Original Article

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Evaluation of Physical Parameters of Skin by Consecutive Ultrasonic Image Processing During Ultraviolet Radiation in an Animal Model of Wrinkled Skin

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ABSTRACT

BACKGROUND AND OBJECTIVE: Skin aging is divided into two categories of intrinsic and extrinsic aging. Skin aging due to repeated exposure to ultraviolet radiation (extrinsic aging) is different from aging caused by time (intrinsic aging). The appearance of wrinkles caused by sunlight is due to subcutaneous fat atrophy and reduced production of collagen and elastin, thereby altering the biomechanical properties of the skin tissue. This study was conducted to investigate the skin damage caused by ultraviolet B (UVB) radiation by consecutive ultrasonic image processing with high resolution.

METHODS: In this experimental study, we evaluated the skin injury process among 25 C57BL/6 mice in healthy group (zero dose), and case group exposed to UVB radiation at 0.03 milliwatts per square centimeter (5 times a week for 5 weeks) due to differences in skin characteristics. Physical parameters of dermal and epidermal layers were measured and evaluated weekly from day 7 to day 35 using ultrasonic image processing.

FINDINGS: The thickness of the dermal and epidermal layers obtained by ultrasonic processing during the process of ultraviolet radiation injury in the mouse model significantly increased during the 5 – week study ($p<0.05$). In addition, the percentage of changes in the thickness of the epidermis layer (from 0.22±0.01 mm on day zero to 0.37±0.02 mm on the thirty-fifth day) and the dermal layer (from 0.57±0.05 on day zero to 0.90±0.08 mm on the thirty-fifth day) showed 68% and 57% increase, respectively.

CONCLUSION: The results showed that UVB radiation increased the thickness of the skin layers.

KEY WORDS: *Consecutive Ultrasonic Image Processing, Ultraviolet B (Uvb) Radiation, Dermal And Epidermal Thickness, Mouse Model.*

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Introduction

Nowadays, various imaging methods are designed to investigate the properties of human skin with advantages over traditional methods such as noninvasiveness and high accuracy (1). The use of these methods is very important to evaluate the efficiency of the treatment of skin diseases, the effects of topical medications and cosmetic products. Today, ultrasonic image processing can provide objective information about the depth of skin tissue (2).

Skin aging is divided into intrinsic and external aging. Skin aging due to repeated exposure to ultraviolet radiation (extrinsic aging) differs from aging caused by time (intrinsic aging) (3). The appearance of skin wrinkles caused by sunlight is due to subcutaneous fat atrophy and decreased collagen and elastin production, thereby altering the biomechanical properties of skin tissue (4). UVB (Ultraviolet B) is one of the main causes of aging that directly harms skin cells and indirectly affects them through inflammation and production of reactive oxygen species (5).

In the epidermis, the expression of epidermal growth factor receptor (EGFR) is reduced by synthesis of UVB-induced free radical, causing hyperplasia and disruption in keratinization of epidermis. On the other hand, UVB in the dermis causes an imbalance in the production and destruction of extracellular matrix components, and causes loss of skin elasticity and formation of wrinkle (6). In this study, the effect of UVB radiation on physical parameters of skin was investigated. This study was conducted to track the skin damage caused by UVB radiation using high–resolution ultrasonic image processing as a noninvasive method. Due to the sensitivity of the physical and mechanical parameters of the skin during the process of lesion formation, a non-invasive imaging technique is needed to evaluate the desired parameters and provide an effective index in skin-related research (7).

Therefore, in the present study, the effect of UVB radiation on physical parameters of skin was first investigated. Then, the physical parameters of the skin were evaluated based on high – resolution ultrasonic image processing as a noninvasive method to track and detect skin damage caused by UVB radiation.

Methods

In this experimental study, after being approved by the Ethics Committee of Tarbiat Modares University (code of ethics: IR.MODARES.REC.1396.4284), 25 male C57BL/6J mice aged 4 – 5 weeks and weighing 15 – 20 g were purchased from Pasteur Research Institute (Karaj, Iran). Before the experiment, animals were kept in special cages for laboratory animals for two weeks in a suitable environment and away from direct light. During this period, they had free access to standard food, water, and standard temperature, and were kept in 12h light/12h dark cycle at animal laboratory of Tarbiat Modares University to adapt to the new environment. One day before the imaging, the animals' hair was completely shaved. To achieve an animal model of wrinkle in the skin, rats were exposed to UVB radiation at 30 microwatts per square centimeter 5 times a week for 5 weeks (measurement of radiation intensity at different intervals was done using UV meter model UV-3) (8) (Fig 1).

B A

Figure 1. A) The way the mice are placed in the UVB radiation box, B) The UVB lamp and the UV detector

Histological study was performed on day 14 (after 405 mJ/cm2 radiation) and on day 35 (1890 mJ/cm2) to ensure skin damage. The formation of wrinkles occurred by UVB radiation on day 35. B-mode ultrasonic images and photography were obtained before radiation and during the radiation process (Figs 2, 3). To evaluate the process of skin lesion formation, histological study was performed using hematoxylin eosin and Masson's trichrome staining to correctly identify fibroblast cells and collagen fibers at the end of day 14 and day 35 of radiation.

High–frequency B-mode ultrasonic imaging (SonixTouch Ultrasound System, Ultrasonix Medical Corp., Richmond, Canada) was performed with a linear probe of 1.5 cm×0.5 cm, and 40 MHz central frequency range, so that the dermal and epidermal layers are perfectly visible.

After calibrating the ImageJ software based on the resolution of the ultrasonic images, the thickness of the dermal and epidermal layers of the skin were measured before and after UVB radiation during the creation of wrinkles.

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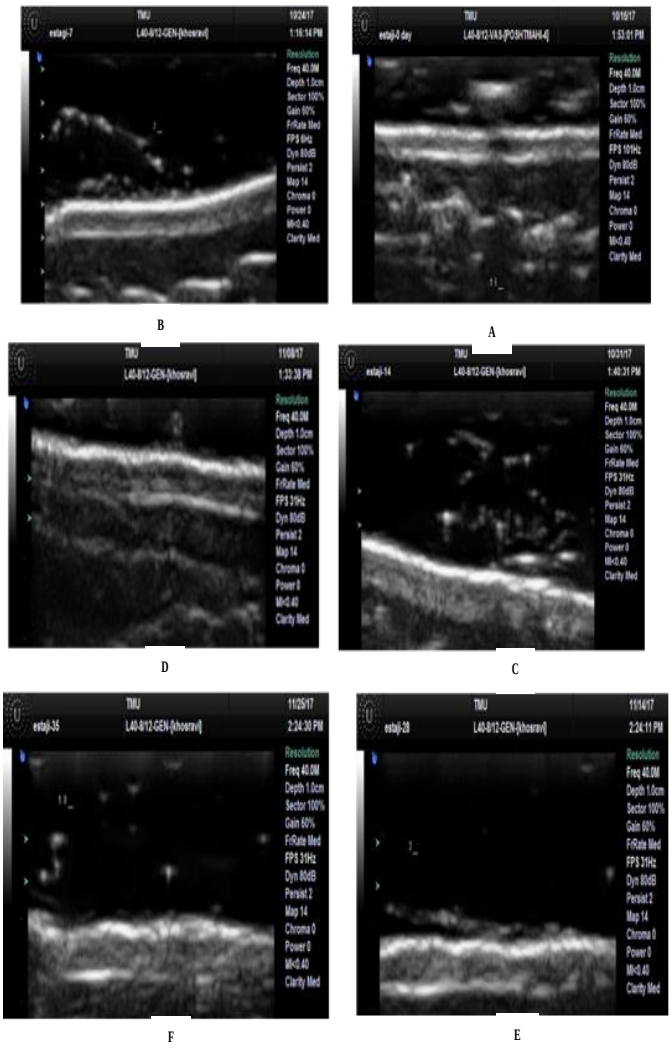

Figure 2. Ultrasonic images of skin layers during the process of skin wrinkling a) healthy, b) day 7 of radiation, c) day 14 of radiation, d) day 21 of radiation, e) day 28 of radiation, f) day 35 of radiation.

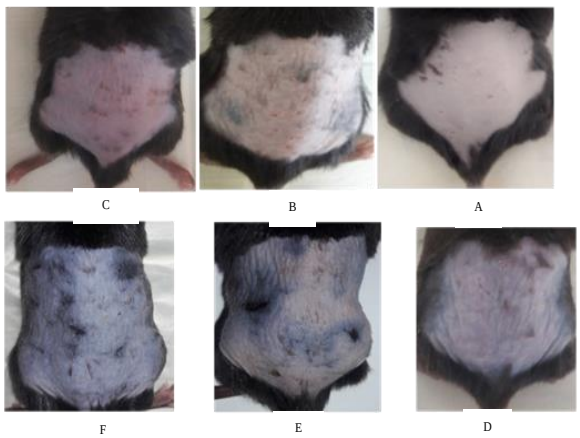

Figure 3. Photography of skin layers during the process of skin wrinkling a) Healthy, b) day 7 c) day 14, d) day 21, e) day 28, f) day 35 of radiation.

The image registration protocol was performed weekly to examine the physical parameters of the skin layers during the lesion process. The results of physical parameters of dermal and epidermal layers were reported as mean and standard deviation. To examine the statistical differentiation of parameters during the weeks of injury formation, data were analyzed by SPSS software version 16, ANOVA, and LSD (Least Significant Difference). $P<0.05$ was considered significant.

Results

There was no significant difference in the mean thickness of the epidermal layer, dermal layer and their total thickness before radiation (day zero, healthy skin) in study groups. On day 7 of UVB radiation, a significant increase in the mean thickness of the epidermal layer, dermal layer and their total thickness was observed compared to healthy skin after UVB radiation injury ($p<0.05$).

There was no significant difference in the thickness of the epidermal layer on day 14 compared to day 7 ($p=0.185$), but there was a significant difference in the thickness of the dermal layer and their total thickness ($p=0.00$). In addition, the thickness of the epidermal layer, the dermal layer and their total thickness on day 21 significantly increased compared to day zero ($p=0.00$). Results showed that the thickness of the epidermal layer, the dermal layer and their total thickness on day 28 of radiation also showed a significant difference compared with day zero ($p=0.00$). Comparing the mean thickness of the epidermal layer, the dermal layer and their total thickness also showed a significant increase ($p=0.00$) on day 35 of UVB radiation compared to day zero (Table 1). The results of photography, ultrasonic imaging, and histologic examination of cutaneous lesions after day 35 (day 62) are shown in Fig 4.

As the radiation process continued, skin lesions were created on the back of the mice (Fig 4a), and the ultrasonic images show that the dermal layer and the epidermal layer are destroyed (Fig 4b). Examination of the microscopic sections in the radiation group revealed a small corneal ulcer in the superficial dermis. Microscopically, many separate epithelial islands and cystic structures in different sizes were observed. There was creatine in the central part of most of the epithelial islands, and in some of them there were low to moderate amounts of melanin pigment. The periphery of the

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islands was covered with basal-like polyhedral cells with euchromatin with pale eosinophilic cytoplasm (Fig 4c). Therefore, the wrinkle model was approved by day 35. On days zero, 14 and 35 of UVB radiation, the samples were examined histologically to confirm the skin lesion as modulus of skin wrinkling. The results of sections stained with Hematoxylin and Eosin (H&E) (Fig 5) and Masson's trichrome (Fig 6) in radiated samples were compared with healthy skin.

On day zero (healthy skin), very fine collagen and elastic fibers were visible in the connective tissue of the dermis. The epidermal surface was without wrinkles and clefts with the presence of one row of basal cells, and two to three rows of keratinocyte cells with a fine granular layer (Fig 5a).

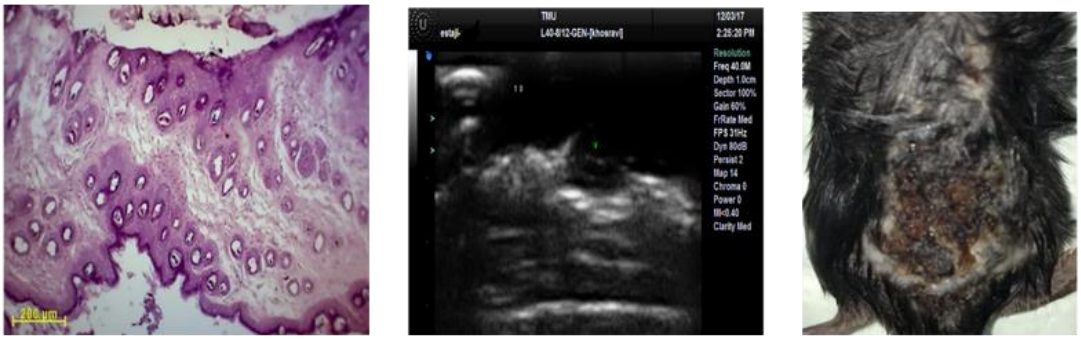
On the day 14 of radiation, fibers thicker than normal collagen and elastic fibers were seen in the tissue. In addition, 3 to 5 rows of basal cells and keratinocyte cells were also seen in the epidermal layer, and the epidermal surface was completely wrinkled along with the stratum corneum (Fig 5b). On the day 35, density of the thick collagen fibers was much more than the healthy group and day 14, and the epidermal wrinkles and clefts were also deeper and greater than the two preceding groups. The densities of the elastic fibers were lower than the two preceding groups. On the other hand, the cell density of the epidermis was also noticeable compared with the two preceding groups. The creatine layer is visible on the outer surface of the epidermis (Fig 5c).

The results showed that on day zero (normal), very fine collagen and elastic fibers were visible in the dermal connective tissue. Epidermal surface without wrinkles and clefts with a row of basal cells, and two to three rows of keratinocyte cells were observed with a fine granular layer (Fig 6a).

On day 14 of radiation, relatively short thick fibers were seen in the dermis in blue (Fig 6b). On day 35 of radiation, very short thick fibers with a significant density of collagen fibers were seen in the dermis in blue. The dominance of the fibers in the dermis was for thick collagen fibers (Fig 6c).

Table 1. The mean thickness of the epidermal layer, the dermal layer and their total thickness (mm) during lesion development from day zero (healthy) to day 35 of UVB radiation (damaged skin).

Thickness	Epidermal layer (mm)	Dermal layer (mm)	Total thickness (mm)
Day zero (healthy)	0.22±0.01	0.57±0.05	0.79±0.05
Day 7	0.30±0.03	0.65±0.06	0.95±0.06
Day 14	0.32±0.03	0.72±0.07	1.04±0.08
Day 21	0.33±0.02	0.76±0.07	1.09±0.07
Day 28	0.35±0.02	0.85±0.07	1.21±0.08
Day 35	0.37±0.02	0.9±0.08	1.26±0.13
P-value	0.000	0.000	0.000



C B A

Figure 4. A) Photographic image of cutaneous lesions created by continued exposure to radiation until day 62 in C57BL/6J mice; b) Ultrasonic image of cutaneous lesions in C57BL/6J mice by 40 MHz ultrasound; c) Histological image with Hematoxylin and Eosin staining of the skin ulcer reveals a small corneal ulcer in the superficial dermis.

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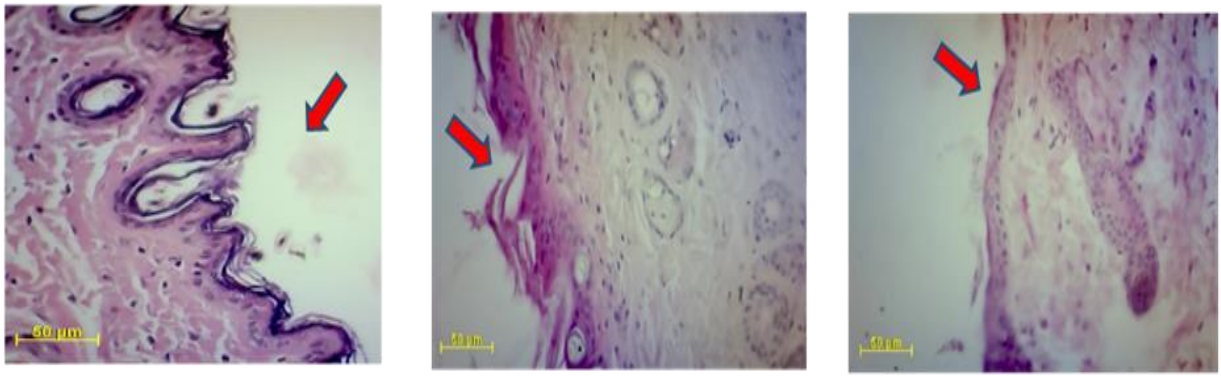
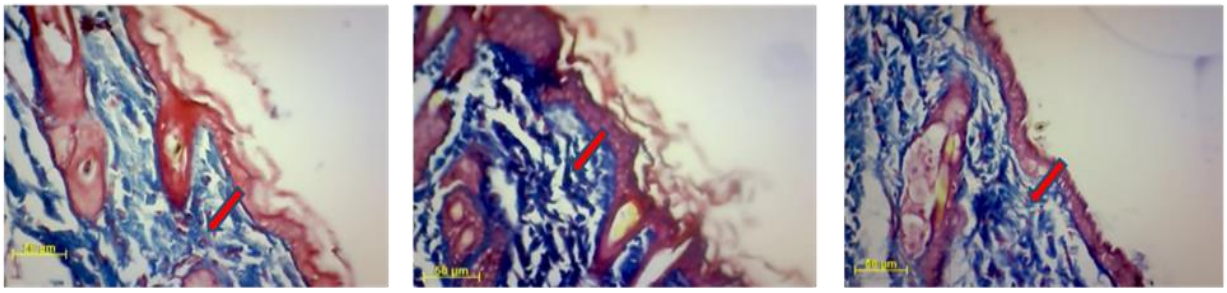


C B A

Figure 5. Histological images of skin (stained with Hematoxylin and Eosin); a) day zero, b) day 14, c) day 35 of UVB radiation. Arrows in the figure show changes in epidermis; the wrinkles and clefts in the epidermis on day 35 of radiation are deeper and more intense than in the two previous groups.



C B A

Figure 6. Histological images (stained with Masson's trichrome), a) day zero, b) day 14, and c) day 35 of UVB radiation. Arrows in the figure show collagen fiber degradation, twisting, and breaking down during the radiation process.

Discussion

In this study, the animal model of skin aging was confirmed with photographic and histological imaging based on the extraction of physical parameters from ultrasonic images as well as the appearance of skin wrinkles, and a non-invasive method was also proposed to evaluate the process of skin injury. Epidermal changes in C57BL/6 mice show a significant increase in the thickness and size of epidermal cell (9). Increased epidermal thickness was reported in the study of Che et al. on C57BL/6J mice exposed to ultraviolet radiation (intensity of 120 mJ/cm2 for three minutes, three times a week, and for three weeks) (9).

Sharma et al. examined the thickness of the epidermis in three types of C57BL/6J, SKH1, and Balb/c mice exposed to UV radiation, and the results showed that exposure to extreme UV radiation increased epidermal thickness in C57BL/6J and SKH1 mice and the model is similar to the aged human skin. In Balb/c mice, the epidermis became significantly thicker after the UV radiation period compared to the healthy control group. The findings of the present study also confirm the results of previous studies, according to which exposure to UV radiation increased the thickness of the epidermis and dermis as a model of wrinkle formation in C57BL/6J mice. In the present study, the thickness parameters of the epidermal layer, the dermal layer and their total thickness on day 35 of UV radiation showed a significant difference compared to day zero (healthy mice). The percentage of increase in the mean thickness of the epidermal layer on days 7, 14, 21, 28, and 35 were 36, 45, 50, 59 and 68%, respectively.

Moreover, the average thickness of the dermal layer in these days increased by 14, 26, 33, 49 and 57% compared to day zero (healthy rats), respectively. The mean thickness of the epidermal and dermal layer of the skin on day 35 of UV radiation was measured to be 59% more than normal skin. It can be concluded that in the

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process of UV damage to the skin and induction of premature aging, the thickness of the skin layers increases due to the degradation, twisting, and breaking down of collagen fibers, the accumulation of non-functional fibers and the increase of inflammatory cells. Increased thickness of the epidermis of the skin appears to be a clear reaction that reduces the penetration of UV radiation and can also be known as a sign of skin damage, and B-mode ultrasonic images can now extract the thickness of skin layers. However, most clinical studies are limited to the qualitative and superficial examination of skin changes, which are not highly valid due to qualitative observation and examination. UVB radiation causes a significant increase in the thickness of the skin layers. Non-invasive evaluation of skin lesions was provided by UVB radiation for 5 weeks and weekly evaluation by consecutive ultrasonic image processing and extraction of the thickness of dermal and epithermal layers. In this study, the increase in thickness after UV radiation as an indicator of aged skin and the creation of wrinkles in the animal model of mice were confirmed by extracting physical parameters from ultrasound processing, photographic imaging and histological studies.

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