Research Article

Eliminating HPV DNA Positive Result with Large Loop Excision of the Transformation Zone (LLETZ)/Loop Electrosurgical Excision Procedure (LEEP) in Precancerous Cervical Lesions

Rizal Sanif, Patiyus Agustiansyah, Raissa Nurwany, Irawan Sastradinata, Theodorus, Wisman Agustian

Departement of Obstetrics and Gynecology
Faculty of Medicine Universitas Sriwijaya
Dr. Mohammad Hoesin General Hospital
Palembang

Abstract

Objective: To determine the use of LLETZ/LEEP to eliminate HPV DNA positive result in patients with cervical precancerous lesions at General Hospital dr. Mohammad. Hoesin, Palembang.

Methods: A case series with cervical precancerous lesions was undertaken at the Oncology Polyclinic of dr.Mohammad Hoesin Hospital Palembang from January to October 2022. There were 24 samples with positive HPV DNA before LLETZ/LEEP. Samples then checked for HPV DNA after LLETZ/LEEP. The effectiveness of LLETZ/LEEP therapy was analyzed using the Mc Nemar test. Comparison of HPV DNA outcomes (positive or negative) based on procedure, HPV DNA type and histopathological type was analyzed using Fisher Exact and Pearson Chi Square tests. All data were analyzed using SPPS version 22.0.

Results: In this study, it was found that the average age of patients with cervical precancerous lesions was 40.25 ± 7.67 years (28 - 57 years). Based on the diagnosis, 8 samples were found with High-grade Squamous Intraepithelial Lesion (HGSIL) and 16 samples with Low-grade Squamous Intraepithelial Lesion (LGSIL). All samples in this study were housewives and the majority were multiparas (75.0%). History of abortion in the patients in this study was only found in 5 samples (20.8%). The results showed that there were significant differences in the HPV DNA before and after LLETZ/LEEP therapy (p = 0.000). In addition, the results showed that there was no difference in the outcome of HPV DNA based on the procedure (p = 1.000) and the type of HPV DNA (p = 0.643). After LLETZ/LEEP therapy was carried out, it was found that only 1 subject has positive HPV DNA result and the HPV DNA virus found was type 52 and (high risk) and 42 (low risk).

Conclusion: It can be concluded that LLETZ/LEEP therapy is effective in eliminating HPV DNA positive results in cervical precancerous lesions

Keywords: cervical cancer, HPV DNA, LLETZ/LEEP, precancerous lesions, RCT

Correspondence author. Rizal Sanif. Department of Obstetrics and Gynecology.
Faculty of Medicine Universitas Sriwijaya. Dr. Mohammad Hoesin General Hospital. Palembang.
Email; rizalsanif@yahoo.com

INTRODUCTION

Cervical cancer is the fourth common cancer in women worldwide with 341.831 death in 2020. This mortality is mainly related to the delay in diagnosis, where most cervical cancer stages (70% of cases) are diagnosed at invasive, advanced and even terminal stages.[1-4]

Pathogenesis of cervical carcinoma is well known and begins with human papilloma virus infection and progresses to precancerous lesions. Over 50-80% sexually active women was infected by HPV during their life with 20% progressed to cervical cancer. Cervical precancerous lesions will develop into invasive carcinomas over a long period of time, between 10-20 years. Therefore, prevention of this disease is important and can be done by carrying out currently available vaccinations and early detection. Cytology examination with high false negative results

has been screening standard for cervical cancer over 50 years. HPV DNA examination has the advantage of very high negative predictive value is projected to be the new standard.[5,6]

Early detection of cervical precancerous lesions leads to various types of procedures. In developing countries there are three treatment options for cervical precancerous lesions, namely cryotherapy, large loop excision of the transformation zone (LLETZ or LEEP), and cold knife conization (CKC). The recommendations for treatment are for those with confirmed High Grade Squamous Intraepithelial Lesion (HGSIL) or adenocarcinoma in situ (AIS) with cryotherapy, loop electrosurgical excision procedure (LEEP)/ large loop excision of the transformation zone (LLETZ), or cold knife conization (CKC).[5]

The success rate of hot knife conization such as LLETZ/LEEP in cervical precancerous lesions therapy using LLETZ/LEEP is effective if the HPV DNA in patients with precancerous lesions decreases significantly.

This study aims to determine the effectiveness of LLETZ/LEEP in cervical precancerous lesions therapy by examining HPV DNA at dr. Mohammad Hoesin Hospital, Palembang.

METHODS

A case series study to determine the use of LLETZ/LEEP to eliminate HPV DNA positive result in patients with cervical precancerous lesions was undertaken at Dr. Mohammad Hosein Hospital Palembang from January to November 2022. Obtained 30 patient with positive pap smear for cervical precancerous lesions, 6 subjects were excluded due to negative HPV DNA results. The independent variable in this study was LLETZ/ LEEP while the dependent variable was HPV DNA.

The collection of HPV DNA test specimens was carried out by taking cervical mucus with a disposable broom-type cervical brush into the cervical canal before and after LLETZ/LEEP procedure. The direct examination was carried out in Prodia Palembang laboratory. Examination of the HPV genotype using the QIAamp Mini DNA Kit (Qiagen) DN. LLETZ or LEEP were chosen by oncologist based on clinical appearance of the cervix during the procedure.

Data processing used SPSS 22.0. Descriptive analysis was carried out to assess general characteristics and HPV DNA data. The effectiveness of LLETZ/LEEP therapy was analyzed using the Mc Nemar test. Comparison of HPV DNA outcomes (positive or negative) based on procedure, HPV DNA type and histopathological type was analyzed using Fisher Exact and Pearson Chi Square tests.

RESULTS

In this study, it was found that the average age of patients with cervical precancerous lesions was 40.25 ± 7.67 years (28 - 57 years). All samples in this study were housewives and the majority were multiparas (75.0%). History of abortion in the patients in this study was only found in 5 samples (20.8%) (Table 1).

Table 1. General Characteristics of Research Subjects

Variable	Frequency	(%)
Age (years old)		
Mean ± SD	40.25 ± 7.67	
Median (Min- Max)	40 (28 – 57)	
Age at Marriage		
Mean ± SD	23.63 ± 3.93	
Median (Min- Max	23.5 (17 – 30)	
Age, n (%)		
≥ 35	19	79.2
< 35	5	20.8
Occupation, n (%)		
House wife	24	100
Parity, n (%)		
Nulliparous	1	4,2
Primiparas	5	20.8
Multipara	18	75.0
Abortion History, n (%)		
Yes	5	20.8
No	19	79.2
Diagnosis, n (%)		
HGSIL	8	33.3
LGSIL	16	66.7
Anatomical Pathology Results, n (%)		
HGSIL	9	37.5
LGSIL	15	62.5
Procedure, n (%)		
LEEP	12	50.0
LLETZ	12	50.0

Explanation
HGSIL : High-Grade Squamous Intraepithe-Lial Lesion
LGSIL : Low-Grade Squamous Intraepithe-Lial Lesion
LEEP : Loop Electrosurgical Excision Procedure
LLETZ : Large Loop Excision of The Transformation Zone (LLETZ)

Based on the diagnosis, 8 samples were found with High-grade Squamous Intraepithelial Lesion (HGSIL) and 16 samples with Low-grade Squamous Intraepithelial Lesion (LGSIL). Meanwhile, based on the results of anatomical pathology, there were 9 samples with HGSIL histopathological type and 15 samples with LGSIL

histopathological type. There was 1 sample that was initially diagnosed as LGSIL but based on the PA results it was HGSIL (Table 1).

Table 2. Characteristics of HPV DNA

Characteristics	Frequency	%
HPV DNA before, n (%)		0
Positive	24	100.0
HPV DNA Type, n (%)		
High Risk	13	54.2
Low Risk	10	41.7
Other Type	1	4.2
HPV DNA After, n (%)		
Positive	1	4.2
Negative	23	95.8
Alteration, n (%)		
Yes	23	95.8
No	1	4.2

In this study, before LLETZ/LEEP therapy was performed, all 24 subjects (100%) with cervical precancerous lesions had positive HPV DNA, consisting of 13 subjects (54.2 %) with high risk HPV DNA types, 10 subjects (41.7.3%) with low risk HPV DNA types and 1 subject (4.2%) with other types of HPV (Table 2).

After LLETZ/LEEP therapy was carried out, it was found that only 1 subject has positive HPV DNA result. Using the McNemar test, the results showed that there were significant differences in the HPV DNA result before and after LLETZ/LEEP therapy (p = 0.000) (Table 3). Patients who still found positive HPV DNA virus after LLETZ/LEEP therapy had the histopathological type HGSIL, were treated with the LLETZ procedure and the HPV DNA virus found was type 52 and (high risk) and 42 (low risk). This patient was then undergoing hysterectomy.

Table 3. The Effectiveness of LLETZ/LEEP Therapy in Patients with Cervical Precancerous Lesions

Characteristics	before	after	*P-value*
HPV DNA			
Positive	24	1	0.000*
Negative	0	23	
Total	24	24	

DISCUSSION

Cervical precancerous lesions are preceded by HPV infection and are influenced by several factors that increase the risk of precancerous lesions including Early age at sexual debut, multiple sexual partners, history of genital warts and smoking.[7] These risk factors will play a role in the process of carcinogenesis, thereby changing normal cells into abnormal cells that lead to cervical cancer.[8] Most cases of precancerous cervical lesions are diagnosed in women between the ages of the third and fourth decades of life with the peak incidence of cervical cancer being in the age group 40-49 years.[9,10] Women aged 40-49 years have a 2.4 times higher chance of developing precancerous lesions compared to those aged 30-39 years.[5]

In this study, it was found that the average age of patients with cervical precancerous lesions was 40.25 ± 7.67 years and the majority were aged ≥ 35 years (79,2%). These results are in line who reported the majority of patients aged > 35 years.[10-12] Early marriage which lead to early sexual debut is also a risk factor for cervical precancerous lesions. Having sexual intercourse for the first-time during puberty, which is less than 17 years old, is a risk factor for cervical precancerous lesions because the transformation zone and metaplasia tend to mutate easily. In this study, it was found that the average age of patients with cervical precancerous lesions when married ranged between 15-30 years with an average of 23.63 years. These results similar to other studies that found the majority of the age at marriage in patients with precancerous cervical lesions was <30 years (86.3%), aged 16-20 years as many as 41.3%; aged 21-25 years as much as 23.6%; and aged 26-30 years as much as 23.4%.[6,8,9]

The majority of patients with cervical precancerous lesions in this study were multipara (73.47%). This result is in line with the study which reported that the majority of patients with cervical precancerous lesions were multiparas.[6,8] Several studies state that high parity have a significant effect. Women who often give birth (or give birth to many children) automatically experience injuries to their reproductive organs including the cervix, especially in women with short birth intervals, these injuries often include a higher risk of HPV infection.[13,14]

HPV is a member of the Papillomaviridae family which is divided into 2 subfamilies with more than 50 genera, however, only 5 genera (classification based on L1 sequence) are associated with infection in humans namely Alpha-, Beta-, Gamma-, Nu-, and Mu- papillomavirus. In this study, 24 samples (100%) of patients with cervical precancerous lesions had positive HPV DNA, with a high-risk type of 54.2% and a low risk of 41.7% and 4.2% other types of HPV. The most

high-risk HPV DNA types were 18 and 52, while the most low-risk HPV DNA types were 70. These results are in accordance with a study by Kabir et al in 2019 which reported that the five high-risk HPVs that were most often detected in either single or multiple HPV infections were HPV. 16, 18, 45, 51 and 52. At the end of the study after the procedure, it was found that only 1 sample had positive HPV DNA with multiple infections, namely high-risk DNA types 52 and low risk 42.[15]

In this study, both of these procedures had effectiveness in the treatment of cervical precancerous lesions which could be assessed from changes in the HPV DNA. A total of 24 samples that initially had positive HPV DNA after the procedure there was only 1 sample that still had positive HPV DNA.

Research conducted by Petrillo et al in 2020 on 182 (60.7%) women who were vaccinated with the HPV vaccine within 4 weeks after LEEP and 103 (34.3%) women who were not vaccinated. Recurrence of cervical dysplasia following the LEEP procedure occurred in 30 (10.5%) women, of whom 17 were unvaccinated and 13 were vaccinated. Administration of HPV vaccine after LEEP appears to reduce the risk of recurrence, suggesting that HPV vaccination may act as an adjunctive treatment after LEEP.[16]

CONCLUSION

From these results it can be concluded that LLETZ/ LEEP therapy is effective in eliminating HPV DNA positive results in cervical precancerous lesions. After LLETZ/LEEP therapy was carried out, it was found that only 1 sample was still found to have HPV DNA positive.

ACKNOWLEDGEMENT

The authors would like to thank Sriwijaya University for funding the studies. This research was also supported by Prodia Laboratory where the HPV DNA was examined.

REFERENCES

1. GLOBOCAN. The Global Cancer Observatory: All Cancer. 2020. https:// gco.iarc.fr/today/data/factsheets/ cancers/39-all-cancers-fact-sheet.pdf.
2. GLOBOCAN. The Global Cancer Observatory: Cervix Cancer. 2020. https://gco.iarc.fr/today/data/factsheets/ cancers/23-Cervix-uteri-fact-sheet.pdf
3. GLOBOCAN. The Global Cancer Observatory : Cancer Today. Estimated number of new cases in 2020, Indonesia, both sexes, all ages. 2020. https://gco.iarc.fr/today/online-analysis-map?v=2020&mode=population&mode_population.
4. Nurwany R, Sanif R , Novaliani A, Theodorus. The Efficacy of Chemotherapy in Advanced-Stage Cervical Cancer on Vitamin A Serum. Indones J Obstet Gynecol. 2022; 10(2);95–101.
5. Arbyn M, Anttila A, Jordan J, Ronco G, Schenk U, Segnan N, et al. European Guidelines for Quality Assurance in Cervical Cancer Screening. Summary of the supplements on HPV screening and vaccination. Eur J Cancer. 2015; 1: 22-31
6. Chandra R, Rauf S, Malinta U. Human Papilloma Virus Selftesting as an Alternative Method for Cervical Cancer Screening. Indones J Obstet Gynecol. 2017 Apr 5(2);114–19.
7. Wudtisan J, Tantipalakorn C, Charoenkwan K, Sreshthaputra RA, Srisomboon J. Factors Associated with Development of High-Grade Squamous Intraepithelial Lesions of the Uterine Cervix in Women Younger than 30 Years. Asian Pac J Cancer Prev. 2019 Apr 29;20(4):1031-6.
8. Basu P, Taghavi K, Hu SY, Mogri S, Joshi S. Management of cervical premalignant lesions. Curr Probl Cancer 2018;42(2):129–36. from:https://doi.org/10.1016/j. currproblcancer.2018.01.010
9. Teame H, Addissie A, Ayele W, Hirpa S, Gebremariam A, Gebreheat G, et al. Factors associated with cervical precancerous lesions among women screened for cervical cancer in Addis Ababa, Ethiopia: A case control study. PLoS One. 2018;13(1):1–13.
10. Budiana ING, Artha IBRK. Characteristics of Cervical Precancerous Lesions at a Tertiary Hospital in Bali. Indonesia. Indones J Cancer. 2020;14(4):117
11. Getinet M, Taye M, Ayinalem A, Gitie M. Precancerous Lesions of the Cervix and Associated Factors among Women of East Gojjam, Northwest Ethiopia, 2020. Cancer Manag Res. 2021;13(September):9401–10.
12. Wabo B, Nsagha DS, Nana TN, Assob CJN. Prevalence and risk factors associated with precancerous cervical lesions among women in two cities in Cameroon. Pan Afr Med J. 2022;41.
13. Kusuma LF, Yarso KY. Hormonal contraceptive use as risk factor for breast cancer in young Javanese women. J thee Med Sci (Berkala Ilmu Kedokteran). 2016;48(04 (Suplement)):19–19.
14. Ashar H, Kusrini I, Musoddaq A, Asturiningtyas IP. First sexual intercourse and high parity are the most influential factors of precancerous cervical lesion. Maj Obstet Ginekol. 2020;28(3):113
15. Kabir A, Bukar M, Nggada HA, Rann HB, Gidado A, Musa AB. Prevalence of human papillomavirus genotypes in cervical cancer in Maiduguri, Nigeria. Pan Afr Med J. 2019;33:1–11.
16. Petrillo M, Dessole M, Tinacci E, Saderi L, Muresu N, Capobianco G, et al. Efficacy of HPV vaccination in women receiving LEEP for cervical dysplasia: A single institution's experience. Vaccines. 2020;8(1):1 -8.