the hysterectomy specimen, and 3/10 HPF in the curettage specimen had both lung and brain metastases occurred during follow-up. Metastatic disease was detected in one of the three ETT patients. She had a normal pregnancy 8 years before the diagnosis, the tumor had a deep myometrial invasion, with a mitotic count of 12/10 HPF had lung metastases three years after diagnosis. The second patient who had a high mitotic count (10/10 HPF) and a high Ki-67 proliferation index (80%) was treated with EMA/CO regimen and three months after that, hysterectomy was performed in another hospital the tumor was reported as CC. We don't have the clinical follow-up.

In our study, mitotic activity was significantly increased in patients with metastasis in both tumor groups, and this was correlated with an increased Ki-67 proliferation index. Also, two of the three patients with metastasis in the tumor groups had a time interval of more than 48 months since antecedent pregnancy and deep myometrial invasion. These findings showed that mitotic count, Ki-67 proliferation index, the long interval between antecedent pregnancy and diagnosis, and deep myometrial invasion may have was central to predicting tumors with a potential for metastasis. Be aware that the mitotic count in curettage specimens may be less than in hysterectomy specimens, as seen in case 3.

To our knowledge hitherto, there have been some molecular studies related to PSTT and ETT, particularly focused on the Y chromosome[22,23]. Most of the cytogenetic analyses were performed by comparative genomic hybridization and concluded the absence of the Y chromosome in these tumors[24,25]. In NGS studies, we identified some similar point mutations in both the PSTT and ETT cases, besides several distinct mutations. KIT and TP53 mutations were detected only in PSTT, whereas ETT revealed missense mutations in PIK3CA, RB1, and SMARCHB1. However, as we performed NGS in just 2 cases, the specificity and importance of these observations must await further studies with larger series.

Study Limitations

The strengths of this article are that it is a different cohort of GTN of IT, which are rare in the literature, that it contains immunohistochemical and morphological supporting data that can help in the differential diagnosis, and that it contains molecular data on the molecular mechanisms involved in the pathogenesis of these tumors, although it has been done in two cases. The weaknesses are the lack of follow-up information in some patients and the fact that molecular studies can be performed in few cases.

Conclusion

As a result, nodular pattern, geographic necrosis, and extracellular eosinophilic globules were peculiar to ETTs, vessel wall affinity, marked pleomorphism, intranuclear pseudo-inclusion, spindle tumor cell, and vacuolar degeneration were seen more commonly for PSTTs in our series. An immunohistochemical panel of p63, hPL, and CD146 was helpful for the exact typing of the tumor. However, while interpreting the immunoreactivity of tumor cells, the percentage and heterogeneity of the staining should be considered cautiously. In the prediction of metastasis, the long interval between antecedent pregnancy and diagnosis, deep myometrial invasion, mitotic count, and ki67 proliferation index were involved rather than other histomorphological parameters, but none of the parameters seems to be an absolute predictor of the metastasis.

Ethics

Ethics Committee Approval: The Hacettepe University Non-interventional Research Ethics Committee approved this study (the ethics board approval number is 2022/12-13).

Informed Consent: All patients consented to the research and publications.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: F.Ö.A., F.G., G.E.T.K., A.C.G., Y.G.G., A.U., Concept: F.Ö.A., F.G., G.E.T.K., A.C.G., Y.G.G., A.U., Design: F.Ö.A., F.G., G.E.T.K., A.C.G., Y.G.G., A.U., Data Collection or Processing: F.Ö.A., F.G., G.E.T.K., A.C.G., Y.G.G., A.U., Analysis or Interpretation: F.Ö.A., F.G., G.E.T.K., A.C.G., Y.G.G., A.U., Literature Search: F.Ö.A., F.G., G.E.T.K., A.C.G., Y.G.G., A.U., Writing: F.Ö.A., F.G., G.E.T.K., A.C.G., Y.G.G., A.U.

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