

Frozen Section Evaluation of the Cone Margin Status in High-Grade Cervical Intraepithelial Neoplasia

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Background: Meticulous examination of frozen section of cone specimens is required to precisely evaluate the resection margin status and rule out invasion in cases with high-grade cervical intraepithelial neoplasia (CIN).

Objectives: The aim of the present study was to determine the role of frozen section examination (FSE) of the cone specimen in the evaluation of the resection margin status and to rule out invasion in patients with high-grade CIN.

Patients and Methods: This cross-sectional study was performed on 38 patients with high-grade CIN undergoing conization biopsy at Imam Hossein hospital in Tehran from April 2012 through May 2013. Then, FS examination was performed for all patients and the results obtained were compared with those of permanent paraffin sections.

Results: Thirty-three (86.8%) out of 38 patients had the same results in frozen and permanent sections of cone biopsy margin specimens ($P=1$). Two out of 33 (6.1%) patients had frozen and permanent positive margins and 31 (93.9%) patients had negative frozen and permanent margins. Among the other 5 patients (13.2%), 2 had positive frozen margins and negative permanent margins and 3 patients had negative frozen margins and positive permanent margins. Diagnostic accuracies in cone margin and lesion grading were 63.1% and 44.7%, respectively.

Conclusions: In conclusion, in high-grade CIN, FS examination was a rapid, reliable and cost-effective means of evaluating cervical conization specimens.

Keywords: Conization; Frozen Sections; High-Grade Intraepithelial Neoplasia

1. Background

In the US, cervical cancer is the most prominent type of cancer of genitalia in women next to endometrial and ovarian cancers (1). However, in some parts of Iran, cervical cancer is prevalent and appears as the second common gynecological cancer, next to endometrial or ovarian cancer (2).

Worldwide, cervical carcinoma continues to be a significant health care problem. In developing countries, where health care resources are limited, cervical carcinoma is the second most frequent cause of cancer death in women (3). Invasive cervical cancer has a long pre-invasive state, which is amenable to treatment, because of currently available cervical cytology screening programs (4). All CIN 2 and 3 lesions require treatment in women 21 years of age and older (5). This recommendation is based on a meta-analysis study, where CIN 2 progresses into carcinoma in situ (CIS) in 20% and into invasive carcinoma in 5% of cases.

Although CIN can be treated with a variety of techniques, the preferred treatment for CIN 2 and 3 is loop

electrosurgical excision procedure (LEEP) (6). Inadequate and inaccurate excision of CIN that varies from 15 to 50% may cause problem, unless it is accompanied by sufficient follow up (7). Invasive carcinoma appears in most of the patients as a result of incomplete treatment. The role of FS examination (FSE) in CIN has not received much attention. Some researchers have discussed high concordance with the definitive paraffin examination in grading the lesion, but there are few studies evaluating the role of FSE of resection margin status (7-9). In addition, there are conflicting reports on the efficiency of FSE of the cone specimen in evaluating the margin status.

2. Objectives

The aim of the current study was to choose the most efficient mode of treatment with the least probable recurrence. Therefore, the aim of present study was to evaluate the role of FSE of the cone specimen in the margin status and to rule out invasion in this high-risk group of patients.

3. Patients and Methods

3.1. Participants

This cross-sectional study was conducted in Imam Hossein teaching general hospital in Tehran, Iran, from April 2012 to April 2013 in accordance with good clinical practice guidelines. The ethics committee of Shahid Beheshti University of Medical Sciences reviewed and approved the protocol of this study, which was carried out after obtaining written informed consents from all the participants. Based on the results of previous study (7), and having assumed a 74% sensitivity, a sample size of 33 positive cases was calculated for a maximum 15% error in estimating the sensitivity with a confidence level of 95%. Sampling was done conveniently, since all the samples studied met the inclusion criteria. The inclusion criteria was high-grade CIN (CIN2 or CIN3) based on cervical biopsy. Exclusion criteria were pregnant women, adolescent patients and patients with previous treatment. The patients with biopsy-proven high-grade CIN underwent a cervical cone biopsy procedure to evaluate the frozen sections. The cone specimens were excised in one piece under general or local anesthesia and immediately sent for FSE. The specimens were then opened longitudinally at the 12 o'clock position. The tissue was then divided into 12 parts and the fragments from suspicious area were selected by the pathologist in the colposcopic images for FSE whereby the results of frozen and permanent sections were compared.

3.2. Statistical Analysis

The statistical analysis of the data were carried out using mean, standard deviation, median, range and SPSS version 17 (SPSS Inc. Chicago, Illinois, USA). The accuracy of the methods was determined using sensitivity and specificity, positive and negative values and diagnostic accuracy index, and $P < 0.05$ was considered as statistically significant. The differences between permanent and frozen status were evaluated by Wilcoxon Signed-Rank and MacNemar tests.

4. Results

The patients aged from 24 to 48 years with mean age 38.8 years. The mean gravidity and parity of patients was 3 (range, 0 - 9). Among married patients, the mean age of marriage was 18.8 ± 4.7 years. Eighteen patients (47.4%) had a history of taking the contraceptive pills. Fourteen patients (36.8%) were passive smokers. Thirty-eight patients were referred for colposcopy including 7 (18.4%) patients with abnormal cervical appearance; 12 cases (31.5%) with an abnormal pap smear, 4 subjects (10.5%) with chronic vaginal discharge, 7 patients (18.4%) with inter menstrual bleeding and 8 patients (21.1%) with post coital bleeding. As shown in Table 1, of 38 patients 24 (63.2%) had normal Pap smear, 7 (18.4%) had ASCUS

(atypical squamous cells of undetermined significance) Pap smear, 5 (13.2%) cases had HSIL (high-grade squamous intraepithelial lesion), one patient (2.6%) had LSIL (low-grade squamous intraepithelial lesion) and one (2.6%) had AGC (atypical glandular cell).

Table 2 showed the results of FSE and the final pathological results of conization specimens and margin status. Comparing the results of frozen and permanent sections revealed no significant difference in lesion ($P = 0.37$) and margin status of cone specimens ($P = 0.65$).

Table 1. General Characteristics of Studied Patients ^a

Variables	Values ^b
Age, y	38.8 ± 8.3
Gravidity	3.0 ± 1.9
Parity	2.7 ± 1.8
Marital age, y	18.8 ± 4.7
Hx.OCP	18/38 (47.4)
Smoking	1/38 (2.6)
Passive smoking	14/38 (36.8)
Multipartner	12/38 (31.6)
Chief complaint Abnormal cervical appearance	
Abnormal cervical appearance	7 (18.4)
Abnormal Pap smear	12 (31.6)
Chronic vaginal discharge	4 (10.5)
Intermenstrual bleeding	7 (18.4)
Postcoital bleeding	8 (21.1)
Total	38 (100.0)
Pap smear AGC	
AGC	1 (2.6)
ASCUS	7 (18.4)
HSIL	5 (13.2)
LSIL	1 (2.6)
Normal	24 (63.2)
Total	38 (100.0)

^a Abbreviations: AGC, atypical glandular cells; ASCUS, atypical squamous cells of undetermined significance; Hx. OCP, history of ocp consumption; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion.

^b Results are presented as mean ± SD or frequency (%).

Table 2. Comparison Between the Results of Frozen and Permanent Sections and the Final Pathological Findings of Margin Status of Cone Specimens ^a

Count	Lesion		Margin	
	Frozen	Permanent	Froze	Permanent
3	CIN II	CIN II	No	No
1	CIN III	AIS	Yes	Yes
6	CIN II	CIN I	No	No
1	CIN III	CIN III	No	No
1	No	CIN II	No	No
7	CIN I	CIN I	No	No
2	CIN I	CIN II	No	Yes
2	CIN II	No	No	No
2	No	CIN I	No	No
3	CIN I	CIN II	No	No
1	CIN III	CIN III	Yes	Yes
4	No	No	No	No
1	CIN III	CIN II	No	No
1	CIN III	CIN II	Yes	No
1	CIN II	CIN II	Yes	No
1	AIS	CIN III	No	Yes
1	CIN III	CIN I	No	No
P	0.37 ^b	0.37 ^b	0.65 ^c	0.65 ^c

^a AIS, adenocarcinoma In Situ; CIN, cervical intraepithelial neoplasia.

^b Based on Wilcoxon Singed-Rank test.

^c Based on MacNemar test.

Among 38 patients, 17 (44.7%) cases had the same lesion grade in permanent and frozen sections of cone biopsy specimens with diagnostic accuracy 44.7%; in 12 patients (31.6%) lesion grade in frozen section was higher than that of permanent one, and in 9 patients (23.6%) lesion grade in frozen section was lower than that of permanent section (Table 4).

The evaluation of margins, showed comparable results in frozen and permanent sections of cone biopsy specimens in 33 of 38 patients (86.8%) (P = 1). Positive cone margins were observed in only 2 (6.1%) of 33 patients either in frozen or on permanent sections. In addition, five patients (13.2%) had the same results in frozen and permanent sections of cone biopsy specimens, of whom 3 patients had negative frozen and positive permanent sections and frozen and permanent sections were found in 2 cases (Table 3).

Table 3. Margin Status by Frozen Section and Definitive Examination of Paraffin Sections ^{a,b}

	Frozen Margin		
	No	Yes	Total
Definitive Paraffin Exam			
No	31 (93.9)	2 (6.1)	33 (100.0)
Yes	3 (60.0)	2 (40.0)	5 (100.0)
Total	34 (89.5)	4 (10.5)	38 (100.0)

^a Based on MacNemar test (P=1).

^b Values are presented as No. (%).

Table 4. Comparison of Intraepithelial Lesion Diagnosis by FSE and Definitive Examination of Paraffin Sections ^{a,b}

	Frozen Section Examination				
	No	CIN I	CIN II	CIN III	AIS
Definitive paraffin exam					
No	4 (66.70)	0 (0.00)	2 (33.30)	0 (0.00)	0 (0.00)
CIN I	2 (11.80)	8 (47.10)	6 (35.30)	1 (5.90)	0 (0.00)
CIN II	1 (9.10)	5 (45.50)	3 (27.30)	2 (18.20)	0 (0.00)
CIN III	0 (0.00)	0 (0.00)	0 (0.00)	2 (66.70)	1 (33.30)
AIS	0 (0.00)	0 (0.00)	0 (0.00)	1 (100.00)	0 (0.00)
Total	7 (18.40)	13 (34.20)	11 (28.90)	6 (15.80)	1 (2.60)

^a Abbreviations: AIS, adenocarcinoma In Situ; CIN, cervical intraepithelial neoplasia.

^b Values are presented as No. (%).

FSE from patients with lower grade lesion showed no cases of invasive carcinoma in permanent sections, which was a clinically significant finding. In the current study, the sensitivity and specificity of FS in positive cone biopsy specimen margins were 30.3% (95% CI: 24.7, 36.5) and 91% (95% CI: 87.0, 93.9), respectively; the positive and negative predictive values and diagnostic accuracy were 74.2% (95% CI: 64.5, 82.0), 60.6% (95% CI: 55.7, 65.2) and 63.1% (95% CI: 58.8, 67.2), respectively (Data not shown).

5. Discussion

The current study demonstrated that with high-grade CIN, the FSE was a rapid, reliable and cost-effective means of evaluating cervical conization specimens. Using the FS techniques in patients with gynecologic malignancies can influence the types of surgery by differentiating the benign versus malignant lesions before surgery. In addition, in breast and prostate cancers, FSE was also used to assess the margin status of the lesion (10). Following conservative CIN treatment, the two most undesirable conditions are the positive margins and the undiagnosed invasive disease. In these two situations, the treatment is considered incomplete and the risk of recurrence is very high (11).

In a study (10), recurrence was seen in 8.7% of patients showing oxygenal margin compared with those lacking margin involvement (2.3%). FSE can provide precise and rapid evaluation of the cone margin status in high-grade CIN. Moreover, waiting for the results of the permanent section pathology can impose stress on patients and also the cervical edema make the next surgery difficult for the patients. Several studies have shown different reasons for discrepancies in the results of FSE compared with those of permanent paraffin sections. For instance, some investigators compared the accuracy of FSE with that of permanent paraffin section, and reported an accuracy rate of 75 - 100% of FS (7 - 8, 11). Ren et al. (4) showed that FSE accuracy rate was 100% among patients with biopsy-proven CIS (Carcinoma In Situ). Furthermore, FSE accuracy rates of 93% (4) and 92% has been reported by others (8). Behtash et al. (11) reported that the diagnosis by the FSE was in agreement with paraffin section in 26 out of 30 cases (87%), and paraffin section confirmed 3 invasive cases diagnosed intra-operatively. In addition, Hasanzadeh et al. performed FSE on conization biopsy samples from 20 patients with high-grade CIN. Of these, they found comparable results from examination of frozen and permanent sections of cone biopsy specimens in 15 (75%) patients (7). The accuracy of FSE has long been discussed. Although the diagnostic precision and value of FSE on CIN has been established, the discrepancies still remains. Many researchers have come to the conclusion that patients must undergo appropriate surgical operations despite existing discrepancies. However, since a discrepancy may no longer be detectable on inaccurate therapy, most gynecologists and pathologists do not agree with applying FSE after CKC (Cold Knife Conization). Carvalho et al. diagnosed two cases of micro-

invasive carcinoma as CIN with less than 2 mm stromal invasion by FSE (8). In the study by Hasanzadeh et al. (7) the discrepancy rate was 25% (5:20), where FSE revealed CIN 3 in one patient, whereas paraffin section showed invasive carcinoma. The discrepancies cause unsuitable treatment, thus it is most important to find the root cause and minimize such differences. According to some research, regular review of cervical smears before operation is important and can help surgeons find atypical cells and increase the efficiency and accuracy of FSE (12). Gu et al. (12) suggested that two items are responsible for erroneous diagnoses during FSE. The first factor is the quality of the section and the second is the limited time available to make a diagnosis. However, we believe that the first factor is preventable or minimized if knowledgeable and experienced technicians perform the section using appropriate equipments, making sure that preparations are satisfactory. Regarding available time to make diagnosis, gynecologists should be given information about the process of freezing CKC specimens and be patient enough for an interpretation of the results. The discrepancy may arise due to an incorrect selection of fragments for FSE. Consequently, it seems that for exact diagnosis and treatment of cervical lesions using FSE, pathologists should receive adequate information from gynecologists before surgery. The information provided can include the results from previous smears and biopsies performed by colposcopy. In addition, during surgery, clamping of the cervical erosion zone should be avoided, and the acetowhite test and Lugol's iodine should be applied before CKC. On the other hand, marking of suspect sections of cervical conization specimens seems to be necessary. Furthermore, as highlighted by Gu et al. (12), gynecologists should be patient when waiting for FSE results. It is worth mentioning that final results can be affected by the experience of pathologists and the use of advanced equipment.

Some limitations need to be taken into account in the interpretation of our findings. First of all, FSE in the present study was limited by lack of facility including disc used to take frozen specimens from cervix at 12 hours position and inadequate space for performing FSE. Simultaneous preparation of frozen sections from different patients of different hospital wards was hampered, especially by large number of specimens. This issue may result in low sensitivity of FSE.

The sample used for FSE should be identified in 12 o'clock position using a suture; otherwise, pathologist cannot access to the margins of excision, considering the correct orientation by a surgeon. In this study, diagnostic accuracies in cone margin were 63.1%.

Having evaluated the cone margins by FSE, Bretelle et al. (13), arrived at a conclusion that frozen section was helpful in conization and effectively decreased residual or recurrent high-grade disease. This finding makes cervical pathology more manageable. In fact, this management helps achieving immediate clear margins in majority of conizations. This is of great importance because large

numbers of patients are lost to the follow-up. Rouzier et al. (10), concluded that FSE of the end cervical margin of cervical specimen performed throughout CKC was highly accurate. Ren et al. (4). Concluded that definitive examination of margin status using paraffin sections was consistent with FSE findings in all cases (4).

In conclusion, in high-grade CIN, FSE was a fast, reliable and cost-effective method of evaluating cervical conization specimens. FSE can be applied to diagnose frank invasion, a procedure adequate for starting treatment. Besides, it is a reliable method to identify clear resection margin as high rates of patients are lost to follow-up. Since discrepancies do exist and may result in an inappropriate treatment, further research should be undertaken to improve FSE in detecting all cases.

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Authors' Contributions

Zahra Vahedpoorfard contributed in conception, design, statistical analysis and drafting of the manuscript. Tahereh Ashraf Ganjooei, Maliheh Arab, Farah Farzaneh, Maryam Sadat Hosseini and Mehdi Yaseri contributed in conception, data collection and manuscript drafting. Zahra Vahedpoorfard contributed in conception, design and statistical analysis. All authors read and approved the final version of the paper.

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