RESEARCH ARTICLE

Editorial Process: Submission:10/21/2021 Acceptance:03/14/2022

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

Haleh Ayatollahi[1], Solmaz Solmaz Ershadimoghaddam[1], Siamak Naji[2], Zahra Yekta[3], Zahra Jalali[4]*

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.

Keywords: Colposcopy- cervical intraepithelial neoplasia- trichloroacetic acid- human papillomavirus

Asian Pac J Cancer Prev, **23 (3)**, 947-952

Introduction

Current management options for cervical intraepithelial neoplasia (CIN) vary from active surveillance to surgical interventions and mainly depend on the CIN grading, HPV subtype, patient's age and preference (Garcia et al., 2012). Patients with low-grade CIN are commonly subject to simple watchful waiting and are routinely required to undergo a periodic examination to ensure remission or detect potential progression of the present lesion. Women aged under 25 years and with a normal immune system are assumed to benefit more from the conservative approach (Massad et al., 2013). Yet the successful conservative treatment comes at the expense of psychological distress, as individuals with initially abnormal cytology experience a considerable amount of anxiety, while waiting for the follow-up examination (Sharp et al., 2013). On the other hand, surgical interventions are reserved for high-grade CINs, recurrent or persistent cases who fail to improve despite watchful management. Patients treated with different techniques of either ablative or excisional therapy, have demonstrated cure rates of 90% – 95% with minimal adverse events in short- term, consisting mainly of self- limiting pain and bleeding (Kyrgiou et al., 2006b). In the long-run, however, women with a previous history of invasive interventions (e.g., cold knife conization) are found to have 30% to 35% increased rates of preterm labor when compared to random controls (Kyrgiou et

*[1]Department of Obstetrics and Gynecology, Urmia University of Medical Sciences, Urmia, Iran. [2]Mahzad Hospital, Urmia University of Medical Sciences, Urmia, Iran. [3]Department of Community Medicine, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, Iran. [4]Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, USA. *For Correspondence: zjalai@bidmc.harvard.edu*

al., 2006a; Arbyn et al., 2008; Kyrgiou et al., 2016). In an attempt to avoid such undesired events, various methods have been proposed as potential alternative treatments for CIN. Given the well-established role of HPV in the pathogenesis of CIN, agents with promising effects on the eradication of mucocutaneous warts have been at the center of attention in many studies. Among them, trichloroacetic acid (TCA) is a caustic chemical frequently used in the cosmetic industry for its keratolytic characteristics as it causes coagulative necrosis and protein precipitation 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) and 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<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)

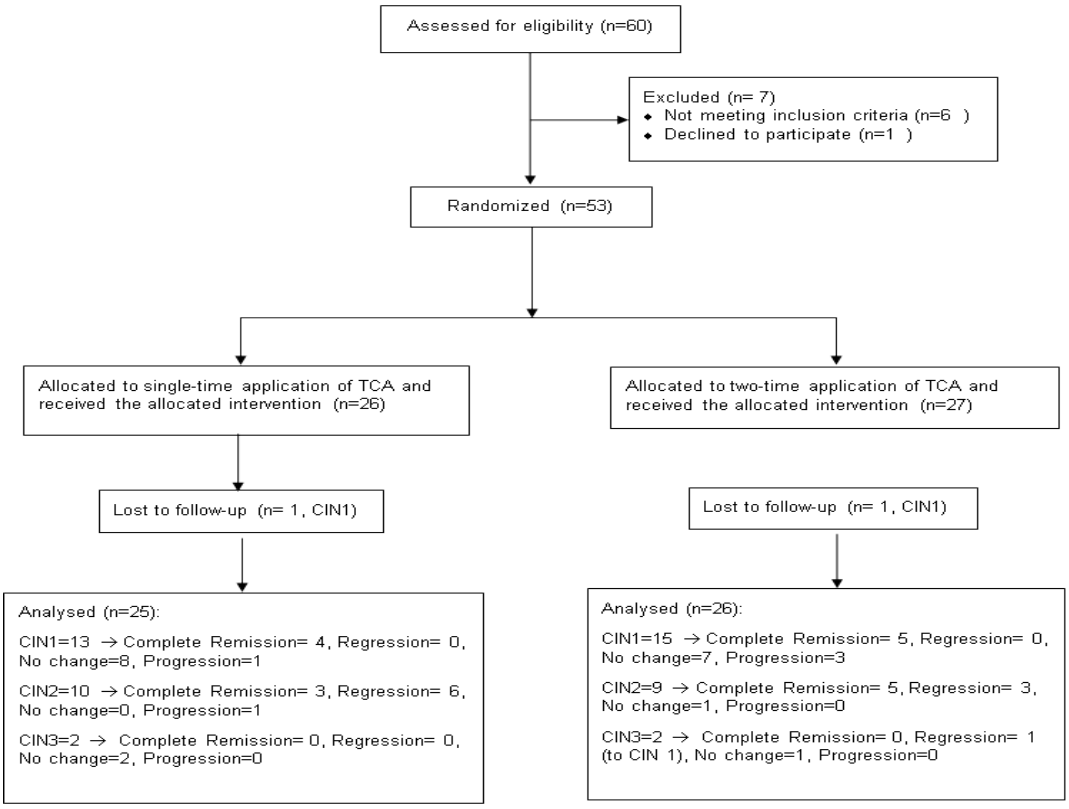

Figure 1. Study Flow-Chart; Patient Enrollment and Allocation is Summarized

and histology (p-value=0.915). In both groups, grade 1 CIN comprised the majority of cases at the initial evaluation (52% in group1; 57.7% in group 2), followed by CIN 2 (40% in group1; 34.61% in group2) and CIN 3 (8% in group1; 7.6% in group2), 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

Table 1. Base- Line and Follow-up Characteristics of Analyzed Patients

Variable	All Patients	Group1 (Single application of TCA)	Group2 (Double application of TCA)	p-value
Participants, n	51	25	26	
Age, mean(SD)	33.64 (8.4)	34.32 (10.76)	33 (6.2)	0.431
Baseline Lesion grading, n (percentage of column)				
Low grade	42 (82.35)	19 (76)	23 (88.46)	0.243
High grade	9 (17.64)	6 (24)	3 (11.53)	
Baseline Histopathology, n (percentage of column)				
CIN1	28 (54.9)	13 (52)	15 (57.69)	0.915
CIN2	19 (37.25)	10 (40)	9 (34.61)	
CIN3	4 (7.84)	2 (8)	2 (7.69)	
Treatment outcome, n (percentage of column)				
Remission	17 (33.33)	7 (28)	10 (38.46)	0.761
Regression	10 (19.6)	6 (24)	4 (15.38)	
Unchanged	19 (37.25)	10 (40)	9 (34.61)	
Progression	5 (9.8)	2 (8)	3 (11.53)	
Adverse events, n (percentage of column)				
None	31 (60.78)	18 (72)	13 (50)	0.371
Pain	7 (13.72)	3 (12)	4 (15.38)	
Spotting	5 (9.8)	2 (8)	3 (11.53)	
Vaginal discharge	8 (15.68)	2 (8)	6 (23.07)	

Table 2. Response Rate to the Single vs. Double TCA Application; Treatment Groups are Categorized Based on Their Base-Line CIN Grading

			Response to Treatment				
Baseline Pathology		N	Remission	Regression	Unchanged	Progression	p-value
Low grade, n	Total	42	14 (33.33)	9 (21.43)	16 (38.1)	3 (7.14)	
(percentage of row)	1st group	19	4 (21)	6 (31.57)	8 (42.1)	1 (5.26)	0.313
	2nd group	23	10 (43.5)	3 (13)	8 (34.8)	2 (8.7)	
High grade, n	Total	9	3 (33.3)	1 (11.1)	3 (33.3)	2 (22.2)	
(percentage of row)	1st group	6	3 (50)	0	2 (33.3)	1 (16.6)	0.829
	2nd group	3	0	1 (33.3)	1 (33.3)	1 (33.3)	
CIN1, n	Total	28	9 (32)	N/A	15 (54)	4 (14)	
(percentage of row)	1st group	13	4 (31)	N/A	8 (61)	1 (8)	0.594
	2nd group	15	5 (33)	N/A	7 (47)	3 (20)	
CIN2, n	Total	19	8 (43)	9 (47)	1 (5)	1 (5)	
(percentage of row)	1st group	10	3 (30)	6 (60)	0	1 (10)	0.483
	2nd group	9	5 (56)	3 (33)	1 (11)	0	
CIN3, n	Total	4	0	1 (25)	3 (75)	0	
(percentage of row)	1st group	2	0	0	2 (100)	0	>.9
	2nd group	2	0	1 (50)	1 (50)	0	

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 group1 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, CIN3; 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 CIN1 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 internal 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.

Acknowledgments

Funding Declaration

This study was funded by Urmia University of Medical Sciences. It is derived from the second author thesis for completion of her OB/GYN specialty training.

Ethics approval and consent to participate

This study was approved by the Research Ethics committee of Urmia University of Medical Sciences under the codeIR.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 IRCT20171128037651N1.

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.

References

Arbyn M, Kyrgiou M, Simoens C, et al (2008). Perinatal mortality and other severe adverse pregnancy outcomes associated with treatment of cervical intraepithelial neoplasia: meta-analysis. *BMJ*, **337**, a1284.

Boothby RA, Carlson JA, Rubin M, et al (1990). Single application treatment of human papillomavirus infection of the cervix and vagina with trichloroacetic acid: a randomized trial. *Obstet Gynecol*, **76**, 278-80.

Cranston RD, Baker JR, Liu Y, et al (2014). Topical application of trichloroacetic acid is efficacious for the treatment of internal anal high-grade squamous intraepithelial lesions in HIV-positive men. *Sex Transm Dis*, **41**, 420-6.

Demars L, Valea F, Fowler W, et al (1992). Trichloroacetic acid as first line treatment in HPV-associated low-grade dysplasia. *Gynecol Oncol*, **45**, 85.

Garcia F, Hatch K, Berek J (2012). Intraepithelial disease of the cervix, vagina, and vulva. Berek & Novak's gynecology. 15th ed. Philadelphia (PA): Lippincott Williams & Wilkins, pp 592-604.

Geisler S, Speiser S, Speiser L, et al (2016). Short-term efficacy of trichloroacetic acid in the treatment of cervical intraepithelial neoplasia. *Obstet Gynecol*, **127**, 353-9.

Hillemanns P, Garcia F, Petry KU, et al (2015). A randomized study of hexaminolevulinate photodynamic therapy in patients with cervical intraepithelial neoplasia 1/2. *Am J Obstet Gynecol*, **212**, 465.e1-7.

Jayaprasad S, Subramaniyan R, Devgan S (2016). Comparative evaluation of topical 10% potassium hydroxide and 30% trichloroacetic acid in the treatment of plane warts. *Indian J Dermatol*, **61**, 634-9.

Kyrgiou M, Athanasiou A, Paraskevaidi M, et al (2016). Adverse obstetric outcomes after local treatment for cervical preinvasive and early invasive disease according to cone depth: systematic review and meta-analysis. *BMJ*, **354**, i3633.

Kyrgiou M, Koliopoulos G, Martin-Hirsch P, et al (2006a). Obstetric outcomes after conservative treatment for intraepithelial or early invasive cervical lesions: systematic review and meta-analysis. *Lancet*, **367**, 489-98.

Kyrgiou M, Tsoumpou I, Vrekoussis T, et al (2006b). The up-to-date evidence on colposcopy practice and treatment of cervical intraepithelial neoplasia: the Cochrane colposcopy & cervical cytopathology collaborative group (C5 group) approach. Cancer treatment reviews, 32, 516-23.

Lin H, Huang E-Y, Chang H-Y, et al (2005). Therapeutic effect of topical applications of trichloroacetic acid for vaginal intraepithelial neoplasia after hysterectomy. *Jpn J Clin Oncol*, **35**, 651-4.

Malviya Vk, Deppe G, Pluszczynski R, et al (1987). Trichloroacetic acid in the treatment of human papillomavirus infection of the cervix without associated dysplasia. *Obstet Gynecol*, **70**, 72-4.

Massad LS, Einstein MH, Huh WK, et al (2013). 2012 updated consensus guidelines for the management of abnormal cervical cancer screening tests and cancer precursors. *J Low Genit Tract Dis*, **17**, 1-27.

Pezeshkpoor F, Banihashemi M, Yazdanpanah MJ, et al (2012). Comparative study of topical 80% trichloroacetic acid with 35% trichloroacetic acid in the treatment of the common wart. *J Drugs Dermatol*, **11**, e66-9.

Sharp L, Cotton S, Carsin AE, et al (2013). Factors associated with psychological distress following colposcopy among women with low-grade abnormal cervical cytology: a prospective study within the Trial Of Management of Borderline and Other Low-grade Abnormal smears (TOMBOLA). *Psychooncology*, **22**, 368-80.

Singh JC, Kuohung V, Palefsky JM (2009). Efficacy of trichloroacetic acid in the treatment of anal intraepithelial neoplasia in HIV-positive and HIV-negative men who have sex with men. *J Acquir Immune Defic Syndr*, **52**, 474.

Zhu WY, Blauvelt A, Goldstein BA, et al (1992). Detection with the polymerase chain reaction of human papillomavirus DNA in condylomata acuminata treated in vitro with liquid nitrogen, trichloroacetic acid, and podophyllin. *J Am Acad Dermatol*, **26**, 710-4.