

## COMPARISON OF CERVICAL BIOPSY USING PUNCH BIOPSY FORCEPS VERSUS LOOP ELECTRODE

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### ABSTRACT

**Context:** The biopsy of cervix can be obtained by various methods with availability of newer modalities like loop electrode. **Objectives:** To compare the histo-pathological parameters and clinical outcome of cervical biopsy obtained using punch biopsy forceps versus loop electrode. **Methods:** Women attending OPD were screened for cervical pathology, and colposcopy was done for those who screened positive. Patients who required cervical biopsy after colposcopy were allocated into 2 group; one undergoing LEEP biopsy and other half biopsied with Punch forceps. During procedure patients were evaluated for the intra-op pain and bleeding and their severity. The histo- pathological diagnosis was carried out and the sample was studied for its size, adequacy, and presence of any thermal or crush artefacts. **Result:** The two methods of biopsy were comparable in intra-op parameters, except for the increased requirement for additional haemostasis in LEEP biopsy. There was no case of bleeding from biopsy site at the follow-up visit. LEEP biopsy was associated with continued vaginal discharge more often than punch biopsy. An adequate sample for histopathological diagnosis was obtained in 91.25% of all cases. The comparative findings were reflective of comparable efficacy of both methods in providing an acceptable tissue sample for diagnosis. **Conclusion:** After analysing and comparing the aforementioned parameters, we opined that neither method can be deemed clearly superior to the other as a cervical biopsy procedure.

**KEYWORDS:** Punch biopsy forceps; Loop electrode; Cervical biopsy.

### INTRODUCTION

The diagnosis of preinvasive cervical neoplasia rests on the traditional histologic interpretation of submitted samples after screening and colonoscopy guided biopsy.[1] Traditionally, punch biopsy has been used for cervical biopsy.[2,3] The technique for treatment of CIN by using diathermic current i.e. Loop electrotherapy can be used for obtaining cervical biopsy for diagnostic purposes by using the small electro-surgical wire loop.[4]

Cervical biopsy is not associated with serious complications.[5] Varying degree of pain is commonly experienced by the patients. Minor bleeding from site of biopsy is another common complication. Infection in the cervix or an ascending endometritis, parametritis, or salpingitis can occasionally be seen following LEEP, but they are rare and usually represent a flare-up of an already existing subclinical infection.[6] The accuracy of histologic interpretation and diagnosis by a cervical biopsy is strongly governed by the quality of tissue pro-

vided, and proper handling and processing of the specimen.[7]

In punch biopsy specimens, many factors lead to unsatisfactory specimens, such as crush artefacts, denudation of the mucosa and failure to provide abnormal tissue of sufficient amount and depth. Diagnostic problems of LEEP specimens are most often caused by thermal damage. Prolonged contact between the loop and the tissue results in broad zones of thermal damage, coagulative necrosis, and tissue distortion that preclude an accurate diagnosis of the lesion and the status of excision margins. This study aimed at comparing the two methods Punch vs. LEEP for obtaining cervical tissue. There are no studies which have compared the quality of tissue sample provided by punch biopsy versus that by loop electrode. There is also lack of evidence to establish the safer biopsy method in comparison of per-operative and post-operative outcome.

### MATERIALS AND METHODS

**Study design:** Analytical observation prospective study.

**Ethical approval:** Study was approved by the institutional ethics committee.

**Study time frame:** This was a study carried out between Nov 2014 to February 2016.



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**Study place:** Department of Obstetrics and Gynecology in conjunction with Department of Pathology, VMMC and Safdurjung Hospital, New Delhi.

**Sample size:** 80 patients, randomly allocated into 2 groups of 40 each.

**Inclusion criteria:** A total of 2500 women were screened by cytology/VIA and those screened positive were taken up for biopsy (n=260). Women with abnormal colposcopic findings were selected for cervical biopsy and included in the study population.

**Exclusion criteria:** Active cervical infection and Pregnant were excluded.

**Grouping:** Patients who required biopsy were allocated into 2 groups.

**Group A:** Procedure using loop electrode (LEEP).

**Group B:** Procedure using Tischler's punch biopsy forceps.

Biopsies were conducted under aseptic settings as an outpatient procedure.

**Methodology:**

**Group A underwent biopsy by LEEP:** LEEP was done by the QL/LEEP High Frequency Gynaecologic Therapy Equipment. The biopsy site selected by colposcopy was identified by applying lugol's iodine. A current was selected at a setting of between 22-30 W which is suggested according to the size of the loop of 1 cm. The power settings were adjusted in accordance with the electrode tip size, the consistency of the cervical tissue, and any fibrous scarring from previous procedures. The wire loop was pushed perpendicularly into the cervix just lateral to the biopsy site at the depth of 5 to 8mm, drawn across it, and then pulled out perpendicularly on the other side. After biopsy sample was obtained, the site was examined for any excessive bleeding. In case of prominent bleeding, the site was cauterized with a 5 mm ball electrode at 30 to 50 W.[8]

**Group B underwent the conventional punch biopsy:** A standard Tischler forceps was used to obtain biopsy from the specific site on the transformation zone. It has stainless steel shaft with a pistol grip handle and an oblong bite measuring 7\*3\*1.5 mm. The site of biopsy was noted for any significant bleeding.

The sample obtained with the biopsy were preserved with 10% formalin and sent to the Department of Pathology for analysis after careful labelling.

During procedure patients were evaluated for pain and bleeding. Patients were asked to rate their pain at the time of acquiring the sample from 0 to 10. The pain scale used was the Numerical Rating Scale by NIH.[9]

Bleeding from the biopsy site was observed and the need for any accessory method for hemostasis was recorded if any. Minor bleeding was controlled by pressure with a large, cotton-tipped swab. If direct pressure failed to tamponade the bleeding, cauterisation was tried in the LEEP group and vaginal packing in Punch biopsy group. In case of severe bleeding or the failure to achieve hemostasis by above methods, a suture ligation was done with 2-0 or 3-0 absorbable suture.

Bleeding was classified into 3 groups:

**Mild:** Controlled by pressure with swab for 3 minutes.

**Moderate:** when there was requirement of cautery or vaginal packing.

**Severe:** When hemostatic sutures were required, active bleeding seen or patient required hospital admission.

The histopathological diagnosis was carried out and the sample was also described under the following parameters: Size, Adequacy, Presence of any thermal or crush artefacts and presence of blood clots with the sample.

Patients were carefully followed up at intervals of 1 week and 4 weeks.

History pertaining to any symptoms such as pain, bleeding, discharge per vaginum was noted. The histopathological report was discussed with the patient and treatment was offered as per the diagnosis further.

**RESULTS**

**Demographics:** The mean age of our study population was 34.8 years. Maximum numbers of patients were in 30 to 40 years of age group. Both groups were comparable in demographic parameters. The mean parity was 2.7 in our patients. Majority of patients in the study were multiparous (78%). The mean age at the time of first delivery was 21.06 years. Most of the patients included in the study were literate (74%) and resided in urban areas. The distribution of residence, marital status, and parity were similar in the 2 study groups.

**Pain:** The severity of pain at the time of obtaining the biopsy was graded on a scale of 0 to 10 according. A majority of patients (81%) had mild pain, scoring be-

**Table 1. The diagnosis on biopsy was compared with the initial colposcopic impression for each patient.**

COLPOSCOPIC IMPRESSION	Biopsy findings		
	BENIGN	CIN1	CIN2/3
Low grade (n=56)	27	23	4
High grade(n=24)	17	1	6

tween 1-3. 15% patients had pain score between 4 to 6. There was no cases of severe pain (score more than 7). In our study the mean pain score in the biopsy forceps group was  $2.23 \pm 1.11$ ; and that during LEEP biopsy was  $2.18 \pm 1.24$ . The difference in the pain scores was not statistically significant ( $p$  value = 0.118). [Table 4]

**Bleeding:** In our study, mild bleeding from biopsy site was noticed in 65% cases that was controlled by applying pressure by gauze. Moderate degree of bleeding was observed in 8 (2%) cases of punch biopsy that was controlled by vaginal packing. In the loop biopsy group, moderate amount of bleeding was seen in 20 (50%) cases that was controlled by electro cautery during the procedure ( $p < 0.001$ ). There were no cases of prolonged bleeding in our study that required a post procedural electrocautery. There was one case of severe bleeding after the procedure that was treated with vaginal packing and conservative treatment. There were no cases that would warrant haemostatic sutures or blood transfusion.

**On follow up:** Patients were advised follow up at 1 week and 4 weeks. All 80 patients had at least one follow up visit.

**Vaginal discharge:** Serous discharge was seen in 33% of LEEP group and 8% of punch biopsy group. Thus the difference was found to be statistically significant ( $p$  value = 0.007) between the two groups. We did not find any patients of purulent or sanguinous discharge in our study.

**Bleeding:** There were no cases of bleeding from the biopsy site on the follow up visit.

**Status of healing:** The morphological appearance of the cervix was completely normal in 60 patients. Raw healthy granulation tissue could be seen at the biopsy site in 8 cases. In 12 patients of the LEEP group, there was discolouration at the site. There were no cases of infected or friable appearance of the cervical surface in either group.

**Histo-Pathological parameters: Adequacy:** An adequate sample for histopathological diagnosis was obtained in 91.25% of all cases. Amongst the punch forceps group, an inadequate sample was reported in 3 cases (8%) as compared to 4 cases in LEEP biopsy group. The difference is not statistically significant. ( $p=1.0$ ). [Table no 5]

A repeat biopsy was carried out using the same method for the 7 cases of inadequate sample in the first procedure. The second biopsy was adequate in providing a diagnosis in all 7 cases.

**Size:** On comparison of the size of sample obtained, the difference between the 2 groups was statistically significant ( $p$  value  $< 0.001$ ) with the mean size of tissue obtained in punch forceps group being  $0.35 \pm 0.13$  cm and the mean size obtained by LEEP was  $0.77 \pm 0.34$  cm. The average size of tissue obtained by LEEP was significantly greater than by punch forceps. However the adequacy of the sample in providing a diagnosis as the number of adequate samples were found to be comparable in both groups.

**Thermal artefact:** In our study there were 10 cases of thermal artefacts present in the LEEP biopsy samples. That signified that 25% of LEEP samples had some degree of thermal artefact. However the histological diagnosis was possible in 96.2 % of these. [ Table 6]

**DISCUSSION**

The study was designed to evaluate the clinical and histo-pathological outcomes of conventional punch biopsy and the LEEP biopsy as diagnostic procedures after evaluating screened positive patients with colposcopy.

In our study there was 35% agreement between colposcopy and histopathological diagnosis of CIN. Colposcopy tended to overestimate the disease in 57% cases and underestimated the disease in 8% cases.

Studies on colposcopy have shown a high sensitivity ranging from 62 to 100%, and a specificity ranging be-

**Table 2. Comparison between colposcopic impression and biopsy result**

Study	Sample size	Agreement	Overestimation	Underestimation
Chappatte et al[10]	100	44%	32%	24%
Korkolopoulou et al[11]	108	-	-	45%
Kierkegaard et al[12]	813	-	-	25%
Massad et al[13]	2825	37%	-	-
Present study	80	35%	57%	8%

**Table 3. Comparison of sensitivity and specificity of colposcopy in various studies with the present study**

Authors	No. of cases	Sensitivity (%)	Specificity (%)
Kierkegaard et al[12]	813	62-72	-
Massad and Collins[13]	2112	89	52
Present study	80	96	50

**Table 4. Comparison of pain during biopsy procedures**

Study	Group	Sample size	Mean pain score	Remarks
Schmid et al [21],2007	With local anaesthesia	34	1.5	Difference was not significant (p=0.47)
	Without L.A.	34	1.9	
Church L et al[22], 2001	Topical anaesthesia	24	2.63	Difference was not significant
	Placebo	26	3	
Present study, 2016, without L.A.	Punch biopsy	40	2.23	(P= 0.11), difference not significant
	LEEP biopsy	40	2.18	

tween 48 and 99%.[14,15,16] The present study results fall within the range of previously reported studies, with a sensitivity of 96% and specificity of 50% for all CIN. The large variation in results amongst different studies is due to different grading systems used for colposcopic findings.

**Clinical parameters:** The pain sensation from the cervix is carried by the pudendal nerve and the hypogastric plexus of nerves. The pain perception is quite subjective and varies to an extent with patient anxiety and apprehension. For the sole purpose of taking a biopsy, local anesthesia has not been recommended. [17,18] In the studies that have studied LEEP as a therapeutic procedure, a lower incidence of pain has been documented. In a cervical screening and treatment study done by Rema et al in India in 2008, mild-to-moderate pain during or immediately after treatment was reported by less than 5% of women who underwent LEEP.[19] In a 2012 study, Duessing et al reported that about 5.4% patients experienced severe pain undergoing LEEP.[20] The difference may be attributed to the fact that for a therapeutic LEEP procedure, local anesthesia in form of paracervical block or pudendal block is usually administered.

Mild degree of bleeding has been reported as a frequent complication in LEEP and punch biopsy. Significant bleeding from biopsy site is rare, reported in only about 0.2 % procedures.[23,24] One LEEP study done by Chirenje ZM et al in 2001 reported that clinically significant early bleeding occurred in 2% of women.[25] In our study, mild bleeding from biopsy site was noticed in 65% cases that was controlled by applying pressure by gauze. Moderate degree of bleeding was ob-

served in 8 cases of punch biopsy that was controlled by vaginal packing. In the LEEP biopsy group, moderate amount of bleeding was seen in 20 cases that was controlled by electrocautery during the procedure. Pfendler et al did a LEEP study in Zambia in 2008 amongst HIV-infected women and reported that use of electrocautery were needed to control haemorrhage in up to 3.3% of women during the first 24 hours post-treatment and in 1.5% to 5.2% of women during the following days and weeks.[26] There were no cases of prolonged bleeding in our study that required a post procedural electrocautery. A study done in Chiang Mai Hospital in 60 patients reported that only one (1.7%) woman had severe intraoperative haemorrhage requiring suturing was comparable to our study. There were no cases in our study that would warrant haemostatic sutures or blood transfusion. There have been few reports regarding need for hysterectomy to control bleeding after LEEP excision. Sankaranarayanan et al indicated that hysterectomy was performed to manage haemorrhage in 1 woman out of the first 50 treated by LEEP. Studies are deficient regarding severe bleeding during LEEP when used as a diagnostic procedure from a specific limited site on the cervix.

On follow up: In our study, 80% of patients did not have any post procedural vaginal discharge at follow up and the difference was found to be statistically significant (p value = 0.007) between the two groups. There is scarce data regarding post procedural discharge when it comes to LEEP as a biopsy method, however a few studies have documented the vaginal discharge after excisional treatment by LEEP. Chirenje et al reported a high incidence of discharge, in 79% patients after treat-

ment by LEEP.[25] In a 2005 study in Zimbabwe, the average duration of discharge was reported to be 14 days.[27]

In the current study, there were no cases of bleeding from the biopsy site on the follow up visit. There have not been many studies quantifying the same. Pfaendler et al have reported an incidence of bleeding after LEEP procedure to be between 1.5% to 5.2% in Zambia in 2008 amongst HIV-infected women.[26]

Literature does not offer comparisons between the status of healing at the biopsy site in either types of cervical biopsy. In our study the biopsy site was examined at the follow-up visit in all patients. The morphological appearance of the cervix was completely normal in 60 patients. Raw healthy granulation tissue could be seen at the biopsy site in 8 cases. In 12 patients of the LEEP group, there was discoloration at the site. There were no cases of infected or friable appearance of the cervical surface in either group. However, a comparative analysis could not be done between the 2 groups as the follow up period ranged between 1 to 6 weeks and the 2 groups did not have uniform distribution of the follow-up visit.

**Table 5. Comparison of adequacy of sample**

Study	Procedure	Inadequacy
Byrom et al [28]	Cervical punch biopsy	5.3%
Present study	Punch biopsy	8%
	LEEP biopsy	10%

**Histo-pathological parameters:** The adequacy of a biopsy sample is judged by the ability to make a histological diagnosis, which was comparable in both groups in this study. In 2006, Byrom et al had found the rate of inadequacy in cervical biopsy samples to be 5.3% in a paired punch biopsy and LLETZ group.[28] Zuchna et al

had studied the diagnostic accuracy of guided cervical biopsies in 488 cases. They reported that the number of specimens influences the sensitivity of cervical biopsies. The maximum grade of CIN on the first and second biopsy compared with CIN on the first biopsy yielded significant improvement.

Wright et al reported that the size of the loop used is important, and suggested that large loops (10-20mm) appeared superior to small loops (3-7mm) with regard to histological accuracy and success rates.[29] In our study, the same loop size of 10 mm was used for all biopsies. For the punch biopsy group, a Tischler forceps with dimensions of 7\*3\*1.5 mm was used. Further studies may be carried out to evaluate the implications on adequacy by reducing size of biopsy surface.

The main concern about LEEP specimens is the effect of thermal artefact on critical histologic evaluation.[30] The high rate of surgical-margin thermal destruction, with related limitation of interpretability, may represent a serious diagnostic and therapeutic limitation of the LLETZ procedure when considered as an alternative to cold knife cone biopsy. An effort was made in 2004 by Nagar et al to determine whether the pure cut setting results in less thermal artefact than the traditional blend setting when performing a large loop excision of the transformation zone (LLETZ). No significant difference was detected in terms of grading of thermal artefact, the presence of dysplasia at the specimen margins, or in positive follow-up smears.[31] Although there was less thermal artefact at the deep stromal margin, cautery at this margin does not generally interfere with pathological assessment of the specimen and the pure cut setting does not produce a clinically significant decrease in the degree of thermal artefact.

In our study, a blend cut setting was used in all cases. Studies have reported that endo-cervical specimens suffer the most thermal injury.[32] In our study, 70% of specimens with thermal injury, the injury was limited to endocervical area.

**Table 6. Comparison of the thermal artefacts reported**

Study	Number of cases	Procedure	Incidence of thermal artefact	Remarks
Montz et al[33]	50	LLETZ	18%	
Messing MJ[34]	46	LLETZ	Slight – 34% Moderate –39% Severe –26%	Margins involved in 37% cases
loff et al[35]	100	LLETZ	100%	Margins involved in 28%
Present study	40	LEEP biopsy	25%	In 70%, limited to endocervical area.

## CONCLUSION

The two methods of biopsy were found to be comparable in intra-op pain, but there was increased requirement for additional haemostasis in LEEP biopsy. LEEP biopsy was associated with continued vaginal discharge more often than punch biopsy. The comparative findings were reflective of comparable efficacy of both methods in providing an acceptable tissue sample for diagnosis. After analysing and comparing the aforementioned parameters, we opined that neither method can be deemed clearly superior to the other as a cervical biopsy procedure.

**Strength:** This is the one of the few study compared the clinical outcome in patients undergoing cervical biopsy by these two methods. Moreover the outcome was also compared in terms of histo-pathological sample. Also this study complimented our efforts at screening for cancer cervix and creating awareness for the same. All the cervical biopsies were conducted by the same gynaecologist and all the pathological specimens were studied by one pathologist thus eliminating observer bias.

**Limitations:** weakness of the study was the non-uniform pattern of follow-up in our study population.

**Suggestions:** Continued efforts are required to create awareness and increase outreach to target population.

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