ORIGINAL RESEARCH

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Correlation Between Frozen Section and Definitive Diagnosis in High-Risk Endometrial Carcinoma: Retrospective Analysis

Mine ÖZŞEN[a], Selin YİRMİBEŞ[a], Fatma ÖZ ATALAY[a]

[a]Department of Pathology, Bursa Uludağ University Faculty of Medicine, Bursa, Türkiye

ABSTRACT Objective: This study aims to evaluate the correlation of intraoperative and definitive diagnosis in high-risk endometrial carcinomas, and percentages of compliance or non-compliance of intraoperative examination in high-risk endometrial carcinoma cases. **Material and Methods:** In this retrospective study, the pathology archives of our center were evaluated and 90 high risk endometrial carcinoma cases that were examined intraoperatively, between 2005-2017 were analyzed. The following criteria were used to identify high-risk endometrial carcinoma cases: Grade 3 endometrioid carcinomas with lymphovascular invasion and/or myometrial invasion of more than half, tumors of stage 2, 3, 4 and non-endometrioid endometrial carcinoma. **Results:** Histopathological subtyping was accurate in 52 of the 57 cases of endometrioid carcinoma, 3 of the 20 cases of serous carcinoma and 1 of the 4 cases of clear cell carcinoma. None of the patients diagnosed with mixed carcinoma were accurately diagnosed in frozen section. When results of intraoperative and permenant evaluations were compared; 74.4% of the cases were compatible for histopathological subtype, 76.7% for grade, and 77.7% for myometrial invasion. **Conclusion:** In patients with high-risk endometrial carcinoma without a preoperative diagnosis, intraoperative evaluation is essential for determining prognostic parameters and performing surgical staging intraoperatively. Despite the careful evaluation of pathologists, the error rate is high in intraoperative evaluation of high-risk endometrial carcinomas. In order to minimize the error rate, both gross and microscopical assessment should be performed with utmost care.

Keywords: Clear cell carcinoma; endometrioid carcinoma; frozen section; intraoperative evaluation; serous carcinoma

Intraoperative evaluation has a significant role in identification, management, and treatment planning of gynecological malignancies. A majority of gynecological intraoperative evaluations consist of ovarian and endometrial cancers. Although preoperative biopsy is performed for endometrial tumors, biopsy or curettage materials are limited in terms of histopathological subtyping, grading, depth of invasion, extent of tumor and the status of lymph nodes. This makes intraoperative diagnosis important and necessary for endometrial cancers, which require a multidisciplinary approach in treatment strategies.[1,2] Intraoperative surgical staging and determining an early treatment plan is an opportunity enabled by frozen evaluation in high-risk endometrial cancers and is effective in determining the prognosis.[3]

This study aims to evaluate the correlation of intraoperative and definitive diagnosis in high-risk endometrial carcinomas, and percentages of compliance or non-compliance of frozen examination in high-risk endometrial carcinoma cases.

MATERIAL AND METHODS

The study included 90 high-risk endometrial carcinoma cases which were evaluated intraoperatively in our center, between 2005 and 2017. The following criteria were used to identify high-risk endometrial carcinoma cases: Grade 3 endometrioid carcinomas with lymphovascular invasion and/or myometrial invasion of more than half, tumors of Stage 2, 3, 4 and non-endometrioid endometrial carcinoma. Cases with

Correspondence: Mine ÖZŞEN
Department of Pathology, Bursa Uludağ University Faculty of Medicine, Bursa, Türkiye
E-mail: m.isikoglu@hotmail.com

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slides that are not suitable for evaluation or cases whose slides and blocks could not be obtained, were excluded from the study.

Demographic information and tumor size of the cases were obtained from the patient files and pathology reports in the electronic hospital database. Hematoxylin-eosin stained slides prepared with a thickness of 4-5 micrometers, were re-examined in terms of histopathological diagnosis, grade (based on the Federation of Gynecology and Obstetrics grading system) and depth of myometrial invasion. Results of intraoperative and permanent evaluations were compared. The results were grouped under three error categories; compatible diagnosis (no error), minor error and major error. The diagnosis was considered as compatible, if the intraoperative and permanent evaluation results were the same. Different diagnosis in two evaluations, still meeting the criteria for high-risk endometrial carcinoma was accepted as minor error. Different diagnosis that did not meet the criteria for high-risk endometrial carcinoma was considered major error. Intraoperative evaluations were performed by pathologists specializing in other fields than gynecopathology. The evaluation of the permanent sections was made by a pathologist experienced in gynecopathology.

The study was approved by the Uludağ University Faculty of Medicine Clinical Research Ethics Committee (date: November 11, 2020, no: 2020-20/21) and was conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was taken.

RESULTS

Mean patient age was 63.5±8.6 (range 44-86) years. General characteristics of the cases are summarized in Table 1. Of the 90 cases, final diagnosis was endometrioid carcinoma in 57 (63.4%), serous carcinoma in 20 (22.3%), clear cell carcinoma in 4 (4.4%), and mixed carcinoma in 4 (4.4%), undifferentiated carcinoma in 4 (4.4%) cases and carcinocarcinoma in 1 (1.1%) case.

Of the 57 cases with the definitive diagnosis of endometrioid carcinoma, 52 cases were evaluated as endometrioid carcinoma intraoperatively while 4 cases were reported as high grade malignant tumor. In 1 case, the diagnosis could not be made from intraoperative evaulations and it was reported that definitive diagnosis would be given in permanent sections.

Of the 20 cases with definitive diagnosis of serous carcinoma, only 3 were diagnosed with serous carcinoma intraoperatively. Twelve cases were evaluated as endometrioid carcinoma, 2 cases as high grade malignant tumor, 1 case as adenosarcoma and 1 case as adenosquamous carcinoma intraoperatively (Figure 1A). One case was indefinitive in frozen sections and reported that definitive diagnosis would be given in permanent sections.

One of the 4 cases of clear cell carcinoma had the identical diagnosis in the intraoperative evaluation, while 2 cases were reported as endometrioid carcinoma and 1 case as high grade malignant tumor intraoperatively (Figure 1B). All 4 of the mixed carcinoma cases were reported as endometrioid carcinoma. Three of the 4 undifferentiated carcinoma cases were reported as high-grade carcinoma while 1 patient was evaluated as malignant mesenchymal tumor in frozen sections.

The single carcinosarcoma case was diagnosed as high-grade malignant tumor in intraoperative evaluation.

Grade of the tumor was not reported during the intraoperative evaluation in 17 cases. Seventy three cases had grade given in the intraoperative evaluation and 21 of them had different grade reported in the final diagnosis. While 7 cases had higher grades on frozen section compared to final diagnosis, 14 had lower grades. Cases with discordant tumor grade were as follows: 14 endometrioid carcinomas, 4 serous carcinomas, 2 mixed carcinomas and 1 clear cell carcinoma (Table 2).

Myometrial invasion depth was discordant in 13 cases. In 11 cases, a lower invasion depth was given, while in 2 cases a higher invasion depth was reported in intraoperative examination. Invasion depth was not reported in 3 cases and in one case the pathologist indicated it would be determined after permanent sections (Figure 2) (Table 3).

TABLE 1: General characteristics of the cases (n=90).

	Variable	% (n)
Final diagnosis	Endometrioid carcinoma	63.4 (57)
	Serous carcinoma	22.3 (20)
	Clear cell carcinoma	4.4 (4)
	Mixed carcinoma	4.4 (4)
	Undifferentiated carcinoma	4.4 (4)
	Carcinosarcoma	1.1 (1)
Myometrial invasion depth (final)	<50%	44.4 (40)
	>50%	55.6 (50)
Grade (final)	1-2	44.4 (40)
	3	55.6 (50)
Frozen diagnosis	Endometrioid carcinoma	77.7 (70)
	Serous carcinoma	3.3 (3)
	Clear cell carcinoma	1.1 (1)
	Mixed carcinoma	0 (0)
	Undifferentiated carcinoma	0 (0)
Myometrial invasion depth (frozen)	<50%	52.2 (58)
	>50%	47.8 (32)
Grade (frozen)	1-2	44.4 (40)
	3	37.7 (34)

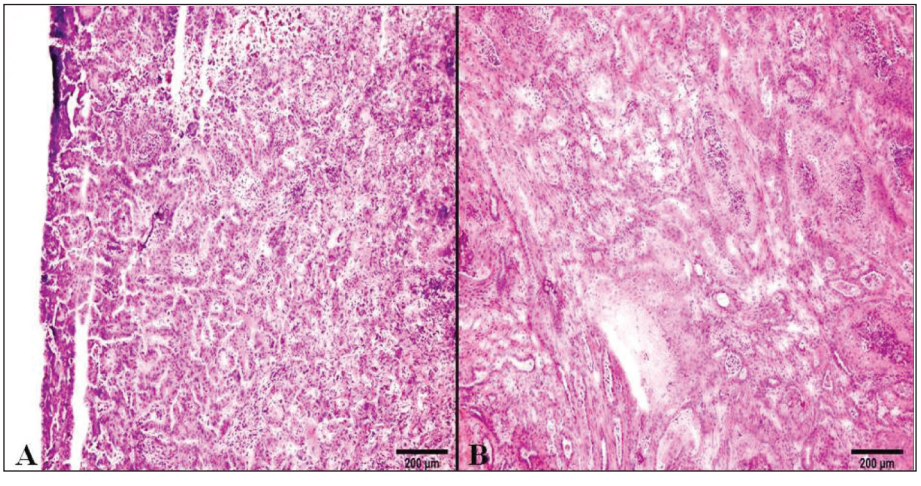

FIGURE 1: A) Serous carcinoma, Grade 3 in final evaluation, by a pathologist specializing in gynecopathology. The same tumor was evaluated as endometrioid carcinoma, Grade 1 in intraoperative section by a pathologist not specializing in gynecopathology. It is thought that this evaluation error is due to the failure of careful evaluation of the nuclear atypia of serous carcinoma (H&E, x200). **B)** Clear cell carcinoma, Grade 3 in final evaluation, by a pathologist specializing in gynecopathology. The same tumor was evaluated as endometrioid carcinoma, Grade 3 in intraoperative section by a pathologist not specializing in gynecopathology. It is thought to be due to the fact that the cells appear more eosinophilic in the intraoperative evaluation (H&E, x200).

All tumor diameters were based on the measurements taken during frozen evaluation. Eighty seven cases had available information on tumor diameter and the mean diameter was 4.9±2.1 (range 2-12) cm.

When intraoperative and permanent evaluations were categorized according to error categories; 74.4% of the cases were compatible in terms of histopathological subtype, 76.7% in terms of grade, and 77.7% in terms of myometrial invasion.

TABLE 2: Diagnostic concordance between frozen and and final diagnosis: tumor grade.

	PREOPERATIVE VS FINAL TUMOR GRADE		
	Concordance (n=52)	**Discordance (n=21)**	**Total (n=90)**
	76.7%	23.3%	100%
Frozen grade			
G1-G2	22	18	40
G3	30	4	34

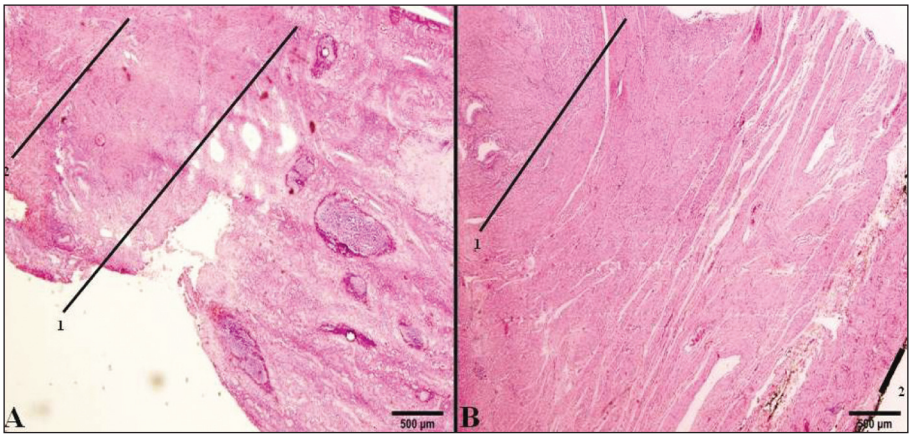

FIGURE 2: A) The slide evaluated less than half in intraoperative section for myometrial invasion depths (The first line represents the endometrial line, while the second line represents the depth of invasion.) (H&E, x40). **B)** The same tumor evaluated more than half in final evaluation for myometrial invasion depths (The first line represents the depth of invasion, while the second line represents the serosal surface.) (H&E, x40).

TABLE 3: Diagnostic concordance between frozen and final diagnosis: depths of myometrial invasion.

	PREOPERATIVE VS FINAL TUMOR: DEPTHS OF MYOMETRIAL INVASION		
	Concordance (n=73)	**Discordance (n=13)**	**Total (n=90)**
	77.7%	22.3%	100%
Frozen			
<50%	36	11	47
>50%	37	2	39

When compared frozen and definitive diagnoses in terms of risk degree, 19 cases (20.8%) had minor error with risk levels of both diagnoses being the same but difference in terms of histological subtype, grade or myometrial invasion depth was present; 11 cases (12.08%) had major error that affected risk degree of patients.

DISCUSSION

Intraoperative consultation using frozen sectioning has an important role in pathology practice. PubMed search with the keyword “frozen section” revealed about 1,288 studies published in the English literature between 1945 and 2019.

The research included only diagnostic studies without distinguishing between benign and malignant. Few of the studies is about high-grade endometrial cancers. Our study, which includes 90 high-grade endometrial carcinoma cases that were examined with frozen section between 2005 and 2017, is thought to contribute to the literature at this point.

Endometrial carcinoma, which can be encountered in different histological types and grade, is the most common gynecological malignancy. These tumors are frequently diagnosed with preoperative curettage or biopsy. However, considering that curettage materials may not represent the whole tumor regarding histological type, grade and tumor extent, frozen examination becomes valuable in patient management, especially in high-risk tumors.[1,4] Di Cello et al. compared preoperative and postoperative diagnoses in high-risk endometrial carcinomas and found that 68.6% of the cases with postoperative diagnosis of Grade 3 endometrioid carcinoma were identified as a lower grade tumor in the preoperative stage.[5] In another study by Gilks et al., cases of high-grade endometrial carcinoma were reevaluated by three different pathologists and in 35.8% of the cases were discordant among the pathologists. Histological subtype was discordant in 30.4% and grade in 5.4% of the cases.[6]

For endometrial tumors, frozen evaluation which allows intraoperative staging, is most commonly used for deciding whether or not to perform pelvic lymph node dissection by the evaluation of tumor size, histological type, grade, myometrial invasion depth, lymph node involvement and cervical spread in tumors with the most frequent rate of lymphatic system metastasis.[5,7,8] Even if there are no present risk factors for the patient, there is a 2.8% chance of pelvic and less than 1% chance of paraaortic lymph node metastasis. This rate increases almost three times in Grade 3 endometrioid carcinomas or non-endometrioid carcinomas, almost four times in tumors with cervical or adnexal spread, and almost five times when tumor invades more than half of myometrium.[1,9]

Endometrioid carcinomas are relatively easy to recognize when they show classical morphological patterns. However, if they lose their differentiation, manifest a different architectural pattern (papillary, microglandular-like, carcinosarcoma-like or mixed) or show cytological changes (clear cell, spindle cell or mucinous differentiation) this poses difficulty in the diagnosis. The distinction between Grade 3 endometrioid carcinoma and non-endometrioid carcinoma can be extremely difficult, especially in the artifacted frozen sections.[10] In a study by Mandato et al., intraoperative and permanent diagnostic compatibility was evaluated, and compatibility was found to be 95% in Type 1 endometrial carcinoma and 76% in Type 2 endometrial carcinoma.[11] In another study, the highest diagnostic compatibility (97.2%) was found endometrioid type endometrial carcinoma.[4] In our study, accuracy rate of intraoperative evaluation was 91.2% for the 57 endometrioid carcinoma cases. Accuracy rates were 15% in serous carcinoma and 25% in clear cell carcinoma cases. Of the 17 serous carcinoma cases whose specific diagnoses were not given in frozen sections, grade was undetermined in 3 cases, 5 cases were mistakenly reported as Grade 2 endometrioid carcinoma, and 7 as Grade 3 endometrioid carcinoma. This may be because differentiating serous carcinoma from endometrioid carcinoma is difficult, as the histological features (nuclear atypia, pleomorphism) becomes ambiguous due to frozen artifacts and some serous tumors show abundant gland formation. 1 clear cell carcinoma was reported as Grade 2 endometrioid carcinoma, 1 case as Grade 3 endometrioid carcinoma, and 1 case was reported as high grade malignant tumor intraoperatively. The reason for the difficulty in diagnosing clear cell carcinomas in frozen section is that clear cell carcinomas are less common than endometrioid carcinomas, and clear cell changes that may also occur in endometrioid carcinomas are not well recognized, especially among pathologists who have no specification in gynecopathology. Endometrial tumor grade is directly associated with lymph node metastasis therefore, accurate grading intraoperatively is important for guiding the surgical procedure. Some studies reported intraoperative grading accuracy as 30-86% and considered the main cause of error for low grading due to insufficient sampling.[1,4,12-14] Karalok et al. reported 5.9% of the cases with higher grade at the final diagnosis and 0.9% with a lower grade.[15] In our study, 7.7% of the cases had lower tumor grade and 15.5% had higher tumor grade at de-

finitive diagnosis. Endometrioid carcinomas comprised 66.6% of these cases. We believe that this is the result of difficulty in assessing nuclear grade due to frozen artifacts, insufficient sampling, or increased solid areas in serial sections.

Another important parameter that effects lymph node metastasis rate and prognosis is myometrial invasion depth.[16] Cirisano et al. found lymph node metastasis in 11 cases out of 36 Grade 3 endometrioid carcinoma with tumor invasion more than half of the myometrium while only 1 of 21 cases with less than half myometrial invasion showed lymph node metastasis.[17] In a study by Li et al., it was shown that the risk of metastasis to the pelvic lymph nodes was significantly increased in patients with non-endometrioid type tumor or Grade 3 tumor or with myometrial invasion of more than half.[18] The deepest point of the tumor, total wall length and the tumor's distance from the serosa should be measured when determining myometrial invasion depth. Accuracy of macroscopic evaluation of myometrial invasion depth is 83-91% and it should be kept in mind that accuracy decreases as tumor grade increases.[2] In various studies evaluating intraoperative and permanent sections in terms of myometrial invasion, the rate of compatibility varies between 54% and 96.6%.[19] In our study, the depth of myometrial invasion was discordant with the depth of invasion reported in the definitive diagnosis in 17.7% of the cases. Of them, 13.3% had lower invasion depth and 4.4% had greater invasion depth in the final report. We believe that this is due to insufficient specimen (usually 1-3 samples taken from the tumor) in intraoperative consultation or sections prepared for the permanent slides may show a region with deeper invasion of the tumor.

It is stated in most of the published cases that the risk of metastasis increases with tumor size over 2 cm.[20,21] However, some studies report that tumor diameter is not an independent prognostic factor.[22]

CONCLUSION

Intraoperative frozen evaluation is a necessary procedure to determine local prognostic parameters and to perform simultaneous staging in high-risk endometrial carcinoma cases without preoperative diagnosis. Surgery completed without frozen evaluation may cause the patient to be assessed as high-risk in the postoperative period and result in insufficient surgery, additional surgical procedures or unneeded radiotherapy. Furthermore, inaccurate intraoperative diagnosis might lead to prolonged surgery thus, increased risk of complications (lymphedema, thrombosis, pulmonary embolism).[23] Despite the careful evaluations of pathologists, studies show that there is high error ratio in frozen assessment of high-risk endometrial carcinomas. In order to minimize the error rate, both macroscopical followed by microscopical assessment should be performed with utmost care.

Source of Finance

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Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: *Mine Özşen, Fatma Öz Atalay;* ***Design:*** *Mine Özşen, Fatma Öz Atalay;* ***Control/Supervision:*** *Fatma Öz Atalay;* ***Data Collection and/or Processing:*** *Mine Özşen, Selin Yirmibeş;* ***Analysis and/or Interpretation:*** *Mine Özşen, Selin Yirmibeş;* ***Literature Review:*** *Mine Özşen, Selin Yirmibeş, Fatma Öz Atalay;* ***Writing the Article:*** *Mine Özşen, Selin Yirmibeş;* ***Critical Review:*** *Fatma Öz Atalay;* ***References and Fundings:*** *Mine Özşen, Selin Yirmibeş, Fatma Öz Atalay;* ***Materials:*** *Fatma Öz Atalay, Mine Özşen, Selin Yirmibeş.*

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