Comparison between Single Versus Twice Application of Topical 85% Trichloroacetic Acid in the Treatment of Cervical Intraepithelial Neoplasia; A Randomized Clinical Trial on Efficacy and Tolerability

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Abstract

Purpose: To compare the efficacy and safety of up to two-time administration of 85% TCA, as a promising alternative therapy to conservative and surgical management of grade one to three CINs. Methods: In this two-armed randomized clinical trial, a total of 53 patients with biopsy-proven CIN lesions were allocated to two groups of TCA treatment. The first group (n=26) received a single dose of local therapy with 85% TCA while the second group (n=27) was treated on two separate occasions with a two-week interval. Two participants (one in each group) were lost to follow-up. At the two-month follow-up after TCA application, a colposcopy-guided biopsy was performed for all patients and the pathological specimens were studied by a single experienced pathologist to determine the post-intervention grading of CIN. Results: Two groups were comparable in terms of age and base-line lesion grading, as CIN 1 lesions comprised the majority of cases (54%), followed by CIN 2(37%). While our sample was a poor representative of CIN3 lesions (7%), no significant difference was noticed between the single and twice TCA treated groups with a response rate of 52% and 54% respectively (either complete remission to normal histology or regression to any low-grade lesion). Either separate analysis (with respect to the base-line grading within each treatment group) or combined analysis (regardless of CIN sub-group) could not generate any statistical significance. The second dose of TCA did not increase the frequency of reported adverse events. Conclusion: The second dose of topical 85% TCA does not seem to increase the CIN response rate more so than its single dose. However, further controlled clinical trials with larger samples are warranted to verify current findings. The use of TCA was not limited by any major side effect, therefore, the potential to achieve an increased efficacy with more frequent TCA applications is appealing.

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transformation zone and endocervical canal, respectively. Cotton swab and wooden end of the swab for ectocervix-subsequent application of 85% TCA using an acid-soaked precipitate in the epidermis and upper reticular dermis. The lack of systemic absorption of TCA after topical application, no risk of systemic side effects, safety during pregnancy, tolerability and affordable price has made it a good candidate for the treatment of CIN (MALVIYA et al., 1987; BOOTHBY et al., 1990; Demars et al., 1992; Zhu et al., 1992; Pezeshkpoor et al., 2012; Jayaprasad et al., 2016). Patients may experience minimal side effects such as pain, spotting, and increased vaginal discharge but these complications resolve shortly after application (Zhu et al., 1992; Geisler et al., 2016). Previous studies have proved TCA to be effective in the treatment of anogenital pre-cancerous lesions where it was found to have an added value in the treatment of immunocompromised patients with anal intraepithelial neoplasia (AIN) and vaginal intraepithelial neoplasia (VAIN) who cannot tolerate any invasive intervention (Lin et al., 2005; Singh et al., 2009). Topical TCA has not been confirmed as a standard treatment for CIN and very little evidence is available regarding its efficacy, optimal dosage and frequency of application. Herein we compare the treatment outcomes of CIN patients treated with single-time topical application of 85% TCA to those who received the same preparation on two separate occasions with a two-week interval.

Materials and Methods

Methods and Patients

All women with biopsy-verified CIN grade 1, 2, and 3 lesions who presented to the Motahari Gynecological Oncology center, the first affiliated clinic of Urmia University of Medical Sciences, from January 2017 to January 2018 entered this randomized trial. Individuals with abnormal cytology reports of Low grade squamous intraepithelial lesion (LISL) or High-grade squamous intraepithelial lesion (HISL) or unsatisfactory colposcopic impressions were excluded from the study. Informed consent was granted by all participants after a full explanation of the purpose, study protocol, mandatory length of the follow-up period and possible consequences. We ensured that every partaker is particularly informed that conization is the ultimate standard treatment for CIN 2 and 3 (Martin-Hirsch et al., 2013). A total of 53 patients met the inclusion criteria and randomized into two groups of treatment with single versus twice dose of TCA. All patients underwent the first visit which comprised colposcopic examination with acetic acid and subsequent application of 85% TCA using an acid-soaked cotton swab and wooden end of the swab for ectocervix-transformation zone and endocervical canal, respectively. Protein denaturation and precipitation were confirmed by visual observation of the color change to white. The first group was advised against engaging in sexual intercourse for 2 weeks and using bathing tubs for 4 weeks. They were required to return at 8 weeks for the reexamination and the repeat colposcopy-directed biopsy. On the other hand, the second group was reexamined after 2 weeks following the initial visit and received the second dose of topical TCA in the same manner as the first dose. Similar cautionary suggestions were made and they were asked to return for the colposcopy-guided biopsy at 8 weeks after administration of the second dose of TCA. For the cases with invisible original lesion upon the post-intervention colposcopy at the 8-weeks follow-up, blind biopsies were collected from the four quadrants of the cervix. Patients were also asked if they had experienced any adverse events including but not limited to pain, symptoms of pelvic inflammatory disease (PID), spotting, post-coital bleeding and excess vaginal discharge. In order to reduce the potential for bias, the initial intervention and follow-up examinations were all performed by the same gynecologist and the sections were reviewed by a single pathologist who were both blinded to other data. As treatment with neither the single nor double dose of TCA constitutes the standard of care for CIN, three months after the end of the study all patients were reexamined and the optimal therapeutic approach was planned according to the latest standard guidelines.

A Sample size of 24 patients in each group was needed to detect a response rate of 40% and to satisfy the statistical requirements (α=0.05, power=0.8). Allowing for a drop-out rate of 10%, the sample size was increased to 53. A per protocol analysis was carried out and two groups were compared in terms of the rate of complete remission (from any CIN to normal histology), regression (from higher grade to lower grade CIN) and adverse effects using Chi-square test or Fisher’s exact test, when indicated, and two-sided p-value ≤ 0.05 was considered significant.

Results

A total of 53 patients with biopsy-confirmed CIN lesion grading from 1 to 3 were enrolled in the current randomized trial. They were randomly allocated to one of the two intervention groups; either single-time or two-time treatment with 85% TCA (n=26 and 27, respectively). However, two patients with CIN 1 lesion were lost to follow-up (one from each of the parallel groups) and the study was concluded with 51 participants at the 8-week. Twenty-five individuals from group 1 and 26 from group 2 were included in the final analysis as depicted in the study flowchart (Figure 1).

The mean age calculated across all of 51 participants equals 33.64 (SD8.4) years. Mean age of the group 1 who were treated with a single dose of TCA and group 2 who received two application of the same preparation on two occasions with a two-week interval was 34.32(SD 10.76) and 33(SD 6.2), respectively. Background and pathologic characteristics of patients are demonstrated in Table 1. At the baseline, two groups of intervention did not differ from one another in terms of age (p-value=0.431), the frequency distribution of lesion grading (p-value=0.243)
and histology (p-value=0.915). In both groups, grade 1 CIN comprised the majority of cases at the initial evaluation (52% in group 1; 57.7% in group 2), followed by CIN 2 (40% in group 1; 34.61% in group 2) and CIN 3 (8% in group 1; 7.6% in group 2), respectively.

While over the half of patients in both groups denied any uncomfortable experience attributable to the TCA, pain (12%) and excess vaginal discharge (23%) were the most common adverse events complained by the participants of groups one and two, respectively.

Chi-square test was performed to compare the proportions of final treatment outcomes and adverse events.
between the two groups which yielded differences with no statistical significance. Although group 2 showed a larger fraction of remission (38.46%) when compared to group 1 with the remission rate of 28%, the observed dominance did not bear any statistical relevance. The combined rate of remission and regression for the two groups were approximately the same i.e. 52% for group 1 and 53.84% for group 2. According to our findings, lesions in group 1 were not more likely than their counterparts to remit or regress at the follow-up biopsy in any statistically meaningful manner (p-value=0.761). Even the analysis of three subgroups alone (i.e. CIN 1, CIN 2 and CIN 3), failed to show any appreciable association between the two groups of intervention and rates of each outcome (remission, regression, progression and unchanged pathology) (CIN 1; p-value=0.594, CIN 2; p-value=0.483, CIN 3; p-value=0.999) (Table 2).

Likewise, the proportion of subjects who reported adverse effects did not differ by the times (single or twice) of TCA administration (p-value=0.371). In both groups, there was no mention of severe complications and all of the adverse events were of self-limiting nature. Out of seven patients in group one who suffered from side effects, 4 patients were found to have remission or regression in CIN and the remaining 3 patients had no change in grading. While the side effects of group 1 was exclusively reported within one week of the TCA application, in the case of group 2, they were experienced mainly after the second dose of TCA.

### Discussion

Our findings showed that regardless of the baseline CIN grading, repeated treatment with topical 85% TCA was not associated with improved short-term outcome, increased severity of side effects or emergence of any serious complication. If the 51 cases are considered as a whole, only 53% of lesions did either remit or regress, while 37.3% of them remained unchanged and in 9.7% of cases a progression in grading occurred.

These findings were not much accordant with the results of the study conducted by Geisler et al., (2016). In their retrospective case series of 241 women with different CIN grading, who had received a single dose of topical 85% TCA as the first-line therapy, TCA was found to be effective for CIN remission and regression.

As of patients’ baseline features, about 45% of Geisler et al., (2016) study group had a CIN3 lesion and only 17.9% were representative of CIN 1 grading, in stark contrast to our sample where CIN1 lesions comprised the bulk of values (54.9) and only 7.8% of cases were CIN3. Even though they detected markedly high rates of remission or regression in CIN grading, the highest of them being 92.8% (95% CI 81.9–97.3) which corresponds to the rate of regression from grade 2 to 1, the CIN1 patients have exhibited the lowest remission rate (75 %; 95% CI 56.6% –88.5%) in their study. The lower limit of 95% confidence interval for remission rate of this group (56.6%) approximates to the efficacy rate of 53% recorded in our study.

The rationale behind conservative management is that, in the presence of a healthy immune system, low grade cervical precancerous lesions may remit on their own, however, it is an unlikely scenario for patients with high grade lesions. Yet in the only single study demonstrating the efficacy of TCA in treatment of CIN (Geisler et al., 2016), 74% of study subjects had high-grade lesions (vs. 18% of high-grade lesions in our study). The response rate of low-grade lesions (82.3%) was not much different from the high-grade lesions (80.3%) and it was solidly concluded that the observed effect of TCA could not be attributed to the chance. However, the role of spontaneous remission was not sufficiently addressed as a potential contributor to the observed outcomes. Though it is...
possible that unknown confounders and small sample size might be responsible for the difference in response rates between our and Geisler’s study.

The study of Geisler et al. was comprehensive in its investigation of the rates of HR-HPV clearance following TCA therapy, which was revealed to be independent of the HPV type. Current study is consistent with their study in terms of the encountered side effects which were solely limited to minor uncomfortable experiences.

In a randomized trial of 262 women with CIN 1 and 2, the efficacy of a novel treatment (hexaminolevulinate photodynamic therapy) was assessed and response rates between two gradings were divergent. While among the CIN1 patients, the treatment results were comparable to those from the placebo group, in CIN2 patients, the same intervention was found to be statistically superior to placebo (Hillemanns et al., 2015).

In a retrospective cohort of 207 women with low-grade cervical dysplasia, local TCA therapy was significantly effective with 78% of regression rate while spontaneous regression was estimated to be 48% (p-value<0.05) (Demars et al., 1992).

In a retrospective study on 54 men with AIN who were treated with topical 85% TCA, a remission rate of 32% and a regression rate of 29% was found among. These response rates rose high to 71%-73% by taking a per-lesion approach instead of a per-patient one (Singh et al., 2009).

Lin et al., (2005) have shown the 50% TCA to be effective in post-hysterectomy management of 28 patients with various grading of VAIN with a remission rate of 71.4%. Grade 1 VAIN patients were more likely to remit (100%) than their VAIN 2 and 3 counterparts (53%) (p-value=0.009). Congruent with other studies, the reported adverse events were negligible in terms of frequency and severity.

Cranston et al., (2014) has found 72 HIV positive individuals with high-grade anal intraepithelial neoplasia (AIN) to benefit from up to four applications of 80% TCA (response rate of 78.6%). They also reported a recurrence rate of 20.8% during the follow-up period (Cranston et al., 2014).

Inferences from our study are limited for its small sample size, particularly due to the very small number of CIN3 lesions, and the fact that data regarding HPV DNA typing was not collected.

### Author Contribution Statement

HA: Conceptual design, methodology, and supervision.
SEM: Data collection, data analysis, manuscript draft.
SN: Conceptual design and supervision.
ZY: Data interpretation and scientific revision.
ZJ: Data curation and write-up. All authors reviewed the manuscript and contributed intellectually. The final manuscript was approved by all authors.

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**Ethics approval and consent to participate**

This study was approved by the Research Ethics committee of Urmia University of Medical Sciences under the code IR.UMSU.REC.1397.366.

**Study registration**

Registration of current trial protocol under the scientific name of “The Comparison Study of the Effect of One Dose and Two Doses of Trichloroacetic Acid 85% In Cervical Intraepithelial Neoplasia Treatment” has been approved in Iranian Registry of Clinical Trials at under registration reference code of IRTC20171128037651N1.

### Availability of data and materials

The datasets generated and/or analysed during the current study are not publicly available but are available from the corresponding author upon reasonable request.

### Competing interests

Authors declare no conflict of interests.

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