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The Efficacy of Immunocryosurgery with Combined 2% Niosomal Zinc Sulfate Suspension and Cryotherapy in the Treatment of *Verruca vulgaris*

Maryam Khalili [1], Roozbeh Mehrabadi [2], Saman Mohammadi [1], Abbas Pardakhty [3], Rezvan Amiri [1], Mahin Aflatoonian [1*]

1. Pathology and Stem Cell Research Center, Kerman University of Medical Sciences, Kerman, Iran
2. Kerman University of Medical Sciences, Kerman, Iran
3. Pharmaceutics Research Center, Neuropharmacology Institute, Kerman University of Medical Sciences, Kerman, Iran

ABSTRACT

Background: Topical zinc sulfate application has lower efficacy in comparison with intralesional usage, due to less penetration of ionic drug within the epidermis. Niosomes introduce new method of drug delivery with improved penetration and sustained release of medicaments within epidermis. On the other hand, combined cryotherapy with topical immunomodulators can increase the efficacy of cryotherapy with less recurrence rate. In this study, we compared the efficacy of 2% niosomal zinc sulfate suspension with cryotherapy versus combined conventional 2% zinc sulfate and cryotherapy in the treatment of *Verruca vulgaris*.

Methods: This is a triple-blind randomized clinical trial on 60 patients. Patients were randomized in 2 groups including combined 2% niosomal zinc sulfate suspension with cryotherapy versus combined 2% conventional zinc sulfate suspension combined with cryotherapy. The efficacy of treatment was evaluated during the treatment sessions until 12 weeks. Patients were followed for 3 months after the end of the treatment to evaluate recurrence rate. We used chi-square test to evaluate the efficacy of treatment and side effects. Mean number of treatment sessions was evaluated by t-test.

Results: Mean number of the treatment sessions for complete remission was 3.66±0.92 and 4.63±0.66, in niosomal group and conventional group, respectively (P=0.001). The rate of complete remission was higher at the 6^{th} and 8^{th} weeks of the treatment in niosomal group compared to conventional group (P=0.001).

Conclusion: This study demonstrated significant rapid remission of wart lesions treated with cryotherapy plus 2% niosomal zinc oxide suspension in comparison with cryotherapy plus 2% conventional zinc oxide suspension.

Keywords: Niosomes, Zinc sulfate, Cryotherapy, *Verruca vulgaris*

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***Correspondence:** Mahin Aflatoonian; Email: maaflatoonian@gmail.com

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Introduction

Verruca vulgaris is a common viral infection caused by human papilloma virus (HPV). In the majority of cases, spontaneous remission of the lesions occurs, but it may have a protracted duration of time. Furthermore, fear of spreading of lesions (due to autoinoculation and koebner phenomenon) as well as cosmetic concern, especially for the lesions in exposed areas may have profound impact on the quality of life of the patients. Currently, there is no definite cure for wart. Treatment modalities for wart are classified into the 4 groups based upon the mechanism of action, including destructive methods, immunotherapy, anti-proliferative and antiviral agents (1, 2).

Cryotherapy is one of the most common treatment methods for wart. Mechanism of action of this procedure is by direct destruction of HPV-affected keratinocytes through ice crystal formation and thermal shock, as well as indirect cell damage by immunologic stimulation. In previous studies, the efficacy of this treatment modality was estimated between 30% - 70% for hand lesions during 3 months treatment (3, 4).

Zinc sulfate is an immunomodulatory agent that has been used as oral, topical and intralesional formulations in the treatment of wart (5, 6). To date, there is little evidence regarding the efficacy of oral zinc sulfate on wart in individuals with normal serum level of zinc. On the other hand, oral zinc sulfate can result in gastrointestinal (GI) adverse effects (nausea, vomiting, and epigasteric pain) and noticeable number of patients do not tolerate it (5). Currently, topical zinc sulfate has been used in the treatment of wart in several studies, but because of low penetration of drug within the epidermis, it has showed lower efficacy in comparison with intralesional method (5). Studies show better penetration of topical zinc oxide with smaller particle size of approximately 0.1 micrometer (6). New method of drug delivery using niosomes with average size (nanometer to micrometer) has improved penetration and sustained release of medicaments within epidermis (7). Combination of cryotherapy with topical immunomodulators (immunocryosurgery) can increase the efficacy of cryotherapy and reduce recurrence rate as well (8). This study, evaluated the efficacy of 2% niosomal zinc sulfate suspension plus cryotherapy in comparison with 2% conventional zinc sulfate suspension plus cryotherapy.

Material and Methods

Niosome forming materials containing polyoxyethylene (2) cetyl ether (Brij® 52) and cholesterol were purchased from Sigma-Aldrich (USA) and Merck (Germany), respectively. Zinc sulfate ($ZnSO_4$) and polyoxyethylene glycol 6000 (PEG 6000) were also bought from Merck. All other chemicals and solvents were analytical grade and prepared from Merck Company.

Preparation of niosomes

Niosomal suspension was prepared by film hydration method, as described previously (9). Briefly, 300 μmol of surfactant (Brij 52) and cholesterol (70/30 molar percent of surfactant/cholesterol) were dissolved in chloroform in a 50 ml round-bottomed flask. The organic solvent evaporated at 70°C under reduced pressure, by using a rotary evaporator at 150 rpm. The resulting thin lipid film formed on the inner wall of the flask was then hydrated using 5 ml of zinc sulfate (2% w/v) and PEG 6000 (5%) in deionized water for 30 min at 70°C. PEG 6000 was used as viscosity modifier. Mean volume diameter of the prepared niosomes was 7.1±0.3 μm determined by laser light scattering method (Malvern, MasterSizer 2000E, UK). The encapsulation efficiency percent of $ZnSO_4$ was more than 53%, measured by flame atomic absorption method (Shimadzu, Japan).

Size analysis of niosomes

Small aliquots of niosomal suspensions were put in flow-through cell of a dynamic laser light scattering instrument (Malvern, MasterSizer 2000E, UK) and three runs were carried out for mean volume dimeter measurement and particle size distribution curve preparation.

Encapsulation efficiency percent

Niosomal suspension was centrifuged at 14000 rpm for 30 min to separate the entrapped and free Zn^{2+}. The niosomal pellet was dissolved in 1% Triton-X 100 solution to disrupt the lipid bilayers and release of encapsulated Zn^{2+}. Both free (supernatant) and entrapped (pellet) Zn^{2+} were measured by flame atomic absorption (Shimadzu, Japan) using calibration curve.

In vitro release of Zn^{2+} was not measured due to the proving of penetration enhancement effect of niosomes for Zn^{2+} through stratum corneum in previous studies (7, 10).

Preparation of traditional zinc sulfate solution

A cloudy solution of zinc sulfate (2% w/v) and PEG 6000 (5%) was prepared by magnetic stirring of precise weighted of two powders and dissolving in deionized water at room temperature.

Clinical study

This is a triple-blind randomized clinical trial on 60 patients from 2019 to 2020. Based on the results of the previous study (10) (rate of complete remission at 10th week was 53.3% in niosomal zinc sulfate plus cryotherapy compared with 12.3% in placebo with cryotherapy) with a statistical power of 80%, sample size was calculated as 44 patients and in order to increase the power of study, it was expanded to 60 patients. This proposal was approved with ethical code of IR.KMU.AH.REC.1397.135 and with clinical registry code of IRCT20190809044483N1.

Inclusion criterion was patients having *Verruca vulgaris* on hands referred to dermatologic clinic of Afzalipour hospital, Kerman, Iran. Exclusion criteria were age less than 12 years old, duration of the lesions more than 12 weeks, previous treatment of wart lesions, immunosuppression, pregnancy, lactation, history of allergy to zinc sulfate or other compounds of topical suspension and history of cold intolerance, cold urticaria or cryoglobulinemia. First, written informed consent was obtained from all of the participants. Then, demographic features including age and sex were recorded. Patients were randomized into the 2 groups including combined 2% niosomal zinc sulfate suspension plus cryotherapy and combined 2% conventional zinc sulfate suspension plus cryotherapy by simple randomization with Minitab 16 (Mini Tab Inc.). Patients were instructed to apply topical suspension on the surface of the lesions with cotton tip applicator after washing of the lesions with water and soap, twice daily for maximum 3 months or until the lesions fade, whichever occurred earlier. Topical suspension of drugs were put in similar bottles and the patients were unaware of the type of the topical drug.

Cryotherapy with liquid nitrogen was performed every other week in both treatment groups with a cotton tip applicator for 2- freeze-thaw cycle until formation of 2 millimeter white halo at the periphery of the lesion. Size of the lesions was measured by standard scaled ruler at the base-line and every 2 weeks for 12 weeks or until remission of the lesions, whichever occurred earlier. Side effects were recorded at each treatment session. Complete clearance was defined as complete disappearance of lesions. Furthermore, for evaluation of recurrence rate, patients were followed until 3 months after the end of the treatment. Efficacy of treatment was evaluated by reduction of size of the lesions during the treatment sessions. Both evaluator and analyzer were unaware of the treatment groups. For evaluation of the adherence of patients to the treatment, they were asked to return the empty bottle containing therapeutic drug.

Statistical analysis

Data were analyzed by SPSS 16 (software IBM, Armonk, NY, USA). Mean± standard deviation, frequency and relative frequency were used for descriptive data. The efficacy of treatment and side effects were evaluated by chi-square test. Mean number of treatment sessions were evaluated by t-test.

Results

Thirty patients were enrolled into the each of the two treatment groups and all of them completed the treatment course. There was no statistical difference between the two groups regarding demographic features (age and sex). Mean age of the patients was 26.53±8.25 and 28.0±9.52 years in niosomal and conventional groups, respectively (P=0.527). Sixty percent and 56.7% of the participants in niosomal and conventional groups were female, respectively (P=0.793). Mean size of the lesions at the base-line was not significantly different (P=0.16). Both treatment groups demonstrated significant reduction in mean size of the lesions during the treatment course (P<0.001). The difference between mean sizes of the lesions was significant at the 6th week of the treatment (P=0.001), but at the end of the treatment the difference was not significant (P=0.43) (Table 1). Mean number of sessions for complete remission was 3.66±0.92 and 4.63±0.66 in niosomal and conventional groups, respectively and the difference was statistically significant (P=0.001). The difference in the rate of complete remission between the two groups was statistically significant at the 6th (50% vs. 3.3%) and 8th (86.7% vs. 40%) week of treatment (P=0.001), but at the end of the treatment, the rate of complete remission was equal (93.3%) in both treatment groups (Table 2). Furthermore, in both groups, there was no difference between the

two genders regarding complete remission at the end of study (P>0.05). There was no significant difference between the two treatment groups regarding side effects (Table 3). Percentage of recurrence rate was 10% and 13.3% in niosomal and conventional groups, respectively (p=0.69).

Table 1. Size of the lesions during treatment sessions in the two studied groups

Treatment sessions	Mean size of the lesions (mm)		P. value
	Niosomal group	Conventional group	
Base line	8.36± 0.40	7.70±0.24	0.16
2nd week	7.73±0.42	7.26±0.24	0.34
4th week	4.63±0.38	5.36±0.22	0.10
6th week	1.83±0.42	3.06±0.22	0.01*
8th week	0.46±0.24	1.03±0.18	0.07
10th week	0.16±0.11	0.06±0.04	0.43
12th week	0.16±0.11	0.06±0.04	0.43
P. value	<0.001**	<0.001**	

* Statistically significant (P<0.05)
** Highly significant (P<0.001)

Table 2. The percentage of complete remission during treatment sessions in both groups

Treatment sessions	Niosomal group N[1] (%)[2]	Conventional group N[1] (%)[2]	P. value
4th week	1 (3.3)	0 (0)	0.313
6th week	15 (50)	1 (3.3)	0.001*
8th week	26 (86.7)	12 (40)	0.001*
10th week	28 (93.3)	28 (93.3)	1
12th week	28 (93.3)	28 (93.3)	1
P. value	<0.001**	<0.001**	

* Statistically significant (P<0.05)
** Highly significant (P<0.001)
[1] Number
[2]Percentage

Table 3. The frequency of the side effects in the two studied groups

Type of side effects	Niosomal group N[1] (%)[2]	Conventional group N[1] (%)[2]	P. value
Pain	25 (83.3)	24 (80)	0.73
Blister formation	13 (43.3)	14 (46.7)	0.79
Pruritus	12 (40)	6 (20)	0.09
Hypopigmentation	5 (16.7)	5 (16.7)	1
Hyperpigmentation	4 (13.3)	5 (16.7)	0.71

[1] Number
[2]Percentage

Discussion

Zinc deficiency may lead to depletion of the capacity of innate and adaptive immune responses against infections. Decrease in the ratio of T helper 1 (Th1) cells to Th2 cells, reduction in the serum levels of pro-inflammatory cytokines related to TH1 including interlukin-1 β (IL-1β), IL-2, IL-6, IL-8 and tumor necrosis factor- α (TNF-α) have been reported in zinc deficiency (10-12). Furthermore, zinc element is essential for the maximum action of inflammatory cells such as neutrophils, macrophages and natural killer cells against bacterial and viral infections (11, 12).

There are several studies evaluating the efficacy of oral zinc sulfate in the treatment of wart lesions. Mun and colleagues reported 50% of complete remission of wart lesions with oral zinc sulfate (10 mg/kg/day, maximum600 mg/day) for 2 months (13). Mahmoudi and

colleagues demonstrated that there is no significant difference between combined oral zinc sulfate plus cryotherapy versus placebo plus cryotherapy regarding complete clearance of common wart lesions of hand lesions (68.4% each group). Furthermore, high percentage of GI adverse effects (68.89%) led to discontinuation of treatment in 15.56% of the cases (14).

Intralesional zinc sulfate can induce inflammation and necrosis and have cytotoxic effects on contaminated basal keratinocytes by HPV virus. Furthermore, it stimulates immune response via increased levels of interferon (IFN)-α (15-19). Studies revealed high efficacy of intralesional zinc on various types of warts (15-19). Two studies in Egypt reported complete remission of 88%- 98% of common warts using 2% intralesional zinc sulfate during 3 sessions that is higher than the results of this study in the third session of treatment (50%) (15-16). Three other studies demonstrated complete remission of nearly 50-70% of recalcitrant plantar warts with intralesional 2% zinc sulfate with 4 biweekly sessions (17-19). Side effects such as pain of injection, erythema, edema, tenderness, post-inflammatory hyper pigmentation (PIH), hematoma, ulceration and scar formation have been reported with intralesional zinc (15-19).

Studies showed that topical application of zinc sulfate could accompany with lower percentage of adverse effects (10, 20-22). Khattar *et al.* reported 50% complete remission with combined topical 20% zinc oxide ointment and mechanical abrasion (emery stone) at the 12th week of the treatment that was significantly lower compared to the rate found in the present study (93.3%). Khattar *et al.* study has been performed on *Verruca vulgaris* and plane wart lesions, while this study carried out on only *Verruca vulgaris* lesions that are more recalcitrant to treatment relative to plane wart lesions (20). Higher complete remission in our study compared to Khattar *et al.* study [despite lower concentration of zinc (2%)], can be due to niosomal formulation of zinc sulfate that leads to enhanced absorption of zinc through thick stratum corneum and effects on infected basal keratinocytes.

Songsantiphap *et al*. revealed relatively low improvement (43.9%) with combined 15% topical zinc oxide (3 times daily for 4 weeks) on hand wart lesions due to low penetration of topical zinc (21). Sharquie *et al.* reported complete clearance of 11.1% and 4.5% with topical 10% zinc sulfate solution and 5% zinc oxide solution for common wart lesions, respectively. However, the efficacy of topical solution on the plane wart lesions was significantly higher (85.7% with 10% zinc sulfate solution and 42.8% with 5% zinc oxide solution). This demonstrates higher efficacy of topical zinc sulfate on thinner wart lesions due to better penetration of topical drugs (22). Farajzadeh *et al*. in another RCT compared the efficacy of combined 2% niosomal zinc formulation and cryotherapy versus combined placebo and cryotherapy and higher percentage of complete remission with significantly faster response rate was reported in the niosomal group compared to the placebo group (60% and 40%, respectively) (10). In the current study, in order to evaluate the efficacy of niosomal zinc formulation relative to conventional formulation, the efficacy of combined niosomal zinc sulfate and cryotherapy was compared with that of combined conventional zinc sulfate and cryotherapy. Significantly more rapid response rate was obtained in the niosomal group compared to the conventional group. Thus, niosomal formulation can provide faster resolution of wart lesions due to enhanced penetration of niosomal zinc sulfate through thickened stratum corneum and cell membrane of infected keratinocytes by HPV and higher intracellular concentration and bioavaibility compared to the conventional formulation (10, 7). Higher percentage of complete remission (93.3%) during lower mean number of the sessions was achieved in this study compared to Farajzadeh *et al.* study. Dissimilarities in type and size of wart lesions between the two studies can lead to difference in results (10).

Conclusion

This study demonstrates significantly rapid resolution of *Verruca vulgaris* in combination therapy of cryotherapy and 2% niosomal zinc oxide suspension, with acceptable side effects and relatively low recurrence rate. Therefore, this combination can be especially recommended in patients with low compliance and recalcitrant, recurrent and difficult-to-treat wart lesions as well.

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Conflict of interest

The authors declare no potential conflict of interest.

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