A Comparative Study of Glycolic Acid Versus Mixture of Trichloroacetic Acid and Glycolic Acid Peeling for Treatment of Three Benign Epidermal Lesions

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Abstract

**Background:** Chemical peeling is one of the dermatological treatments available for improvement of appearance of skin.

**Objectives:** To evaluate the clinical effects of focal Glycolic acid 70% (GA) peel versus focal mix of Trichloroacetic acid 50% (TCA) and GA 70% peel in epidermal lesions including seborrheic keratosis, actinic keratosis and solar lentigines.

**Methods:** An analysis was done on 60 adult patients with the previous epidermal lesions. They were divided into two groups, group 1 treated by focal GA 70% peel and group 2 treated by focal mix peeling. The peel was repeated every 2 weeks for 6 sessions.

**Results:** In group 1, 50% of seborrheic keratosis showed fair improvement (5/30) and 50% poor improvement (5/30). In actinic keratosis, there were 20% excellent (2/30), 50% good (5/30) and 30% fair improvement (3/30). In solar lentigines, there was 50% fair improvement (5/30) and 50% poor improvement (5/30). While, with group 2 there was 50% excellent (5/30), 40% good (4/30) and 10% fair (1/30) cure of seborrheic keratosis lesions. There was 30% excellent (3/30), 50% good (5/30) and 20% fair (2/30) cure of actinic keratosis lesions. There was 50% fair improvement (5/30) in number and color of solar lentigines and 50% poor improvement (5/30).

**Conclusion:** The focal mix peel method is an effective safer modality for treatment of some epidermal lesions than focal GA peeling.

Keywords: TCA; Glycolic acid; Epidermal lesion

Introduction

Chemical peeling is a procedure used either for cosmetic improvement of skin or treatment of some skin disorders. The chemical exfoliating agent is applied to the skin to destroy portions of the epidermis and/or the dermis with subsequent regeneration and rejuvenation of the tissues [1].

Alpha-Hydroxy Acids (AHA) like glycolic acid have been recognized as important adjunctive therapeutic elements in a variety of skin disorders including actinic keratosis, seborrhoeic keratosis, photoaging, hyperpigmentation and acne [2].

In addition, AHA application on the skin regulates the epidermal thickness, the hyaluronic acid, collagen I, procollagen I and mucopolysaccharides, also reduce the fragmentation of elastic fibers [3].

Glycolic acid (GA) application to actinic keratosis cause a superficial exfoliation by keratinocytes dyscohesion of pathologically sticky cells at the level of stratum granulosum in the epidermis. This allows the pathologic cells to become loose and shed and therefore helps to correct an abnormally thickened stratum corneum [4]. Glycolic acid has effect on the induction of cell cycle arrest and induction of apoptosis in human keratinocytes [5].

Apoptosis is a controlled form of cell death through the interaction of many proteins including Bcl2, Bcl2 gene is an antiapoptotic membrane associated molecule that resides in the nucleus envelope and mitochondria. The expression of Bcl2 prevents apoptotic cell death [6].

Trichloroacetic Acid (TCA) is a non-toxic chemical, which has been used to perform skin peels for over 20 years. When TCA is applied to the skin, it causes the top layers of cells to dry up and peel off over a period of several days to one week. When old skin is peeled off, it exposes a new layer of undamaged skin, which has a smoother texture and lighter color. The application of TCA causes coagulative necrosis of cells through extensive protein denaturation and resultant structural cell death [7].

Trichloroacetic Acid (TCA) Peel has been used to remove superficial blemishes such as post-acne scars, correct pigment problems (melisma) and smooth out fine surface wrinkles. Even been used to remove and prevent the growth of pre-cancerous lesions. TCA has been widely used as a peeling agent for the treatment of a number of hyperkeratotic lesions, including actinic keratosis, solar lentigines, and the signs of photoaging [7].

TCA peels are available in different concentrations. The stronger the solution is, the deeper it penetrates the layers of the skin. Superior

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peels are done with 10 to 35% TCA; medium-depth peels are done with 40 to 50% TCA; a deep peel results from the use of greater than 50% TCA. The depth of tissue coagulative necrosis correlates with the concentration of TCA. Medium level concentrations of 35% to 50% necrosis penetrate between the superficial papillary and midreticular dermis. Over the subsequent 5 to 7 days, the epidermis and superficial dermis slough, carrying away cytologically atypical keratinocytes and structurally compromised dermal connective tissue. As the wound heals by second intention, it is repopulated by deep follicular epithelium and newly generated connective tissue thus, the skin is rejuvenated both clinically and histologically [4].

Benign pigmented lesions, including seborrheic keratosis, solar lentigines, melasma, and freckles, are focally distributed. In order to maximize the effects of TCA and to overcome complications such as scarring, hyperpigmentation, and hypopigmentation, focal application of higher TCA concentrations on the pigmented areas only by using a sharpened wooden applicator. This method has the advantages of more rapid healing time and a lower complication [8].

Aim of the Work

The aim of this study is to compare the effect of focal mix peeling, (glycolic acid and trichloroacetic acid) versus glycolic acid peeling alone in some epidermal skin diseases.

Patients and Methods

Study population

Sixty adult patients (45 males and 15 females) with seborrheic keratosis, actinic keratosis, and solar lentigines (20 patients for each disease) were selected for this study from Dermatological Outpatient Clinic of Zagazig University Hospitals at a period from January 2012 to January 2013. Patients had Fitzpatrick skin types IV–V. The patient ages group ranged from 35 to 70 years (mean age 52.5 years).

A full history was taken from each case including personal history, present history and family history. History of drug intake, irradiation, chemotherapy, cosmetic surgery, sun exposure or previous treatment was also taken. The inclusion criterion for patients requires a clinical diagnosis of seborrheic keratosis, actinic keratosis and solar lentigines.

The exclusion criteria included topical treatment with bleaching agents within the last 2 months and any history of prior cosmetic surgery, previous chemical peeling or dermabration, hypersensitivity to glycolic acid or TCA, keloidal tendencies and systemic tretinoin 1 year before the study. Cases of actinic keratosis with bleeding, induration, rapid growth, or pain were be excluded and directed to do excisional biopsy to exclude squamous cell carcinoma (SCC) [9]. The study was approved by the local ethical committee and all the patients gave written informed consent before enrolment.

Study design

Color photographs were taken before treatment, at follow-up visits (every 2 weeks) and 2 months after the treatment period for each patient, using a Nikon D70 camera. Photographs were evaluated by physicians blinded to type of treatment and the results were analyzed. Patients were divided into two groups each group has the same number of patients with each skin lesion; taking into consideration age and sex matching:

Group 1: 30 patients will be treated with 70% glycolic acid peels (10 patients for each disease).

Group 2: 30 patients will be treated with mix of TCA 50% and 70% glycolic acid peels (10 patients for each disease).

Chemical agents:

1. Glycolic acid 70% (Merck Schuchardt OHG).
2. TCA (SDFCL fine-chem limited India).

TCA 50% was made to order by a local pharmacy.

Treatment procedure:

Skin preparation for peeling: Patients were advised to use retinoic acid cream (0.025%) at bed time for two weeks prior to peeling. During the procedure all the patients were advised to use sunscreen lotions during day time and emollient at bed time.

Focal Application of unbuffered GA 70% to group 1: We followed the technique described by Chun et al. [7]. The face was cleaned by alcohol for defatting. The GA was applied focally with cotton tipped applicator to skin lesions, guarding against direct eye contact with the acid solution. The exposure time was carefully monitored, it ranged from 2-5 minutes depending on the erythema observed. When erythema was observed clinically, it indicated epidermal depth of involvement. Dermal penetration may appear as a mild blanch. After the appropriate exposure time, the GA was neutralized by water. Sun screen was applied and the patient was allowed to leave with instruction to avoid sun exposure. Patients with severe erythema were given mild topical corticosteroids creams. The peel was repeated every two weeks for six sessions.

Application of mix of TCA 50% and glycolic 70% to group 2: The face was cleaned by alcohol for defatting. The mix was focally applied rapidly with cotton tipped applicator to all lesions, guarding against direct eye contact with the acid solution. The exposure time was carefully monitored, until a uniform white frost was achieved and then neutralized with tab water. It ranged from 2-5 minutes depending on the indication and the clinical response observed. When erythema and light frost was observed clinically, it indicated epidermal depth of involvement. Dermal penetration may appear as a gray frost. After the appropriate exposure time, the mix was removed [10]. Sun screen was applied and the patient was allowed to leave with instruction to avoid sun exposure. Patients with severe erythema were given mild topical corticosteroids creams the peel was repeated every two weeks for six sessions.

Assessment of clinical response: The two groups were photographed and assessed clinically every session and 2 months post treatment. The pre-treatment and 2-month post-treatment photographs were evaluated and analyzed for clinical response. The clinical response were graded using a 4-point grading scale determined by Chun [7] and Todd et al. [11], as follows: excellent, with an improvement greater than 70%: good, with an improvement of 50% to 70%: fair, with an improvement of 30% to 50%; and poor, with an improvement less than 30%.

Patient satisfaction rates were recorded from interviews conducted 2 months after the end of treatment. Patients were blinded to treatment type. The physicians evaluated complications such as erythema, edema, permanent hyperpigmentation, hypopigmentation herpes simplex flare-up, scarring, or keloid.

Skin punch biopsies were taken before and at the end of treatment. All biopsies were fixed in paraffin and sectioned into 5 µm thick sections. These sections were subjected to histopathological (H&E) and immunohistochemical examination.
**Immunohistochemical examination:** To evaluate Bcl$_2$ expression in the keratinocytes, the sections were stained by monoclonal mouse antihuman Bcl$_2$ onchoprotein (N1587; DAKO Corporation) using detection system (K0673, LSAB2 system; DAKO Corporation) according to the manufacturer’s instruction [12].

**Results**

All 60 patients (45 males and 15 females) completed the study and evaluated by photography and clinical assessment.

**Clinical improvement:**

**Group 1:** There was 50% fair improvement (5/30) and 50% poor improvement (5/30) of seborrheic keratosis after 6 sessions of focal 70% glycolic acid (Figure 1a and 1b). There was 20% excellent (2/30), 50% good (5/30) and 30% fair (3/30) improvement in rough texture, fine wrinkling, number of actinic keratosis after 70% GA sessions (Figure 1c and 1d). And there was 50% fair (5/30) and 50% poor (5/30) improvement of solar lentigines after 70% GA sessions (Figure 1e and 1f) (Table 1).

Glycolic acid peels moderately smoothens rough skin, improves the texture of sun-damaged skin and decrease pigment problems in actinic keratosis.

**Group 2:** There was a 50% excellent (5/30), 40% good (4/30) and 10% fair (1/30) cure of seborrheic keratosis lesions from face focally treated by mix of TCA 50% and Glycolic acid 70% (Figure 2a and 2b). There was 30% excellent (3/30), 50% good (5/30) and 20% fair (2/30) decrease in rough texture, fine wrinkling, and number of actinic keratosis lesions after treatment by mix of TCA and GA sessions (Figure 2c and 2d).

There was significant improvement in GA treated actinic keratosis. While insignificant improvement were noticed in GA treated seborrheic keratosis and solar lentigines.

**Table 1:** Focal Glycolic Acid 70% after completion of treatment.

The figures in parentheses are percentage.

<table>
<thead>
<tr>
<th>Excellent</th>
<th>Seborrheic. keratosis</th>
<th>Actinic keratosis</th>
<th>Solar lentigienes</th>
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<tbody>
<tr>
<td>2 (7%)</td>
<td></td>
<td>2 (20%)</td>
<td></td>
</tr>
<tr>
<td>Good</td>
<td></td>
<td>5 (50%)</td>
<td></td>
</tr>
<tr>
<td>Fair</td>
<td>13 (43%)</td>
<td>5 (50%)</td>
<td>3 (30%)</td>
</tr>
<tr>
<td>Poor</td>
<td>10 (33%)</td>
<td>5 (50%)</td>
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</tbody>
</table>

Total 30 10 10 10

**Figure 1:** Clinical cases of group 1. Seborrheic keratosis patient before GA70% peel (1a), show poor improvement after GA70% peels (1b). Actinic keratosis patient before GA 70% peel (1c), show good improvement after GA 70% peel (1d). Solar lentigines patient before GA 70% peel (1e), show fair improvement after GA70% peel (f).

**Figure 2:** Clinical cases of group 2. Seborrheic keratosis patient before mix peel (2a), show excellent improvement after peel (2b). Actinic keratosis patient before mix peel (2c), show good improvement after peel (2d). Solar lentigines patient before mix peel (2e), show fair improvement after peel (2f).
number and color of solar lentigines lesions after focal application of mix of TCA and GA (Figure 2e and 2f) (Table 2).

There was significant difference between the two groups. In 1st group, the improvement was poor in seborrheic keratosis, moderate in actinic keratosis.

In 2nd group 90% of seborrheic keratosis was cured. Skin texture and lesions of actinic keratosis were improved in 80% of patients in 2nd group. Both groups showed poor clinical improvement in solar lentigines lesions.

So there were significant improvement in focal mix treated seborrheic keratosis and actinic keratosis. But there was insignificant improvement in focal mix treated solar lentigines.

Adverse events:

Adverse events were reported for both chemical peels, with the highest frequency reported within the first two treatment visits.

In group 1, three patients reported slight erythema and two reported slight edema.

In group 2 two patients reported mild erythema and two patients showed slight edema.

Both chemical peels yielded similar adverse events with the most common adverse events being erythema and edema. The side effects were more with high number of skin lesions that indicate multiple focal applications of chemical peeling agents. Erythema and edema usually faded away within 4 days of topical antibiotic cream.

Light microscopic examination:

**Group 1:** Examination of Hx&E stained sections of Seborrheic keratosis before focal GA peeling showed hyperkeratosis, acanthosis and papillomatosis with a large horn cyst. Histopathological examination of GA treated skin reveals no change in histopathological picture (Figure 3a and 3b).

Examination of Hx&E stained sections taken from actinic keratosis patients before focal GA peeling revealed hyperkeratosis, acanthosis and atypical epidermal cells. After GA peeling, there was moderate reduction in the number of the epidermal cells with slight increased epidermal thickness (Figure 3c and 3d).

Histopathological examination [Hx&E stain] of solar lentigines before focal GA peeling revealed moderate elongation of the rete ridges with increased concentration of melanocytes and melanin pigments in the basal cell layer. GA treated skin showed normal epidermis with decrease of melanin pigments in the basal cell layer (Figure 3e and 3f).

**Group 2:** Histopathological examination of (Hx&E) in Seborrheic keratosis before focal mix TCA and GA peeling showed hyperkeratosis, acanthosis and papillomatosis with a large horn cyst. Histopathological examination of treated skin with mix peeling revealed normal epidermis with increased epidermal thickness (Figure 4a and 4b).

Examination of Hx&E stained sections taken from patients with actinic keratosis before focal mix peeling showed hyperkeratosis, acanthosis and atypical epidermal cells. After mix peeling biopsies showed normal epidermal cells with increased epidermal thickness (Figure 4c and 4d). Hx&E stained section of solar lentigines before mix peeling showed moderate elongation of the rete ridges with increased concentration of melanocytes and melanin pigments in the basal cell layer. Histopathological examination of treated skin with focal mix of TCA and GA showed normal epidermis with decrease of melanin pigments in the basal cell layer (Figure 4e and 4f).

**Immunohistochemical examination:**

**Group 1:** Seborrheic keratosis before peeling revealed increased number of Bcl-2 positive cells in basal layers of the lesion. Seborrheic...
keratosis after GA peeling showed the same Bcl_2 positive cells in the basal and suprabasal cell layers (Figure 5a and 5b).

Actinic keratosis before peeling showed increased number of Bcl_2 positive epidermal cells, and after GA peeling showed very few Bcl_2 positive basal epidermal cells (Figure 5c and d).

Solar lentigines before peeling showed increased number of Bcl_2 positive cells in the basal and suprabasal cell layers. Examination of GA treated skin showed few Bcl_2 positive epidermal cells (Figure 5e and f).

**Group 2: Seborrheic keratosis before mix TCA and GA peeling revealed increased in number of Bcl_2 positive cells in basal and suprabasal layers of the lesion.** Seborrheic keratosis treated by focal mix peeling showed marked reduction in the number of Bcl_2 positive cells which become limited to the basal cell layer (Figure 6a and 6b). Immunohistochemical examination of actinic keratosis before peeling showed increased number of Bcl_2 positive epidermal cells, and after focal mix peeling showed very few Bcl_2 positive cells limited to the basal cell layer (Figure 6c and 6d). Solar lentigines before peeling showed increased number of Bcl_2 positive cells in the basal and suprabasal cell layers. Examination of skin after focal mix peeling showed moderate Bcl_2 positive cells in the basal cell layer (Figure 6e and 6f). Focal mix peels demonstrated more significant decrease in Bcl_2 expression than in focal GA peeling in cases of seborrheic keratosis. Otherwise decrease Bcl_2 expression in actinic keratosis and solar lentigines were the same in both groups.

**Discussion**

Alpha-hydroxy acids (AHA), such as glycolic acid (GA), have been used as therapeutic agents (for epidermal skin diseases) for more than a quarter of a century. It is believed that these AHA agents remodel the epidermis and accelerate desquamation, thus exerting their therapeutic effects in cutaneous diseases like seborrheic keratosis, actinic keratosis and ephlides [13].

**Figure 4**: Haematoxylin and eosin stained sections of group 2. Seborrheic keratosis before peeling show hyperkeratosis, acanthosis and papillomatosis with a large horn cyst (4a), examination of skin after mix peeling reveal normal epidermis with increased epidermal thickness (4b). Actinic keratosis before peeling show hyperkeratosis, acanthosis and atypical epidermal cells (4c) while, after mix peeling skin show normal epidermal cells with increased epidermal thickness (4d). Solar lentigines before peeling show moderate elongation of the rete ridges with increased concentration of melanocytes and melanin pigments in the basal cell layer (4e), while skin after mix peeling show normal epidermis with decrease of melanin pigments in the basal cell layer (4f). (bar scale, 50 µm).

**Figure 5**: Immunoperoxidase Bcl_2 stained sections of group 1. Seborrheic keratosis before peeling reveal increased number of Bcl_2 positive cells in basal layers of the lesion (5a), while, after GA peeling show the same number of positive cells in the basal and suprabasal cell layers (5b). Actinic keratosis before peeling show increased number of Bcl_2 positive epidermal cells (5c), while, after GA peeling show few positive basal epidermal cells (5d). Solar lentigines before peeling show increased number of Bcl_2 positive cells in the basal and suprabasal cell layers (5e), while GA treated skin show few positive epidermal cells (5f) (bar scale, 50 µm).
lesions. A good clinical response was achieved without significant complications at the treatment sites. This technique can avoid scarring and reduce the risk of developing hypopigmentation by sparing the adjacent normal skin and adnexal structures. At our current study, focal chemical peeling technique is applied to skin lesions (seborrheic keratosis, actinic keratosis and solar lentigines) using GA alone and mix of TCA and GA. Focal peeling treatment applied comparing GA (70%) alone and with a mix of TCA (50%), GA (70%). The focal mix peeling was done to increase the efficacy and decrease side effects of peeling. The clinical results of focal GA peel (group 1) showed poor improvement of seborrheic keratosis lesions, moderate improvement of actinic keratosis lesions. In solar lentigines there is poor clinical improvement after focal GA peel.

In group 2, there was marked decrease in number and color of seborrheic keratosis in focal mix peel. There was a marked improvement in skin texture, fine wrinkling in actinic keratosis in focal mix peel. There was poor clinical improvement in solar lentigines observed in mix peeling. In spite of there is moderate improvement in histopathology and immunohistochemistry. This may be due to post inflammatory erythema that followed by hyperpigmentation.

Yamamoto et al. [3] found that treatment with glycolic acid can cause improvements in both the epidermal and dermal components and support the usefulness of AHA for rejuvenating photo-damaged skin. Furukawa and Yamamoto [13] revealed that glycolic acid of the appropriate pH increased the thickness of the treated skin and made the melanin pigments in the basal layer less prominent.

It was demonstrated that higher concentration of TCA (>50%) is more likely to be more effective in treatment but with more side effects specially scarring. It is for this reason the combination products as TCA 35% and Glycolic acid 70% formula have been found to be equally effective in producing the level of damage of TCA>50% without the risk of side effects. It was proved that the combination of TCA 35% and glycolic acid 70% in actinic keratosis is an alternative treatment to sequential chemical exfoliation with topical application of 5- fluorouracil [18,19].

Glycolic acid 70% along with a 35% TCA formula results in a medium-depth chemical peel. GA caused detachment of keratinocytes and epidermolysis when applied prior to TCA, thus allowing deeper and more even penetration of the TCA solution [20].

In the current study, histopathological results of H/E stained biopsies after focal GA (group 1) treatment revealed that Seborrheic keratosis showed no improvement. Actinic keratosis after GA peeling showed moderately normal epidermal cells and increased epidermal thickness. Solar lentigines after GA peeling showed mild epidermal improvement with dispersion of melanin pigments in keratinocytes especially those of the basal cell layer.

In group 2, histopathological results of H/E stained biopsies after focal mix of TCA and GA peeling; revealed in seborrheic keratosis nearly normal epidermis with increased epidermal thickness. Actinic keratosis after focal mix peeling showed normal epidermal cells with increased epidermal thickness. Solar lentigines cases after focal mix peeling showed normal epidermis with dispersion of melanin pigments in keratinocytes especially those of the basal cell layer.

From these findings we concluded that focal TCA and GA mix peeling showed more improvement in histopathological results (both increase the epidermal thickness with dispersion of melanin pigments in keratinocytes especially those of the basal cell layer) than in focal GA peeling alone in seborrheic keratosis, actinic keratosis and solar lentigines.
Rabe et al. [21] mentioned that, focal GA peel improves the skin texture and reduces fine wrinkling and number of actinic keratoses lesions. They also found that GA peel cause thinning of the stratum corneum, as well as increase dermal collagen thickness.

Erbel et al. [22] reported that glycolic acid concentrations of 50–70% decrease melanin deposits in the epidermis as well as acceleration of desquamation.

Yamamoto et al. [3] mentioned that GA in mix peeling had effects to increase the epidermal thickness, the hyaluronic acid, collagen I, procollagen I, and mucopolysaccharides, but to reduce the fragmentation of elastic fibers.

Apoptosis is a particular process that leads to the programmed cell death. Bcl-2 is a novel proto-oncogene that inhibits apoptosis rather than promoting proliferation [23,24]. The physiologic role of Bcl-2 protein is to prevent or delay programmed cell death during proliferation. The distribution of the Bcl-2 in normal photo exposed skin suggests that expression is limited exclusively to the basal cell layer which has been correlated to the formation of the epidermis and adnexal structures. Investigation of Bcl-2 expression revealed that it is not strictly correlated with proliferative rate of keratinocyte but more closely correlates with the differentiation status of the cell. So, immunoreactivity is not found in suprabasal and upper layers of epidermis [25].

In the current study, we evaluated the possible antiproliferative and apoptotic effects of GA and mix of TCA and GA peeling. Bcl-2 protein expression in the keratinocytes was decreased after treatment by GA alone in actinic keratosis and solar lentigines. But with no decrease of Bcl-2 expression in cases of seborrheic keratosis while Mix TCA and GA peel decrease the expression of Bcl-2 in the keratinocytes in all three diseases.

So we suppose that GA and mix peeling had antiproliferative and apoptotic effect (decrease Bcl-2 expression). But in GA treated seborrheic keratosis there was no clinical improvement or decrease in expression of Bcl-2. So it may be a correlation between clinical improvement and decrease expression of Bcl-2. These findings denoting a possible apoptotic effect of TCA peeling.

Our results were supported by Abdel-Daim et al. [26] who stated that repetitive chemical peeling using 35% glycolic acid enhance apoptosis which in turn remove photodamaged keratinocytes.

Hsiao et al. [5] demonstrated that GA has antiproliferative effect. GA induced apoptosis are established through multiple molecular pathways including caspase-dependent and caspase-independent pathways. Also Rendl et al. [27] found that topical GA increased the number of apoptotic cells and increased secretion of vascular endothelial growth factor in human epidermal equivalents.

In summary, both glycolic and TCA chemical peels are safe and well-tolerated procedures. Some patients experienced mild adverse events that were well tolerated. Focal mix peeling was more effective in treatment of seborrheic keratosis, actinic keratosis clinically and histopathologically while there is no clinical improvement in cases of solar lentigines.

References