Ultrasound Evaluation of Cervical Regeneration after LLETZ for Cervical Intraepithelial Neoplasia: A Prospective Observational Study

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Abstract. *Objectives: To assess cervical regeneration after large loop excision of the transformation zone (LLETZ) by ultrasound (US) measurements of cervical length (CL) before conization as well as in the postoperative short- and long-term and to identify factors affecting regeneration. Patients and Methods: This was a prospective observational study including patients under 45 years of age treated by LLETZ for Cervical Intraepithelial Neoplasia (CIN) with repeated measurements of CL by transvaginal US before and just after LLETZ, at 1 and 6 months postoperatively. Results: A total of 83 patients were enrolled, out of which 53 were included in the study. The mean CL was 28.6 mm (±5.7) preoperatively versus 18.3 mm (±4.2) after surgery; 21.8 mm (± 4.4) at 1 month and 25.5 mm (±4.9) at 6 months. The mean cone length estimated by US was 10.3 mm (±3.4). The differences in CL before/after conization and CL after conization/at 6 months were statistically significant (p<0.0001). Cervical regeneration at 6 months was 71% (±20), statistically greater than regeneration at 1 month (32%, ±16) (p<0.0001). Discussion: Post-conization cervical tissue regeneration occurred with almost three quarters of the initial cervical length restored at 6 months. Further studies evaluating obstetric outcomes after LLETZ according to cervical regeneration might subsequently be used in clinical practice to identify high-risk pregnancies by pre- and postoperative US measurements of the cervical length. A rigorous assessment of CIN treatment risks and benefits remains essential when considering treating patients of childbearing age given a potential obstetric risk from conization.*

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Since the 1980s there has been a significant decline in both the incidence and mortality of invasive cervical cancer of the uterus (1) due to Pap screening programs (2). Pre-cancerous lesions detected by Pap smears are common with an estimated annual rate of 412,000 in the United States (3), and 40,000 in France (4). They are graded according to severity as CIN (Cervical Intraepithelial Neoplasia) 1, 2 and 3 after colposcopy and biopsy evaluation. Conization by Large Loop Excision of the Transformation Zone (LLETZ) is by far the most common conservative treatment for CIN 2 and 3 (5), providing a comprehensive evaluation of both the degree of abnormality of the excised tissue and the excision margins while preserving healthy tissue. It is also a simple, quick and inexpensive technique which can be performed as an outpatient procedure under local anesthesia with cure rates approaching 95% (6). As suggested by epidemiological data, the increasing incidence and prevalence of CIN associated with a reduction in the mean age of patients with these lesions (7, 8) and the parallel increase in maternal age (9, 10), require a reflection on the adverse effects of this surgery and primarily the obstetrical outcome of patients following LLETZ. Several recent meta-analyses (11-14) have shown a higher risk of subsequent adverse obstetric outcomes, and mainly of preterm delivery, after conization. The size or depth of the surgical specimen is the main factor considered in the context of preterm delivery (14, 15) but does not account for the significant individual variability in initial cervical length (CL) (16) and changes in the cervix following LLETZ.

Measurement of the uterine cervix just after conization has shown the immediate anatomical impact of LLETZ, with a significant reduction in CL clearly demonstrated by transvaginal ultrasound (US) (16, 17). This examination has proven effective for CL assessment after LLETZ, with a good correlation between the pre- and postoperative US measurements and the histological measurements of the surgical specimen reported in one of our previous papers (16). Few publications are devoted to cervical changes following LLETZ (18-22). The aim of this study was, therefore, to evaluate changes in CL using US measurements of CL before surgery and at different postoperative time points to calculate cervical regeneration, and then to determine the factors influencing regeneration.

Patients and Methods

A single-center prospective observational study including patients under 45 years of age treated by conization between November 2010 and May 2012 was conducted at the University Hospital of Rennes. All participants received verbal and written information about the study objectives and procedure and signed an informed consent form. All the patients enrolled required surgical treatment according to the guidelines of the French health authorities (2) due to the presence of precancerous cervical lesions (mostly CIN2 and 3) diagnosed from cervical biopsies after colposcopic investigation, cytological abnormalities with unsatisfactory colposcopic examinations or a discordance between cytology, colposcopy and histology findings (5). The surgical technique used was LLETZ following a standard colposcopic examination, considered to date as the technique of choice (23). Hemostasis of the operative bed was provided if required for point cauterization.

US CL measurements were performed at 4 different time points *i.e.* preoperative measurement on the day of surgery and 3 postoperative measurements: the same day after surgery, at 1 month and at 6 months. Only patients with available measurements were included. Two examiners (FN and ALR) took these measurements using a high-frequency endovaginal probe (8 MHz) with a simple and reproducible technique based on the one used during pregnancy (24) and after cross-validation of the first 10 measurements. US measurement of the cervix with the bladder empty was the measurement of the functional cervix: distance between the external cervical os (easily visualized at the point of divergence of the anterior and posterior cervical lips) and the internal cervical os. The US examination was well-established. The endovaginal probe was inserted slowly without exerting pressure on the cervix and positioned to obtain a satisfactory sagittal view of the whole uterus. The image was subsequently zoomed in to visualize the cervical canal in its entirety, locating the external os, and the internal os at the intersection of the endometrial line and the cervical canal (uterine flexion forming an open angle at this level in general) using the bladder as a landmark when this limit was poorly visible (retroversion or mid position of the uterus) (Figure 1).

Each US examination included 4 steps: 3 measurements of the cervix and 1 measurement of the uterine corpus (distance between the internal os and fundus), which was used as a reference for locating the internal os for successive scans. The mean of the 3 CL measurements was calculated at each time point.

The excised cone was analyzed after formalin fixing, paraffin embedding and section according to a standard protocol. Lesion diagnosis was established and the highest dysplasia grade and lesion margins were recorded.

Relevant clinical information including key patient medical history were collected such as age, obstetrical history (gravidity, parity, preterm births), gynecological history (history of CIN treated by conization or laser), desire for subsequent pregnancy, smoking, and postoperative complications requiring further surgery.

We calculated the mean CL obtained by US at each time point. Comparisons of US CL were made by paired Student's *t*-test. Cone length calculated from US measurements was: (CL before conization − CL after conization). The proportion of excision was calculated using the following formula: (((CL before conization − CL after conization)/CL before conization)×100).

The primary endpoint was cervical regeneration defined by the proportion of CL restored at 1 month and 6 months' follow-up: (((Cone length − Difference between CL before and at 1 or 6 months)/cone length) × 100) *i.e.*: (((CL before conization − CL after conization) − (CL before conization − CL at 1 or 6 months))/(CL before conization − CL after conization) ×100).

Statistical analyses were performed using the IBM SPSS software (Bois-Colombes, France). The results were presented as mean values±standard deviation (SD). Cervical regeneration at 1 and 6 months was compared using paired Student's *t*-test and we assessed whether cervical regeneration at 6 months varied according to the following parameters: age, parity, smoking, history of treated CIN, histological grade of the lesion, resection margins, CL before conization, cone length and proportion of excision. To achieve this, we performed a univariate analysis to determine the variables to include in a multivariate analysis considering regeneration at 6 months as the dependent variable. Patients whose measurement at 1 month was missing were not excluded since it was not useful for this analysis. Findings were considered significant at 5% level.

Results

Out of the 83 patients initially enrolled in the study, 27 were subsequently excluded for missing data and a further 3 patients were also excluded: two for scans whose quality did not allow proper assessment of the CL and one patient who was pregnant at the time of the postoperative follow-up scan. A total of 53 patients with at least the 3 main measurements (preoperative and immediate postoperative and 6 month postoperative measurements) were included in the study (Figure 2).

Mean patient age was 34±6 years (19 to 45 years) and 42% were active smokers. Thirty-two percent of patients were nulliparous and 58% desired subsequent pregnancy. The majority of patients had high-grade lesions (CIN 2 and 3) on biopsy (79%). Two patients had a history of laser vaporization of the cervix, one patient had a prior cone biopsy and one patient had a history of both laser vaporization and conization. The final histological diagnosis was: Normal (19%), CIN 1 (6%), CIN 2 (15%), and CIN 3 (60%). Resection was in healthy margins in 89% of cases. One patient had revision surgery for genital bleeding treated by wicking.

The mean CL prior to LLETZ was 28.6 mm (±5.7), ranging from 19.3 to 46.3 mm, *i.e.* with a ratio of 2.4 between the extreme values. The mean CL just after LLETZ was 18.3 mm (±4.2), ranging from 7 to 33.7 mm. The cone length calculated as the difference between the two previous measurements was 10.3 mm (±3.4), ranging from 4.7 to 21 mm. The proportion

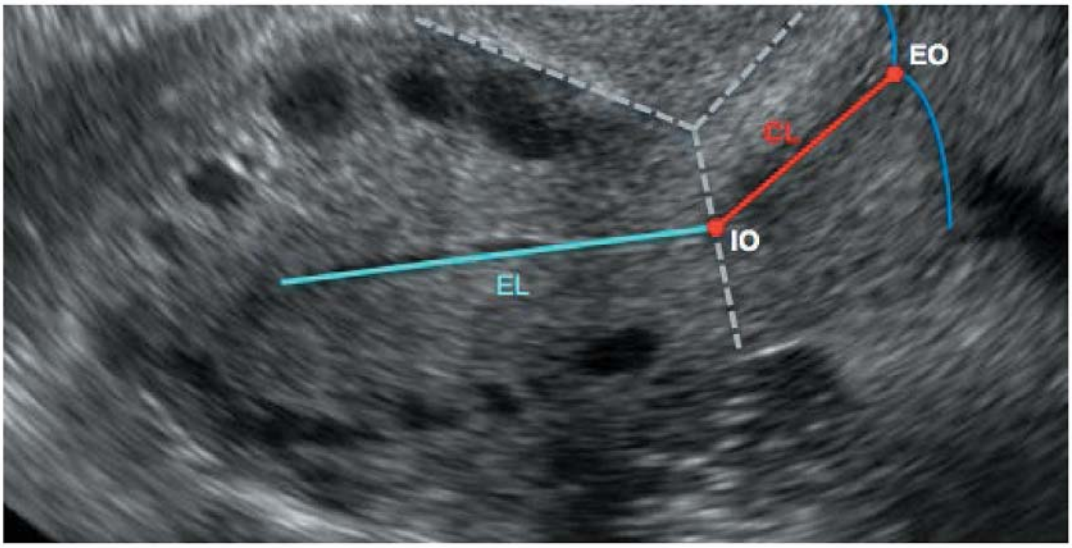

Figure 1. *Ultrasound measurement of cervical length. CL=Cervical length, EL=endometrial line, IO=internal os, EO=external os.*

of excision was 36% (±9), ranging from 18 to 64%. The mean CL at 1-month follow-up was 21.8 mm (±4.4), ranging from 13.3 to 36.7 mm, and 25.5 mm (±4.9), ranging from 18.7 to 40.7 mm at 6 months. The gain in cervical length at 6 months, *i.e.* cervical "re-growth" defined by the difference between the immediate and 6 month postoperative measurements was 7.2 mm (±3), ranging from 2.3 to 15.3 mm (p<0.0001). These results are provided in Tables I and II and in Figure 3.

Cervical regeneration at 1 month was 32% (±16), ranging from 0 to 68%, lower than cervical regeneration at 6 months, which was 71% (±20), ranging from 20 to 112%, with a significant difference of 39% (±23) (p<0.0001) (Table II). We found no statistically significant factors influencing regeneration at 6 months among the variables tested: age, parity, smoking, history of treated CIN, histological grade of the lesion, resection margin status, cone length, CL before conization and proportion of excision. Therefore no multivariate analysis was performed (Table III).

Among the 83 patients initially enrolled, 7 pregnancies were reported after surgery: 2 preterm deliveries, 4 term deliveries and an early miscarriage. The 2 preterm deliveries occurred at 34 and 36 weeks in a patient with a history of 2 preterm deliveries prior to surgery.

Discussion

Our study revealed a cervical "re-growth" process reflecting cervical regeneration, with mean regeneration of 71% at 6 months' follow-up. Five teams in the literature have evaluated regeneration following conization with varying end

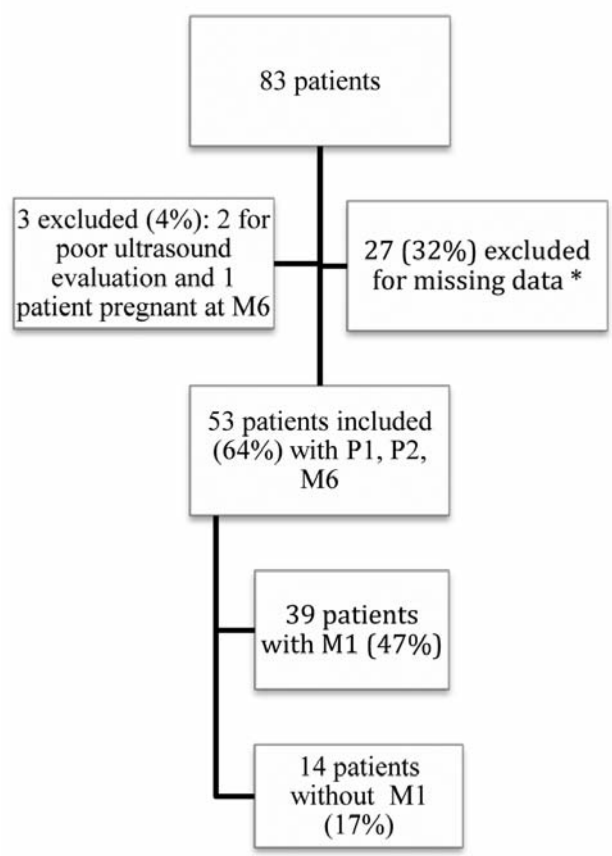

Figure 2. *Study flow chart. *At least one missing data point out of P1, P2 and M6. P1=Preoperative measurement, P2=Immediate postoperative measurement, M1=1 Month postoperative measurement, M6=6 Months postoperative measurement.*

Table I. *Cervical length ultrasound measurements before/after LLETZ, at 1 and 6 months, cone length obtained and cervical regeneration.*

	N	Mean	SD	Minimum	Median	Maximum
Cervical length (mm)						
Before LLETZ	53	28.6	5.7	19.3	27.7	46.3
After LLETZ	53	18.3	4.2	7	17.7	33.7
At 1 month	39	21.8	4.4	13.3	21	36.7
At 6 months	53	25.5	4.9	18.7	24.3	40.7
Cone length (CL before−CL after) (mm)	53	10.3	3.4	4.7	10	21
Proportion of excision (%)	53	36	9	18	35	64
Regeneration at 1 month (%)	39	32	16	0	33	68
Regeneration at 6 months (%)	53	71	20	20	75	112

CL=Cervical length, SD=standard deviation of the mean.

Table II. *Comparison of ultrasound cervical lengths and regeneration according to postoperative period with Student's t-test.*

	Difference (±SD)	*p*-Value
CL before/after LLETZ (mm)	10.3 (±3.4)	<0.0001*
CL before/6 months after LLETZ (mm)	3.1 (±2.5)	<0.0001*
CL just after/6 months after LLETZ (mm)	–7.2 (±3)	<0.0001*
Regeneration between the first /6th months (%)	39 (±23)	<0.0001**

CL=Cervical length. *Cervical lengths are considered as different. **Regeneration at 1 and 6 months is different.

results (18-22). Two of them studied cervical regeneration by two-dimensional transvaginal US. Firstly, by measuring the cervical crater (diameter and depth) 6 months after LLETZ, Paraskevaidis *et al*. showed that the cervical deficit was the same between the group of patients with a small excised cone specimen and the one with a large excised cone specimen (22), but this finding was only indirectly associated with the remaining cervix. Secondly, Gentry *et al*. found no significant difference between cervical length measurements preoperatively and at 3 months postoperatively (20). However, in this study the immediate postoperative measurements were not known, and this limited the interpretation of results. Other authors have focused on cervical volume. For Papoutsis *et al*. cervical regeneration at 6 months was comparable to our findings, between 44 and 97% (median 78%) for cervical length and from 30 to 96% for cervical volume by three-dimensional US (21). In a prospective study including 106 patients, Carcopino *et al*. found a significant reduction in cervical volume (and uterine volume) measured by 3D US between the preoperative and 6-month postoperative measurements (18). Lastly, Founta *et al*. evaluated cervical regeneration by Magnetic Resonance Imaging in 29 patients and showed that increasing the proportion of volume excised by 1% increased the proportional deficit by 0.91% at 6 months (volume at 6 months/initial volume of the cervix) (19).

Our study did not highlight any link between cervical regeneration and cone length assessed by US or with the proportion of excision. This finding was reported in the literature in the Paraskevaidis and Carcopino studies. Showing that the cervical defect was similar regardless of whether a large or small excision was performed, Paraskevaidis *et al*. concluded that cervical tissue regeneration will, at least partly, compensate for the larger tissue loss (22). Likewise, in Carcopino's study there was no significant impact of the surgical specimen dimensions (thickness, length and circumference) on the cervical volume measured 6 months post-treatment (18).

The literature is almost unanimous in defining the height of resection as a key mechanism in the development of functional cervical insufficiency following conization, as evidenced by the lack of an effect of destructive treatments (where less than 5 mm tissue depth is concerned). A meta-analysis published in 2006 (14) noted a significant increase in the risk of preterm delivery (PD) if the excision depth was greater than 10 mm. Another study based on a Danish registry

Table III. *Univariate analysis of the impact of various factors on cervical regeneration at 6 months post LLETZ.*

Factors	Coefficient β*	*p*-Value
Age		
Parity	0.037	0.9406
2 children *vs*. none	–8.485	0.2038
1 child *vs*. none	1.033	0.8858
Smoking no *vs*. yes	–5.46	0.3394
Previous treatment** no *vs*. yes	0.676	0.9497
Lesion severity grade		
CIN 3 *vs*. neither***	–9.263	0.2022
CIN 1, 2 *vs*. neither***	4.659	0.5923
Healthy margins no *vs*. yes	0.329	0.9706
CL before LLETZ	–0.852	0.0827
Cone length	–0.781	0.3542
Proportion of excision	0.101	0.749

CL=Cervical length. *Regeneration (%)=β-Factor + Intercept. **History of previous laser or conization, ***Histologically-normal cervix. No tested variable has a significant impact on cervical regeneration at 6 months with a 5% threshold.

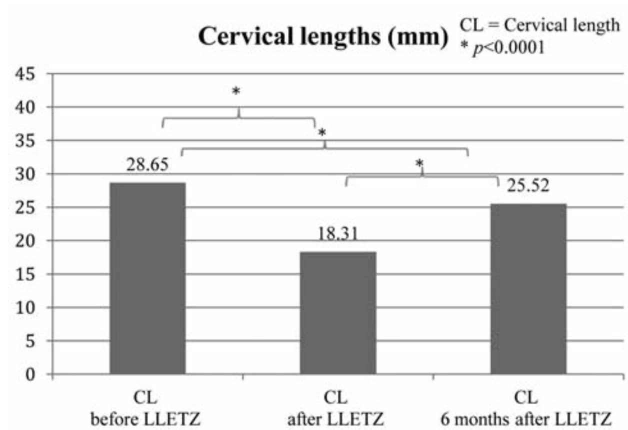

Figure 3. *Histogram of cervical length distribution.*

of 3,605 pregnancies after LLETZ reported an estimated 6% increase in the risk of PD per additional millimeter of tissue excised (15). Recently, in a cohort of 321 women following LLETZ, the authors found an almost tripled risk of PD for excision deeper than 12 mm (25). However, all these figures were derived from retrospective studies.

Our study reveals cervical regeneration of 71% at 6 months, ranging from 20% to 112% (median: 75%) with no other significant impact than increasing therapeutic delay, given that regeneration at 6 months was statistically greater than 1 month. This "regrowth" phenomenon implies that conization is a risk factor for PD although its role remains unclear, since it is not only related to mechanical phenomena (the cervix partly regenerates) but also probably multifactorial. Recent research has highlighted other pathophysiological mechanisms in addition to mechanical phenomena. First, experimental studies using murine models (26, 27) identified modifications to collagen architecture or structural alterations to collagen fibrils with subsequent loss of tissue stiffness during pregnancy as a factor responsible for cervical insufficiency along with a loss in tensile strength. Phadnis *et al*. (28) have also shown highly variable changes in tissue collagen distribution (increase, decrease or no change) in patients who had undergone two successive conization procedures. In addition, immunological factors bringing about changes to the cervico-vaginal flora (29) and a reduction in cervical mucus production (20) may impair the anti-microbial defense mechanisms in the cervix and cause subsequent preterm rupture of the membranes and therefore PD (30). Lastly, certain risk factors for HPV (Human Papilloma Virus) infection causing CIN (age, smoking, sexual behavior, *etc*.) are themselves risk factors for PD and thus represent confounding factors (31). In a meta-analysis in 2011, Bruinsma *et al*. (12) found that the relative risk of PD after excisional treatment was smaller when the comparison was with women assessed in colposcopic units but not treated (RR (Relative Risk): 1.25, 95% CI (Confidence Interval): 1.46-1.58) rather than an external comparison group (RR: 2.19, 95% CI: 0.93-2.49). This shows that patients with CIN have sociodemographic, behavioral and sexual characteristics that increase the risk of adverse obstetric outcomes.

Certain limitations of this study should be noted. The main limitation relates to the lack of evaluation of intra- and inter-examiner agreement between US CL measurements. To reduce the error margin, only 2 operators performed the measurements and cooperated to repeat exactly the same technique, and a mean of 3 measurements at each time point was used. Moreover, the same operator took all the measurements for the same patient in order to reduce bias. Transvaginal US measurement of CL outside pregnancy has been reported as a simple, reproducible and valid method when compared with anatomical measurements of hysterectomy specimens (32). Lastly, the good correlation with a high coefficient (R=0.85) found between the histological length and US measurements of CL before/after LLETZ in a previous study using the same methodology shows it to be a reliable measurement method (16). Another limitation of our study is the inability to determine a regeneration threshold above which the obstetric outcome is safe, and to assess obstetric outcomes following LLETZ depending on regeneration given the small number of pregnancies in our study. Further long-term studies are necessary to determine a regeneration threshold, above which pregnancy could be classified as high- or low-risk. Finally, post-operative measurements were performed at 6 months since the literature reported the process of cervical regeneration to be almost completed at that time point (22). However, we are unable to know whether this process persists beyond that time point from our study and literature review.

Conclusion

Our study demonstrates that LLETZ for CIN treatment leads to a significantly shorter cervical length, which is partially compensated by a tissue regeneration/healing process. However the "functional" quality of the regenerated cervix needs to be evaluated to determine the prognosis for subsequent pregnancies. There is no standardized surveillance protocol for pregnant women following LLETZ and the only recognized risk factor to date is the size of the excised cone which does not address the individual variability of cervical length. Obstetric risks are more likely to be related to variations in cervical regeneration, among other factors, rather than the excision height itself although this should also be assessed. Further prospective long-term studies evaluating obstetric outcome after LLETZ are required to assess the quality of the regenerated tissue in terms of cervical integrity. This would improve assessment of individual obstetric risk due to conization, with measurements of cervical length by transvaginal ultrasound before and 6 months after treatment that would be both useful and easy to implement. The degree of regeneration and patients at risk could therefore be identified.

Prior to the procedure, compliance with the indications of conization (33) and the surgical technique (34) (with pre-operative colposcopic assessment to adjust the cutting height while respecting oncological principles) is fundamental to best preserve the obstetrical future of patients of childbearing age.

Conflicts of Interest

The Authors declare no potential conflicts of interest.

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