

LLETZ Specimen Fragmentation: Impact on Diagnosis, Outcome, and Implications for Training

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Abstract

Purpose This study was designed to assess fragmentation of Large loop excision of the transformation zone (LLETZ) specimens, its influence on short-term cytological outcome, and the risk factors for specimen fragmentation, as well as trainee performance on clinical outcome.

Method This retrospective study was performed at a cancer center. Women who underwent LLETZ for suspected high-grade cervical intra-epithelial neoplasia (CIN) over a 5-year period were included. Patients were identified through a regional database. Data were obtained from hospital and regional databases. Fisher's exact test was used.

Results 75 % of all specimens were obtained intact. When the LLETZ specimen was intact, 89 % of smear tests were reported as negative, against 86 % when the specimen was fragmented. Fragmentation was significantly associated with high-grade smear results at 6 months. Trainee status was significantly related to specimen fragmentation.

Conclusion Fragmentation of LLETZ specimens is associated with an increased likelihood of obtaining a high-grade smear at 6 months post treatment. Enhancing the colposcopy training may help improve clinical outcome.

Keywords LLETZ · Specimen fragmentation · Cytological outcome · Colposcopy training

Introduction

Large loop excision of the transformation zone (LLETZ) has become the standard technique for the management of cervical intra-epithelial neoplasia (CIN), following its initial description in 1989 [1]. The success rate of LLETZ is generally high [1–3]. A Cochrane review of the surgical techniques for the management of CIN concluded that there is no obviously superior technique [4]. Although LLETZ compares favorably against other excisional methods in terms of cost, ease of procedure, and post-operative complications, the principal criticism has been focused on thermal artifact.

Thermal artifact and specimen fragmentation can both impede optimal histologic interpretation. A retrospective analysis and a randomized study comparing needle diathermy versus loop excision concluded that LLETZ is significantly more likely to lead to specimen fragmentation [5, 6]. In the United Kingdom, the National Health Service Cervical Screening Programme (NHSCSP) recommends that at least 80 % of LLETZ specimens should be obtained in one piece as a point of good practice; in addition, as a measure of success of the treatment, at least 90 % of follow-up smear results at 6 months should not demonstrate dyskaryosis and by 12 months, histologically confirmed treatment failures should not exceed 5 % [7]. Currently, there are no reports in the literature that have examined the

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direct relationship between the number of pieces in the LLETZ specimens and the short-term cytological outcome. Additionally, there is scant evidence regarding impact of the colposcopy trainee status on clinical outcome.

This study reviews clinical practice over a 5-year period to determine whether

- Fragmentation of LLETZ specimen is related to the success of CIN treatment as defined by cytological outcome at 6 months following treatment.
- Trainee status influences fragmentation and outcome, as it would have implications for methods of training.
- Demographic factors influence the outcome.

Materials and Methods

We reviewed all women who underwent LLETZ over a 5-year period. Demographic data and details of the LLETZ specimen were gathered from the regional database. The histologic and cytological results were obtained from the hospital database; in addition, the Open Exeter national cervical screening database was interrogated to further improve the follow-up data. It appeared that only a few women had relocated and the reason for a lower than expected follow-up rate is likely due to nonattendance. During the five-year period, 1,804 specimens were obtained. 1,602 specimens were included in the analysis and 202 were excluded (see Table 1). The referral criteria for colposcopy included high-grade dyskaryosis, persistent low-grade abnormalities, and inadequate samples. 60 of the women had two successive loop excisions prior to the inclusion in this study; the reason for the repeat excision was incomplete excision of high-grade lesion in these women over the age of 50.

Colposcopists were either consultants or registered trainees who had completed or were in the process of completing the British Society for Colposcopy & Cervical Pathology (BSCCP) accreditation. The diameters of the loops used were 14, 18, or 22 mm. The referral smear was taken with Ayr's spatula until 2007. The follow-up cytology

was performed using the conventional method until 2007, at which point this was replaced by liquid-based cytology (ThinPrep). Following LLETZ, data regarding cytological results at 6 months post treatment were gathered to study the short-term outcome. The analysis included only those who had presented themselves duly for appropriate cytological surveillance. Those who had a high-grade smear results returned for colposcopy assessment, while those with low-grade or negative results returned for repeat smear at 6 months. Where cytological and histologic samples were studied from the same clinical episode, the more severe result is used. The data were gathered by four junior doctors. The statistical analysis was performed using Fisher's exact test with *p* value of 0.05 setting significance.

Results

The median age of the single- and multiple-piece groups is 31 (range 23–66) and 30 (24–62), respectively. The median parity of both groups is one. 75 % of the LLETZ specimens were obtained intact. The follow-up rate was 86 % at 6 months. The prevalence of smoking was 53 % in the single-piece group and 50 % in the other group. In the single-piece group, 66 % were less than 35 years of age and 67 % in the multiple-piece group. The percentage of nulliparous women who were less than 35 years of age was 37 % and 35 % in the single- and multiple-piece groups, respectively. These features were not significantly different between the two groups.

High-grade dyskaryosis among the referral criteria was noted in 80 % of the single-piece group and 82 % of the multiple-pieces group ($p > 0.05$). A number of other referral features were also studied; but, because the numbers involved are small, we cannot form firm conclusions. Consultants removed the specimen in a single piece in 79 % of cases, whereas trainees managed this in only 62 % of instances ($p = 0.0001$). Table 2 summarizes these results.

Among the specimens excluded, 133 specimens demonstrated no evidence of CIN; a majority of these were reported as normal with no pathology (113), while inflammation and metaplasia accounted for the remainder. Tables 3 and 4

In the multiple-pieces group, the significantly higher number of specimens demonstrate incomplete margins (58 vs 34 %), endocervical margin involvement (73 vs 54 %), and indeterminate margins (12 vs 3 %). At the 6 month assessment, there were no significant differences between the two groups in terms of normal, low-grade, or inadequate smears; however, 2.6 % of the single-piece specimens and 5.7 % of multiple-piece specimens were of a high grade ($p = 0.02$).

Table 1 LLETZ specimen excluded from analysis

Reason for exclusion from analysis	Number of specimens
No histology report regarding LLETZ in the electronic database	14
No cytology follow-up results in the electronic database	22
Cervical cancer diagnosis or treatment	27
No evidence of intra-epithelial neoplasia	133
Ungradable LLETZ	6
Total	202

Table 2 Pre-treatment profile of the two cohorts

	Single pass (%)	Multiple pass (%)	<i>p</i> value
Age (median)	31	30	–
Parity (median)	1	1	–
Smoker status	583/1098 (53)	188/373 (50)	0.34
Women < 35 years	784/1195 (66)	273/407 (67)	0.63
Nulliparous women < 35 years	413/1131 (37)	131/374 (35)	0.62
Trainee colposcopist	271/1195 (23)	168/407 (41)	0.0001
Consultant colposcopist	924/1195 (77)	239/407 (59)	0.0001
High-grade referral smear	960/1195 (80)	335/407 (82)	0.42
Mild dysk ref	176/1195	52/407	0.37
Borderline ref	6/1195	17/407	0.0001
Glandular change	3/1195	8/407	0.0013
Abnormal unclassifiable	7/1195	17/407	0.0001
Inadequate	2/1195	4/407	0.04
Results u/k	4/1195	8/407	0.003
No smear	0/1195	3/407	0.016
Possible invasion	0/1195	1/407	0.25
Negative	1/1195	8/407	0.0001

Table 3 Histologic profile of LLETZ specimen

Histologic feature	Single pass (%)	Multiple pass (%)	<i>p</i> value
High-grade CIN	984/1195 (82)	353/407 (87)	0.04
Incomplete margins	393/1163 (34)	207/359 (58)	0.0001
Endocervical margin involvement	214/393 (54)	151/207 (73)	0.0001
Indeterminate margin	32/1195 (3)	49/407 (12)	0.0001

Table 4 Post-treatment cytology at 6 months

Cytology	Single pass (%)	Multiple pass (%)	<i>p</i> value
Negative	918/1027 (89)	303/352 (86)	0.68
Inadequate	10/1027 (1)	6/352 (2)	0.26
Low grade	71/1027 (7)	23/352 (6.5)	0.81
High grade	27/1027 (2.6)	20/352 (6.2)	0.02

Discussion

LLETZ is a cost-effective treatment which can be performed rapidly and safely [9]. According to the NHSCSP, one feature that would help maintain this as an effective

tool in the management of CIN is to insure that at least 80 % of procedures result in an intact specimen [8]. In our study, this was achieved in 75 % of cases. However, this is a consensus point of good practice rather than an evidence-based recommendation. The principal reason cited for this recommendation is with regard to histologic assessment, particularly if microinvasive disease is diagnosed. Accurate histologic diagnosis is important in counseling the patient, risk assessment, and instituting appropriate treatment or surveillance.

We propose that a number of factors may influence fragmentation of LLETZ specimen. These could be categorized as (a) patient-related factors (habitus and laxity of vagina), (b) operator-related factors (technical/cognitive expertise and quality of the anesthetic block), (c) ergonomic factors (size of the loop, shape of the active element, operating within a confined space, colposcope interposition within the operative axis), or (d) biologic factors (fertility status, referral smear result, presumed morphology of the disease).

There are two principal issues in relation to LLETZ specimen which can influence the pathologist's ability to provide a definitive report; one is thermal artifact and the second is the number of pieces of a LLETZ specimen. The extent of thermal artifact is a function of electrical energy delivered at the tissue and the motion-time sequence of the loop [1]. A degree of electrical artifact is inherent to this technique. In our study, fragmentation is significantly associated with incomplete and indeterminate margins. Earlier studies reported inconsistent findings; however, these studies reported on smaller samples [9–12].

It is well established in the literature that involved margins of LLETZ specimen, in particular positive endocervical margins, are associated with treatment failure. Reports on the consequences of LLETZ specimen fragmentations have not correlated the findings with short-term cytological outcome [1, 12, 13]. In our study, fragmentation is significantly associated with incomplete margins and positive endocervical margins. This is supported by our finding that high-grade smears at 6 months following treatment are significantly more likely in the multiple-pieces group regardless of the histologic findings on the LLETZ specimen. Among the two cohorts of patients in our study, the proportion of those with high-grade referral smears in the two groups is not significantly different. A significantly higher proportion of patients in the multiple-piece group demonstrated high-grade dyskaryosis at 6 month following treatment. Therefore, fragmentation should be viewed as a marker of “technical error.”

The trainee grade of the colposcopist appears to be a significant risk factor in specimen fragmentation ($p = 0.0001$). This is likely to be due to the “technical error” rate associated with the learning curve. The ability to tailor LLETZ treatment

based on pre-procedural data and the colposcopic impression of the morphology must follow a learning curve; currently, this learning curve remains undefined. The current recommendation is to perform the first 50 procedures under direct supervision during training for accreditation. Presently, there is a dearth of evidence regarding the psychomotor skills training and assessment in colposcopy. Ferris et al. advocated that a validated model-based training would confer proficiency in psychomotor, cognitive, and attitudinal skills toward achieving *procedural competence* in colposcopy [14]. This stance is echoed by the concept of *surgical competence*, which is a widely accepted ethos in surgical education [15]. Training methods can be enhanced by deploying a teaching scope, using dynamic images to improve visual perceptive skills and by implementing a validated synthetic or cadaveric model to gain proficiency in psychomotor skills. Simulation-based training will enable the introduction of the “pre-trained” novice to the clinical environment [16]. The potential impact of this could be significant since out-patient LLETZ is an ergonomically, emotionally, and technically demanding procedure. In this regard, patient-focused simulation can yield benefits both for the patient and for the colposcopist during the early learning phase [17]. We believe that selection of trainees for accreditation may assume significance in the future; one criterion for selection could be visuo-spatial aptitude as this can reliably predict psychomotor performance [18, 19]. Better selection of candidates may improve the outcome for patients while the trainee is under direct supervision in the early stages of learning and minimize treatment failure rates among this patient population.

Clinically, the most crucial finding is that in histologically proven CIN2/3, fragmentation of LLETZ specimen significantly increases the probability of yielding indeterminate margins, CIN2/3 on the margins, and finally is associated with high-grade dyskaryosis on cytological assessment at 6 months following treatment (Table 5). This has implications for the patients’ psychologic well-being and resourcing of the care provider.

In neither group did the proportion of negative cytology at 6 months following treatment reach the recommended 90 % benchmark, (single piece 89 % and multiple pieces 86 %); this did not reach statistical significance. Further, fragmentation leads to higher probability of high-grade smear result at 6 months.

Table 5 Features of those who demonstrate CIN2/3 on LLETZ

Feature	Single pass (%)	Multiple pass (%)	<i>p</i> value
Indeterminate margins	30/981 (3)	44/352 (12.5)	0.0001
CIN2/3 on margins	291/981 (30)	152/352 (43)	0.0001
High-grade dyskaryosis at 6/12	24/847 (3)	19/301 (6.3)	0.022

Conclusion

LLETZ is an effective and efficient method of diagnosing and treating CIN. The study reveals that only 75 % of specimens were obtained intact, whereas the benchmark is 80 %. Trainee status of the colposcopists is a significant predictor of specimen fragmentation in our study. Methods of enhancing procedural training and skill transference to the clinical setting must be explored.

Ethical approval Formal ethical approval was not required.

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