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CIN-CLEAR: Long-Term Outcomes after Topical Trichloroacetic Acid Treatment for Cervical Dysplasia

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Keywords:

Human Papillomavirus; Low-Grade Squamous Intraepithelial Lesion; High-Grade Squamous Intraepithelial Lesion; Large Loop Excision of the Transformation Zone; Trichloroacetic Acid

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Abbreviations:

AIS: Adenocarcinoma in Situ; ASC: US Atypical Squamous Cells of Undetermined Significance**;** ASC-H: Atypical Squamous Cells - Cannot Exclude; HSIL**;** HPV: Human Papillomavirus

1. Abstract

1.1. Background

Cervical cancer remains a global health issue. Early detection through screening enables timely intervention to prevent progression. While low-grade squamous intraepithelial lesions (LSIL) often regress spontaneously, high-grade squamous intraepithelial lesions (HSIL) typically require excisional treatment, which may cause complications. Non-surgical therapies, such as topical trichloroacetic acid (TCA), have shown promising short-term efficacy, but long-term outcomes remain unclear. This study evaluates the long-term effectiveness of TCA in treating cervical intraepithelial lesions. Methods: Women treated with 85% TCA for abnormal cervical cytology, including HSIL, LSIL, atypical squamous cell of undetermined significance (ASC-US) or abnormal histology (HSIL, LSIL) at the Academic Teaching Hospital Feldkirch, Austria between 2017 and 2022 were retrospectively analyzed. TCA was applied topically to the transformation zone and endocervix, with follow-up assessments at 8 weeks and then at 6-month and yearly intervals. Outcomes were evaluated using cytology and HPV results at least 3 years post TCA treatment. Remission was characterized as regression to negative for intraepithelial lesion or

malignancy (NILM). HPV clearance was defined as negativity for HPV. Patients undergoing excisional treatment or showing recurrent abnormal cytology (LSIL, HSIL) were classified as nonresponders.

1.2. Results

A cohort of 248 patients was included (median age 29 years). Human papillomavirus (HPV) infection with high-risk types was present in 93.1% of patients prior to treatment. Overall remission after at least 3 years was 79.4% (n=197), including 134 HSIL and 63 LSIL cases. HPV 16 predominated in HSIL (n=90), and high-risk HPV pool A and B were common in LSIL (n=46). HPV clearance at a minimum of 3 years post TCA treatment was seen in 77.5%. Progression to cervical cancer was not detected within the study population.

1.3. Conclusion

Topical 85% TCA achieves high long-term remission in both, LSIL and HSIL, and may represent a promising non-surgical treatment option. It is particularly beneficial for women of reproductive age, offering sustained lesion regression while avoiding risks associated with excisional procedures.

2. Introduction

Cervical cancer continues to be a significant global health burden, with persistent infection by highrisk human papillomavirus (HPV) types identified as the primary etiological factor for the development of cervical squamous intraepithelial lesions and invasive carcinoma [1]. Early detection through cervical cytology screening Papanicolaou (PAP) smears and HPV testing has improved the management of premalignant cervical lesions, al-

lowing for intervention to prevent progression to invasive cancer [2,3]. For low-grade squamous intraepithelial lesions (LSIL), current guidelines recommend an observational approach, as these lesions have a high likelihood of spontaneous regression, particularly in younger women. In contrast, high-grade squamous intraepithelial lesions (HSIL) are associated with a significantly greater risk of progression to invasive cancer and therefore excisional procedures, such as large loop excision of the transformation zone (LLETZ) are recommended [4]. These treatments carry potential risks, especially for women of reproductive age, including cervical insufficiency and obstetric complications such as preterm birth, low birth weight, and perinatal mortality [5]. Hence, there is

growing interest in non-surgical, conservative treatment modalities that can effectively induce regression of cervical dysplasia while minimizing morbidity [6,7]. Previous studies have demonstrated promising short-term efficacy of trichloroacetic acid (TCA) in the treatment of both, HSIL and LSIL [6,7]. Applied topically, TCA induces chemical coagulative necrosis with tissue destruction typically extending through the full thickness of the epithelium of most lesions of cervical intraepithelial neoplasia [8]. However, data on the long-term outcomes are still lacking. Therefore, the primary objective of this study was to evaluate the long-term effectiveness of TCA treatment over an extended follow-up period.

3. Materials and Methods

This retrospective data analysis included all women treated with 85% TCA at the Department of Obstetrics & Gynecology, Academic Teaching Hospital Feldkirch in Austria between 2017 and 2022. All patients were informed in advance regarding the off-label use of TCA and gave their informed consent. The study was approved by the local ethics committee of Vorarlberg (EK-0.04-561). The standardized nomenclature for cytological findings included PAP II/NILM (negative for intraepithelial lesion or malignancy), PAP III/atypical squamous cells of undetermined significance or cannot exclude HSIL (ASC-US, ASC-H), PAP IIID/LSIL, PAP IV/HSIL or endocervical adenocarcinoma in situ (AIS) and PAP V/squamous cell carcinoma (SCC) or AIS [9]. Histologically,

squamous intraepithelial lesions were classified as LSIL or HSIL according to the two-tier system [10]. The molecular testing for HPV at the Department of Pathology of the Hospital was conducted according to a standardized protocol, with HPV detection performed by real-time polymerase chain reaction using the Alinity m System [11]. HPV types 16 and 18 were reported individually. Other HPV high-risk genotypes were categorized into two groups: Pool A, comprising types 31, 33, 52, 2 and 58, and Pool B, including types 35, 39, 51, 56, 59, 66, and 68. For statistical reasons, Pool A and B were subsequently combined and analyzed as HPV high-risk pool (HPV HRP). Criteria for TCA treatment included either cytological or histological abnormalities (ASC-US, LSIL,

HSIL) with or without a positive molecular testing for HPV 16, 18 or HPV HRP. The application of 85% TCA followed a protocol-based procedure: first, a cotton swab is used to apply TCA to the transformation zone and any abnormal areas. Then, a second cotton swab is used for endocervical

application. Initial follow-up was conducted eight weeks post TCA application, including cytological, histological and HPV assessments. Further follow-up examinations were performed after 6 months and then in 12-month intervals. Follow up smears were predominantly collected by referring gynecologists and evaluated by our pathology department. In selected cases with persistent cytological or histological abnormalities (HSIL, LSIL) or persistent HPV positivity at the initial follow-up visit, repeated TCA treatment was offered. Patients who were lost to follow-up before completion of the 3-year observation period were excluded from the final analysis. For the analysis, patients were classified in groups by lesion severity (HSIL or LSIL). In cases where cytological and histological results were discordant, the result indicating the higher-grade dysplasia was used for subsequent analysis. In addition, the presence of HPV infection and the HPV genotypes were assessed. If multiple HPV types were detected, the type associated with the highest oncogenic risk was used for further analysis. These classifications were subsequently used to calculate remission and HPV clearance rates. Remission was characterized by the improvement from HSIL or LSIL to NILM. To evaluate the remission rate, either cytological (PAP) and/or HPV results were used, depending on data availability. The most recent available result served as the outcome. Patients who underwent excisional treatment after TCA or developed recurrent cytological abnormalities (ASC-US or higher) during follow-up were classified as non-responders. HPV clearance was defined as posttreatment negativity for HPV. In cases where HPV testing was not available, repeated NILM

cytology was used as a surrogate marker for HPV clearance. Patients with persistent or recurrent HPV infection or those requiring excisional treatment were considered non-cleared. Women who underwent repeated TCA treatment were also included but analyzed separately. Patients with HPV 16 positivity and histologically HSIL were classified as a high-risk subgroup and also included in a distinct analysis. Data were summarized using descriptive statistics. Statistical analyses were conducted as a perprotocol analysis, including only patients who completed the study and for whom outcome data were available at least 3 years post treatment.

4. Results

A total of 248 patients, who were treated with 85% TCA in the period from 2017 to 2022, were included in this study. The median age was 29.6 years (19-56 years) (Table 1). Based on cytological or histological findings, patients were categorized according to lesion severity, with 175 (70.6%) assigned to the HSIL group and 73 (29.4%) to the LSIL group (Table 1). At baseline cytology, 130 women had HSIL, 105 LSIL, 10 ASC-US, and 3 NILM. Among cases with ASC-US or NILM, HPV positivity and abnormal histological findings were additionally observed

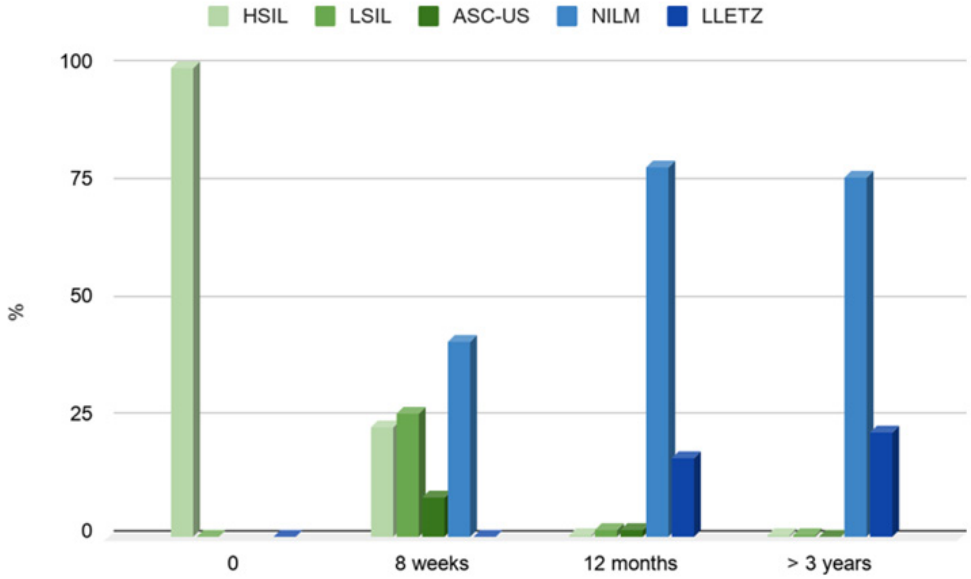
(5 HSIL and 8 LSIL). Among the 130 patients with PAP IV/HSIL cytology, 84.6% also showed HSIL on biopsy, 9.2% had LSIL, and 6.2% showed no dysplasia. In 105 women with PAP IIID/LSIL, 38.1% showed HSIL on biopsy, 54.3% had LSIL confirmed, and 7.6% had no dysplasia. All cases in which the biopsy was free of dysplasia were positive for high-risk HPV. Association with HPV infection was initially seen in 93.1% of patients (n=231) (Table 2). Among patients with HSIL, 90 individuals (51.4%) were infected with HPV 16, 9 (5.1%) with HPV 18, and 66 (37.8%) with other types of HPV HRP. Likewise, in cases of LSIL, 17 patients (23.3%) showed HPV 16, 3 (4.1%) showed HPV 18, and 46 (63.0%) were infected with types of the HPV HRP. HPV types 16 and 18 were predominantly present in patients with HSIL, accounting for 84.1% and 66% of infections compared to 15% and 33% in LSIL, respectively. In contrast, infections with high-risk HPV pool types showed no significant association with lesion grade. Overall, a total remission rate of 79.4% (197/248) was seen (Table 3). Of those in remission, 134 had been diagnosed with HSIL and 63 with LSIL prior to treatment. This corresponded to remission rates of 76.6% (n=175) for HSIL and 86.3% (n=73) for LSIL after 3 years of follow-up. Short-term remission to NILM after 8 weeks in HSIL cases was observed in 41.7%, increasing to 78.9% after

one year. In LSIL cases, remission to NILM was seen in 50.7% at 8 weeks and 78.1% at one year, respectively (Figures 1 and 2). 56 patients (22.6%) underwent repeated treatment with TCA (55 received TCA twice, 1 three times). Of these, 45 (80.4%) were in remission following a minimum follow-up period of 3 years (Figure 3). Among the patients who reached remission after repeated TCA applications, 31 had initially been diagnosed with HSIL and 14 with LSIL, corresponding to remission rates of 77.5% and 87.5%. The HPV high-risk subgroup with histologically confirmed HSIL and HPV 16 positivity before TCA treatment included 81 patients. In 80% of these cases (n = 65), remission to NILM was observed after a minimum of 3 years of follow-up. HPV clearance at a minimum of 3 years post TCA treatment was seen in 77.5% (Table 4). HPV infection 3 years post TCA treatment was observed in 7 patients (2.8%). Of these, 2 patients experienced reactivation of the pretreatment HPV infection over the course of follow-up, 2 patients demonstrated persistent HPV infection and 3 patients showed reinfection with a different HPV type. Among patients with HSIL, clearance rates reached 77.8% for HPV 16, 77.8% for HPV 18, and 72.7% for other types of HRP, while in the LSIL group, clearance was 82.4% for HPV 16, 100% for HPV 18, and 82.6% for HPV HRP types.

During the time of observation, 51 of 248 women (20.6%) were classified as non-responders (Table 3). Of these, 49 patients (19.8%), including 11 who underwent repeated TCA treatment, subsequently received LLETZ. Among the HPV high-risk subgroup (HPV 16 with histologically confirmed HSIL), LLETZ was performed in 15 of 81 patients (18.5%). Prior to treatment, 39 women (79.6%) showed HSIL, while the remaining patients had LSIL (n=10). Postoperative histological examination showed either HSIL (n=26) or LSIL (n=10) in 36 patients, whereas in 22 percent (n=11) of cases, the cone specimen was free of dysplasia. For 2 patients, postoperative histological results were not available.

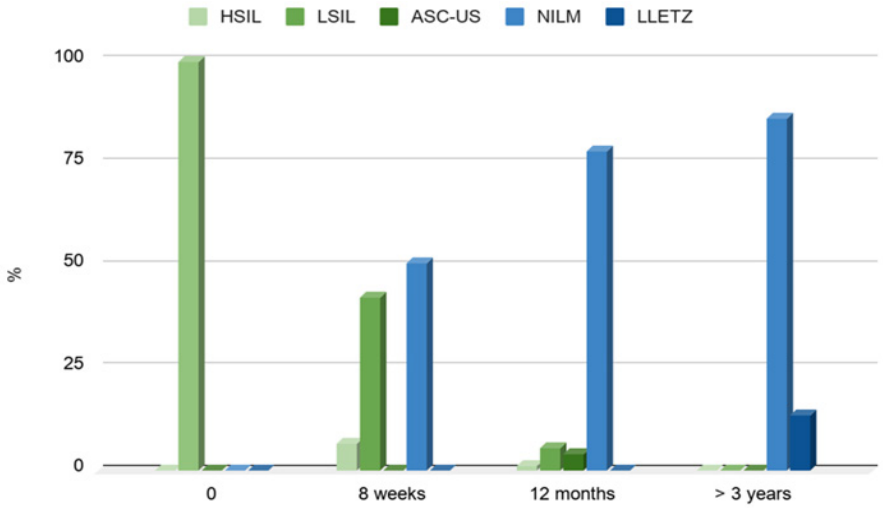
Recurrence of abnormal cytological results was observed in two patients. One case belonged to the HPV high-risk subgroup and showed a recurrence of HSIL 3 years after treatment and short-term remission (8 weeks post-treatment). The second patient initially presented HSIL and HPV HRP positivity. After the first treatment, cytology cleared to PAP II and HPV high-risk testing became negative. 7 years after TCA application, cytology showed PAP IIID whereas HPV testing continued to be negative. The patient is currently under clinical observation. Progression to cervical cancer was not observed in the study population.

Figure 1: Outcomes in 175 women with HSIL after 85% trichloroacetic acid treatment: lesion grade changes and surgical interventions over > 3 years.



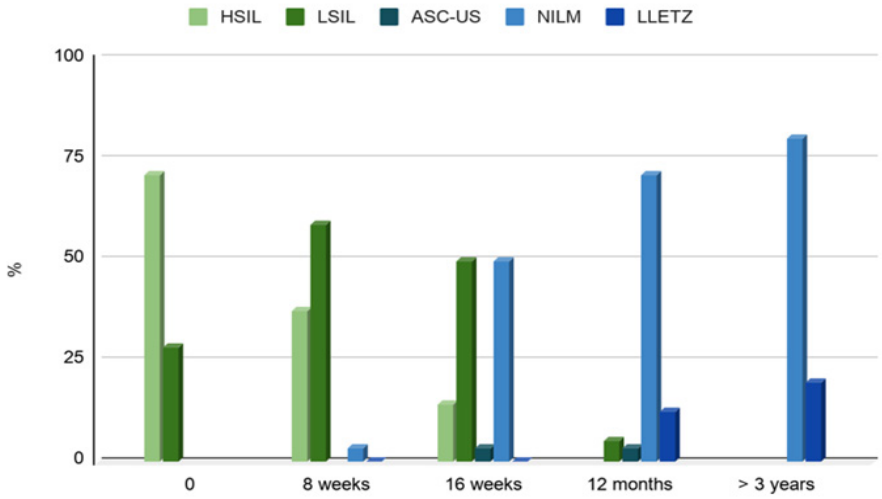
HSIL: high-grade squamous intraepithelial lesion, LSIL: low-grade squamous Intraepithelial lesion, ASC-US: atypical squamous cells of undetermined significance, NILM: negative intraepithelial lesion or malignancy status, LLETZ: large loop excision of the transformation zone.

Figure 2: Outcomes in 73 women with LSIL after 85% trichloroacetic acid treatment: lesion grade changes and surgical interventions over > 3 years.



HSIL: high-grade squamous intraepithelial lesion, LSIL: low-grade squamous Intraepithelial lesion, ASC-US: atypical squamous cells of undetermined significance, NILM: negative intraepithelial lesion or malignancy status, LLETZ: large loop excision of the transformation zone.

Figure 3: Long-term outcomes in 56 patients with repeat 85% trichloroacetic acid treatment: changes in lesion grade and surgical interventions over > 3 years of follow-up.



HSIL: high-grade squamous intraepithelial lesion, LSIL: low-grade squamous Intraepithelial lesion, ASC-US: atypical squamous cells of undetermined significance, NILM: negative intraepithelial lesion or malignancy status, LLETZ: large loop excision of the transformation zone.

Table 1: Baseline characteristics and clinicopathologic findings in 248 women undergoing TCA treatment at study inclusion.

Characteristics	Value
Total	248
Median Age	29.6
Cytology	248
HSIL	130
LSIL	105
ASC-US	10
NILM	3
Histology	248
HSIL	155
LSIL	77
No dysplasia	16
Final classification used for analysis*	248
HSIL	175
LSIL	73

TCA: trichloroacetic acid, HSIL: high-grade squamous intraepithelial lesion, LSIL: low-grade squamous Intraepithelial lesion, ASC-US: atypical squamous cells of undetermined significance, NILM: negative intraepithelial lesion or malignancy status.

*In cases where cytological and histological findings were discordant, the result indicating the higher-grade dysplasia was used for subsequent analysis.

Table 2: Pre-TCA lesion grade and its association with high-risk HPV types in the study cohort (n=248).

Parameter	Total population n=248 (%)	HSIL n=175 (%)	LSIL n=73 (%)
HPV 16	107 (43.1)	90 (51.4)	17 (23.3)
HPV 18	12 (4.8)	9 (5.1)	3 (4.1)
HPV high-risk pools (A+B)	112 (45.2)	66 (37.8)	46 (63.0)
Negative for HPV high-risk types	17 (6.9)	10 (5.7)	7 (9.6)

TCA: trichloroacetic acid, HSIL: high-grade squamous intraepithelial lesion, LSIL: low-grade squamous intraepithelial lesion. HPV: human papillomavirus.

Table 3: Clinical outcomes 3–7 years after TCA treatment in patients with cervical intraepithelial neoplasia: remission and non-response according to lesion grade.

Parameter	Total population n=248 (%)	HSIL n=175 (%)	LSIL n=73 (%)
Remission	197 (79.4)	134 (76.6)	63 (86.3)
Non-response	51 (20.6)	41 (23.4)	10 (13.7)
LLETZ	49 (19.8)	39 (22.3)	10 (13.7)
Recurrence	2 (0.8)	2 (1.1)	0

HSIL: high-grade squamous intraepithelial lesion, LSIL: low-grade squamous intraepithelial lesion. LLETZ: large loop excision of the transformation zone.

Table 4: Overall and type-specific HPV clearance rates according to initial HPV status after at least 3 years of follow up.

Parameter	**Total population n=231**	**HSIL n=165**	**LSIL n=66**
HPV Clearance	77.50%	75.80%	83.30%
HPV 16	78.50%	77.80%	82.40%
HPV 18	83.30%	77.80%	100.00%
HPV high-risk pools (A+B)	75.90%	72.70%	82.60%

HSIL: high-grade squamous intraepithelial lesion, LSIL: low-grade squamous intraepithelial lesion. HPV: human papillomavirus.
Clearance rates are presented as percentages of patients who tested HPV negative at follow-up after treatment with trichloroacetic acid (TCA), with additional calculations performed for type-specific clearance based on the initial HPV genotype (Table 2).

5. Discussion

The results of this retrospective long-term study showed that topical application of TCA appears to be an effective treatment option for cervical intraepithelial lesions. With an overall remission rate of 79.4% after 3 to 7 years of observation, permanent normalization of cervical cytology was achieved. Notably, the remission rate among all women with HSIL reached 76.6% and in the HPV high-risk subgroup, a remission rate of 80% was observed, respectively. Recurrence of cytological abnormalities was rare, observed in only two patients. One case demonstrated recurrence of HSIL following an initial remission 8 weeks post treatment, most likely due to reactivation of the pretreatment HPV 16 infection. In the second case, the recurrence of PAP IIID/LSIL after several years was not consistent with persistence of the original HSIL. It most likely represents a new infection, probably caused by low-risk HPV types (e.g., HPV 6 or 11) that were not included in the HPV high-risk panel.

Other Austrian studies assessing the effectiveness of topical TCA in patients with CIN II and III have demonstrated high short-term remission rates. Geisler et al. [5], documented an 80% remission rate two months post-treatment [6]. Likewise, the prospective TRICIN trial reported a remission rate of 76.9% for CIN II after six months, although TCA was not administered to patients with CIN III [7]. In our analysis, short-term remission rates at 8 weeks were lower than those reported in previous studies, whereas results at 12 months were comparable [6,7]. 6.

6. Despite Lower Early

response rates in our cohort, sustained improvement from 12 months onwards indicates durable regression of dysplastic lesions over time. It also highlights the potential of TCA as a long-term conservative treatment option for patients with HSIL, which usually receive surgical treatment [4,12,13]. In our analysis, 49 patients subsequently underwent LLETZ, including 39 women with HSIL, suggesting that in 77.7% (n=136) no surgical intervention was required. Avoiding excisional procedures not only reduces acute perioperative risks, such as bleeding and infection, but also prevents long-term complications including cervical insufficiency and preterm birth [5,14]. The latter is particularly relevant, given that the median age of our study population lies within the reproductive age range. In addition to the possible adverse effects, it should be mentioned that cervical conization does not always result in complete resolution of the lesion. Non-in-sano resections occur in up to 23% of cases and are associated with an increased risk of recurrence, necessitating repeat surgical interventions or close follow up [4, 15–17]. Furthermore, it is also worth noting that in one fifth of these cases,

histological analysis of the cone specimens showed no evidence of dysplasia, supporting the hypothesis that TCA may have induced complete regression in some patients prior to surgical intervention. However, alternative explanations include spontaneous regression, removal of the lesion during the initial biopsy, or very small lesions missed due to the technical proceeding of the tissue samples. Contributing factors with a positive impact on spontaneous regression include a prolonged interval between biopsy and surgical treatment, HPV negativity prior to surgery and patient age under 25 years [18]. Compared with excisional techniques, TCA offers several advantages. The procedure is minimally

invasive, inexpensive and user-friendly. It can be performed in an out-patient setting, which shows also its resource efficacy. There is no need for an operating room, special equipment or any general anesthesia. As seen in previous studies, treatment with 85% TCA is generally well tolerated, with a median visual analogue scale (VAS) pain score of < 4,0 [6,8]. Possible treatmentrelated side effects included vaginal discharge, spotting, lower abdominal pain, and dyspareunia with a median duration of 5,5 days [7].

Spontaneous regression is most commonly observed in LSIL, therefore, a conservative, observational approach is generally recommended for such patients [4]. In a study by Moscicki et al. [19], a spontaneous regression rate of 61% for LSIL at 12 months was reported [19]. In comparison to that, the short-term remission rate for LSIL following TCA treatment was notably higher at 82.3% and 78.9% [6,7] It is known that active monitoring can lead to psychological distress, including anxiety and uncertainty about potential disease progression and cancer risk, which underscores the importance of thorough patient education and counseling by healthcare practitioners [19-21]. For patients who prefer an active intervention and seek the reassurance of a proactive management strategy, TCA may represent a viable alternative to a purely observational approach in LSIL cases and to avoid a surgical intervention. In fact, patient preference for an active treatment strategy was also the predominant reason for including LSIL patients in our study population.

In contrast to other conservative treatment modalities for CIN, such as topical imiquimod, TCA appears to offer superior efficacy and practicality. Imiquimod has been investigated as a nonsurgical treatment, however, clinical outcomes have been modest. Studies report a total remission rate of HSIL in 37% to 47% after treatment, markedly lower than the remission rates observed with TCA [22, 23]. Moreover, imiquimod therapy requires self-administered topical application over a prolonged period (up to 16 weeks), which demands high patient compliance and is frequently associated with local and systemic adverse effects, including headache, fatigue, myalgia and vulvovaginal pruritus or pain. The occurrence of these side effects has been linked to irregular application or discontinuation of treatment [18, 22]. In contrast, TCA is applied under direct visualization by a clinician, ensuring standardized administration and reducing the risk of non-compliance.

While the results are encouraging, some limitations must be acknowledged. Due to the retrospective design, potential selection and information bias cannot be excluded. The absence of a control group prevents direct comparison with surgical treatment modalities or spontaneous regression. Missing data, including absent HPV results after more than 3 years of follow-up, may have resulted in an overestimation of both remission and HPV clearance rates. Despite these limitations, this analysis provides important evidence supporting the long-term efficacy of TCA for the management of cervical intraepithelial lesions. TCA represents a promising therapeutic option, particularly for young women of reproductive age and for use in low-resource settings. Nevertheless, future prospective, randomized studies with larger cohorts and standardized treatment protocols are needed to confirm these findings.

7. Conclusion

Topical application of 85% trichloroacetic acid demonstrates high long-term remission rates in both LSIL and HSIL. The treatment appears to be an effective non-surgical alternative, particularly beneficial for women of reproductive age, offering sustained lesion regression while avoiding the risks associated with excisional procedures. These findings may support the use of TCA as a conservative treatment option in the management of cervical dysplasia.

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