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LETTER TO THE EDITOR

Chemical peeling and microdermabrasion of the skin: Comparative immunohistological and ultrastructural studies

KEYWORDS
Skin; Peels; Microdermabrasion

Comparative immunohistological and ultrastructural studies were performed on specimens taken before and after the application of chemical peels (Jessner's solution and glycolic acid) and microdermabrasion to the clinically normal facial skin of the post-auricular region. Skin treated with chemical peels showed the immunohistological features of wound healing. Beside these, ultrastructural changes of cell injury were observed. These morphological changes were mild following microdermabrasion.

Chemical facial peelings induce superficial shock to the cells and necrosis of the skin at variable depths. Jessner's solution represents a combination of resorcinol, salicylic and lactic acids in a sufficient quantity of ethanol [1]. Glycolic acid is a form of alpha hydroxyacids that enhances the desquamation of the stratum corneum. Microdermabrasion involves the physical abrading of the skin with a high-pressure flow of crystals [2]. The application of chemical peelings and microdermabrasion is associated with morphological changes reminiscent of wound healing activities in the skin [3]. This study tries to address these issues.

Forty-five healthy adult patients (age range: 14—40 years, including 6 males and 39 females) with cosmetic problems (acne, melasma and freckles) participated in this study. The participants (15 cases in each group) had undergone facial chemical peelings (glycolic acid: 70% concentration, Glyco-70 Isis pharma, France and Jessner's solution: combination of resorcinol 14 mg, salicylic acid 14 mg and lactic acid (85%) 14 ml in a sufficient quantity of ethanol 95% to make 100 cm^3 of solution) and microdermabrasion (Reviderm skin peeler professional, Germany) weekly for 2 months. Punch biopsies were obtained from the clinically normal skin of the right post-auricular region 1-week before treatment. Other punch skin biopsies were obtained 1 week after the end of the treatments from the left post-auricular area. This region was treated in a similar way to the adjacent lesional skin of the face. The specimens obtained before the start of treatment served as the control group while sample obtained at the end of treatment represent the treated groups (Jessner's solution treated, glycolic acid treated and microdermabrasion treated groups).

The epidermal thickness [4], inflammatory cells, fibroblasts [1] and amount of collagen [5] were evaluated following other groups. Immunohistochemical evaluation of microvessel density and cell proliferation was carried out [5]. It was performed for CD34 and CD31 (microvessel density) and PCNA (cell proliferation index). A monoclonal anti-CD34 (Anti-CD34 monoclonal QBEnd/10; Novocastro, UK) was used at a 1/100 dilution for 30 min at room temperature) and anti-PCNA antibodies (clone PC-10; DAKO, Denmark) and antibodies for CD31 (JC/70A, Denmark). Using CD34 and CD31 stained slides; microvessel density was estimated in the skin as described previously [5]. For ultrastructural studies (transmission electron microscopy), skin specimens were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer at 4 °C and pH 7.2 for 24 h and then processed following other groups [1].

Skin treated with chemical peels showed significantly increased total epidermal thickness, dermal vascular ectasia, patchy, high counts of inflammatory cells and densely arranged collagen fibers compared to untreated skin. Skin treated with microdermabrasion showed similar but mild changes, i.e. mild increase in the epidermal thickness and fibroblast count, dermal vascular ectasia, patchy and perivascular inflammation and densely arranged thick collagen fibers. The dermal microvessel density increased following treatment with glycolic acid and Jessner's solution compared to untreated skin. The microves-

Table 1 Morphological changes in the matched untreated skin and Jessner's solution, glycolic acid and microdermabrasion-treated skin samples

Morphological changes	Untreated skin	Glycolic acid-treated skin	Jessner's solution-treated skin	Microdermabrasion-treated skin
A-Histological changes				
Thickness of the stratum corneum	15.50 {1.44}	45.00 {5.62}	65.00 {2.88}	30.00 {4.47}
Thickness of the cellular epidermis	75.00 {2.88}	166.66 {4.21}	206.66 {16.86}	116.00 {4.00}
Total thickness of the epidermis	87.50 {11.90}	211.66 {18.64}	271.66 {25.12}	146.00 {14.6}
Thickness of the rete ridges	75.00 {5.00}	130.00 {6.83}	137.14 {8.08}	130.00 {5.77}
Counts of the fibroblasts	11.60 {0.97}	41.40 {3.20}	30.60 {2.67}	15.66 {0.66}
Density of the collagen bundles	1.11 {0.11}	2.90 {0.10}	2.00 {0.23}	1.69 {0.13}
Counts of the inflammatory cells	4.60 {0.60}	8.0 {1. 52}	10 {0.57}	10 {1.15}
B-Immunohistological changes				
Cell proliferation index (PCNA)	3.53 {0.08}	3.63 {0.12}	3.54 {0.15}	3.67 {0.09}
Microvessel density (CD34)	1.56 {0.17}	1.90 {0.09}	1.81 {0.12}	2.90 {0.21}
Microvessel density (CD31)	1.39 {0.12}	1.63 {0.27}	1.54 {0.15}	1.90 {0.09}
B-Ultrastructural changes				
Features of cell damage				
Loss of the desmosomes	Absent	+	+	–
Widening of the intercellular spaces	Absent	+	+	Occasional
Cytoplasmic vacuolations	Absent	+	+	Occasional and small ones
Fragmentation of the keratohyaline granules	Absent	+	+	–
Collagen bundles with abnormal periodicity	Absent	+	+	–
Collagen bundles with loss of the periodicity	Absent	+	+	–
Paucity of the desmosomes and tonofilaments	Absent	+	+	–
Fibroblasts with fragmentation and apoptosis	Absent	+	+	–
Features of apoptosis				
Cytoplasmic vacuolization	Absent	+	+	Occasional and small ones
Reduced nuclear and cytoplasmic areas	Absent	+	+	–
Condensation of the nuclear chromatin	Absent	+	+	–
Features of increased metabolic activity				
Irregularity of the nuclear membrane	Absent	+	+	–
Large Langerhans cells	Absent	Numerous	Numerous	Numerous

There were increased thickness of the epidermis and density of the collagen fibers, angiogenesis, prominent fibroblasts and high dermal lymphohistiocytic cell infiltrate in the treated skin (chemical peels and microdermabrasion) as opposed to untreated one. The differences in the thickness of the epidermis between the treated and untreated skin reached the levels of statistical significance ($p < 0.003$, <0.229 and 0.1539 for Jessner's solution, glycolic acid and microdermabrasion-treated skin, respectively). The differences in the thickness of cellular epidermis and numbers of fibroblasts between chemical peelings (both Jessner's solution and glycolic acid) and microdermabrasion were statistically significant ($p < 0.001$ and <0.006, respectively). Collagen deposition was statistically significantly high in treated skin compared to untreated skin ($p < 0.0036$, <0.000, and <0.0052 for Jessner's solution, glycolic acid and microdermabrasion-treated skin, respectively). Collagen deposition was statistically significantly high in glycolic acid-treated skin compared to microdermabrasion ($p < 0.001$). A statistically significantly high counts of inflammatory cell infiltrate was seen in the upper- and mid-dermis of the treated skin as opposed to untreated one ($p < 0.0191$ and 0.022 for glycolic acid and microdermabrasion treated skin, respectively). Microvessel density was higher in treated vs. untreated skin. The differences between untreated skin and skin treated with microdermabrasion in the CD34 reached the level of statistical significance ($p < 0.000$). The microvessel number was high following microdermabrasion compared to chemical peelings. The cell proliferation index was slightly prominent in the treated skin as compared to untreated skin. Ultrastructurally, skin treated with chemical peels showed features of cell injury. These ultrastructural changes were mild following microdermabrasion.

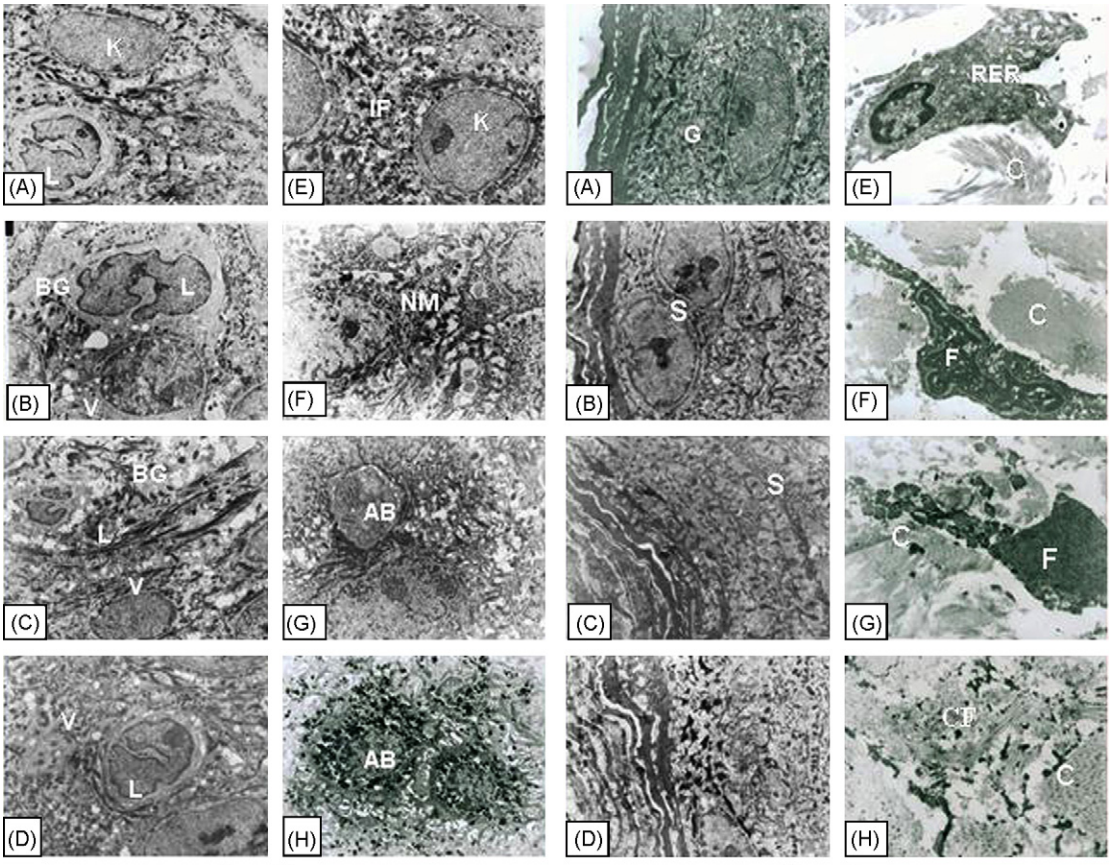

Fig. 1 Morphological changes in the matched untreated skin and Jessner's solution, glycolic acid and microdermabrasion-treated skin samples. Panel 1-I: Ultrastructural changes in the matched untreated skin and Jessner's solution, glycolic acid and microdermabrasion-treated skin samples. (A) Untreated skin: the basal cell keratinocytes were columnar in shape (K) and were seen resting on irregular basal lamina. Langerhans cell (L) were seen among the basal cell keratinocytes. They had characteristic reniform, convoluted nuclei and cytoplasms lacking tonofilaments (5000×); (B) Jessner's solution-treated skin: the keratinocytes (spinous layer) showed cytoplasmic vacuolations (V). Large Langerhans cells (L) with its characteristic Birebick granules (BG) were seen amid the keratinocytes of the spinous layer (5000×); (C) glycolic acid-treated skin: the keratinocytes (spinous and granular layers) displayed cytoplasmic vacuolations (V). Large Langerhans cells (L) with its characteristic Birebick granules (BG) were seen amid the keratinocytes of the granular layer (5000×); (D) microdermabrasion-treated skin: the keratinocytes (spinous layers) displayed small cytoplasmic vacuolations (V). Langerhans cell was also seen (L) (5000×); (E) untreated skin: the keratinocytes (spinous) showing numerous cytoplasmic tonofilaments (IF) and frequent desmosomes joining the cells together (5000×); (F) Jessner's solution-treated skin: the keratinocytes (spinous layer) had highly irregular nuclear membrane (NM) with widening of the intercellular spaces and paucity of the tonofilaments and desmosomes (5000×); (G) glycolic acid-treated skin: the keratinocytes (spinous layer) had irregular nuclear membrane, with widening of the intercellular spaces. An apoptotic body was also seen (AB) among the keratinocytes (5000×); (H) microdermabrasion-treated skin: the keratinocytes (upper part of the spinous layer) with two shrunken apoptotic cells (AB) having convoluted dark nuclei, electron dense cytoplasm and melanin pigments (5000×). Panel 1-II: Ultrastructural changes following chemical peeling and microdermabrasion. (A) Untreated skin: the keratinocytes (upper part of the spinous and granular layers) showing some keratohyaline granules in the cells of the granular layer (G) (5000×); (B) Jessner's solution-treated skin: the keratinocytes (upper part of the spinous and granular layers) showing paucity of the keratohyaline granules and widening of the intercellular spaces (S) (5000×); (C) glycolic acid-treated skin: the keratinocytes (upper part of the spinous and granular layers) with widening of the intercellular spaces (S) (5000×); (D) microdermabrasion-treated skin: the keratinocytes (spinous, granular and horny layers) with normal appearance (similar to untreated skin) (5000×); (E) untreated skin: the reticular dermis with a large branched fibroblast having numerous rough endoplasmic reticulum cisternae (RER) and bundles of collagen fibers (C) maintaining their normal periodicity (10,000×); (F) Jessner's solution-treated skin: the reticular dermis with a fibroblast (F) in the early stage of apoptosis and collagen bundles (C) having abnormal periodicity (10,000×); (G) Jessner's solution-treated skin: the reticular dermis showing a markedly fragmented (apoptotic) fibroblast (F). The collagen bundles displayed complete loss of the normal periodicity (10,000×); (H) Jessner's solution-treated skin: the reticular dermis displaying numerous cellular fragments (CF) and collagen bundles either with abnormal periodicity (C) or complete loss of periodicity (10,000×).

sel density was high following microdermabrasion compared to chemical peelings. The proliferation index was similar following chemical peeling and microdermabrasion-treated skin (Table 1).

Skin treated with chemical peels (glycolic acid and Jessner's solution) showed features of cell injury (widening of the intercellular spaces, cytoplasmic degenerative vacuoles, paucity of desmosomes and collagen bundles with abnormal periodicity) and increased numbers of Langerhan's cells compared to untreated skin. Skin treated with microdermabrasion showed minimal changes indicative of cell injury compared to skin treated with chemical peelings (Table 1 and Fig. 1).

Consistent with previous studies, this investigation reports features of wound healing in the skin treated with chemical peels [1,6]. Inan et al. [1] examined the effects of glycolic acid on rat skin and reported a significant increase in the thickness of the epidermis, prominence of the fibroblasts and increase in the amount of the dermal collagen. The increased density and thickness of the collagen fibers in our series may be reasoned to the ability of the glycolic acid to accelerate collagen synthesis by fibroblasts. Structurally, hydroxy acids (glycolic acid) can increase the thickness of the skin [7] by increasing the synthesis of glycosaminoglycans and collagen fibers [6]. Glycolic acid may also modulates matrix degradation and collagen synthesis through keratinocyte-released cytokines [6].

The histological changes (indicative of wound healing) following microdermabrasion concur with previous reports [2,8]. Following microdermabrasion, Coimbra et al. [8] reported vascular ectasia, dense mononuclear inflammatory cells infiltrate, an increase in the epidermal thickness and organized collagen in the treated skin relative to the control group. These findings suggest that the repetitive intraepidermal and dermal injuries associated with microdermabrasion may stimulate fibroblast proliferation and collagen production, leading to new collagen deposition in the dermis [9].

The presence of high microvessel density in the treated skin concurs with previous studies [10] and suggests that skin peeling can stimulate inflammatory angiogenesis. The presence of dense perivascular and patchy infiltrates of lymphohistiocytic cells in the treated skin suggest that both chemical and physical peelings (skin wounding) involve cellular migration and inflammation. The development of ultrastructural changes indicative of cell damage is in agreement with some studies [1]. Following glycolic acid treatment, Inan et al. [1] found cytoplasmic vacuolization in epidermal keratinocytes and intercellular dysjunctions. The histological and ultrastructural changes associated with microdermabrasion are consistent with a more advanced state of wound healing compared to those treated by chemical peels [2]. These findings may be reasoned to the fact that microdermabrasion is a very superficial abrasive method in which deeper layers are unaffected.

Our study demonstrates variations in the morphological changes between chemical peels (glycolic acid and Jessner's solution) and microdermabrasion, i.e. minimal features of cell injury were associated with microdermabrasion.

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Mahmoud Rezk A. Hussein[1,*]
Department of Pathology, Faculty of Medicine, Assuit University Hospitals, Assuit, Egypt

Eman Elayed Ab-Deif
Department of Histology, Faculty of Medicine, Sohage University, Sohag, Egypt

Amira Ali Abdel-Motaleb
Hatem Zedan
Azza Mahfouz Abdel-Meguid
Department of Dermatology, Faculty of Medicine, Assuit University Hospitals, Assuit, Egypt

*Corresponding author. Present address: Assir Central Hospitals, Assir, Abha, Kingdom of Saudi Arabia. Tel.: +20 93 583 166; fax: +20 88 333 327
E-mail address: mrh17@gawab.com (M.R.A. Hussein)

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LETTER TO THE EDITOR

A novel large *FERMT1* (*KIND1*) gene deletion in Kindler syndrome

KEYWORDS
Blister; Kindlin-1; Kindler syndrome; Poikiloderma; Deletion

Dear Sir,

Kindler syndrome (KS; OMIM 173650) is a rare heritable skin disorder which begins with congenital skin blistering and photosensitivity, which improves with age, and continues with progressive generalized poikiloderma and extensive skin atrophy [1]. Additional clinical features include mucosal involvement, e.g. early and severe periodontitis, and/or esophageal, gastrointestinal and genital involvement [1,2]. The risk of mucocutaneous malignancy is increased [1]. KS results from recessive loss-of-function mutations of the *KIND1* gene (now called *FERMT1*) that encodes the protein kindlin-1, a component of focal adhesions in epithelial cells [3]. Here, we add new data to our previous results, which demonstrated that large deletions in the *FERMT1* gene and DNA rearrangements might account for a significant number of the KS cases in whom pathogenic mutations could not be detected using genomic PCR amplification [4].

Three patients from southern Bulgaria were investigated in this study. Patients 1 and 2 were siblings and unrelated to patient 3. No consanguinity was known; patient 3 had three unaffected brothers and two unaffected sons (described in [6]). Skin biopsy specimens were analyzed with transmission electron microscopy (EM), and with indirect immunofluorescence staining (IIF) as described before with primary antibodies to: kindlin-1 (to the amino acids 541—674; [5]), collagen XVII, laminin β3 chain, α6 integrin and collagen VII [5]. Genomic DNA extracted from peripheral lymphocytes was used for PCR amplification, sequencing and analysis of the entire coding region and exon—intron boundaries of the *FERMT1* gene, as described [4]. The study was conducted according to the Declaration of Helsinki principles, and the participants gave their written informed consent.

The clinical features of the patients are presented in Table 1 and Fig. 1a. EM on a skin specimen of patient 1 demonstrated three levels of separation in the dermal—epidermal junction zone (DEJZ) — within the basal keratinocytes, along the lamina lucida and under the lamina densa — and in the skin of patient 2, reduplication of the lamina densa was prominent (not shown). IIF revealed reduced kindlin-1 staining (Fig. 1b) and positive but interrupted staining pattern for collagen XVII. Antibodies to laminin 332 and collagen VII produced thickened and patchy staining, with interruptions or branching of the DEJZ in the skin of both patients 1 and 2 (not shown). Integrin α6 which is targeted to the ventral surface of basal keratinocytes in control skin was also found at the lateral and apical cell membranes in the patients' skin (not shown). Even if these morphological features were indicative of KS, no *FERMT1* mutations were disclosed using the DNA sequencing strategy successfully used in the past. The fact that no amplicons were obtained for exons 14 and 15 suggested the presence of a homozygous genomic deletion spanning the region. Therefore, we designed several primers located in intron 13 and downstream of the 3′UTR, which allowed us to circumscribe the deleted interval. With primers 13F2:5′-CTTGCACCAGCTACCCTCTC-3′ and 15R3:5′-GGCTGCCAATAATGTTGGTT-3′, an about 2 kb product was obtained with genomic DNA from all three patients, instead of the expected 9.988-kb amplicon (Fig. 1c and d). The 2-kb PCR products were

Abbreviations: KS, Kindler syndrome; EM, electron microscopy; IIF, indirect immunofluorescence; DEJZ, dermal—epidermal junction zone; UTR, untranslated region; FERM, four point one ezrin radixin and moesin; PH, pleckstrin homology.