Comparative Study of Elastic Fiber by Image Analysis System in Exposed and Nonexposed Human Skin

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Background: **The elastic fiber network undergoes profound alterations during the ageing process.**

Objective: **Our study compared two type ageing process of elastic fiber; the first, age associated, is found in skin from a nonexposed area; the second, related to sun exposure, is found in an exposed area.**

Methods: **In order to differentiate these two forms of ageing process, the author tried to compare length, breadth, perimeter, shape factor and amount of elastic fiber between the exposed and nonexposed skin of 240 Korean men using computerized digital image analysis system.**

Results: **Amount and breadth of elastic fibers are significantly different between exposed and nonexposed area in the same age group. After the fifties, the length of each elastic fiber in exposed areas are significantly longer than those of nonexposed area. Breadth is more sensitive than length to photoageing. The amount of elastic fiber is variable in nonexposed areas but increased steadily according to age in exposed areas.**

Conclusion: **The amount and four morphological factors of elastic fiber show there are significant differences between photoageing and chronological ageing process.**

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Key Words: Chronological ageing, elastic fiber, image analysis, photoageing

Elastic fibers are extracellular structural components of the connective tissue of a variety of organs.[1] The term "ageing" is used to describe both chronologic ageing and actinically demaged skin.[2,3] The elastic fiber networks undergo profound alteration during the ageing process.[4,5,6] Sunlight is responsible for the marked elastosis found in exposed areas of the human skin.[7] But sunlight has relatively little effect on the elastic tissue in nonexposed areas of the human skin. The precise quantitation of elastic fiber in the human skin has been difficult, since conventional methods do not lend themselves to accurate quantitation. To our knowledge, there has been no previous description of such a quantitation of the elastic fiber according to ageing process. In order to differentiate these two forms of ageing process, the author tried to compare the various morphological factors and amount of elastic fiber between exposed and nonexposed areas of human skin using a comupterized digital image analysis system.

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MATERIALS AND METHODS

Clinical materials

The specimens of exposed and nonexposed skin were obtaned from 240 Korean men from various age groups, containing a sufficient amount of nor-

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Table 1. Number of subjects in each age group

	0-10	11-20	21-30	31-40	41-50	51-60	over	Total
Nonexposed area	16	23	23	26	14	13	15	130
Exposed area	10	21	26	19	9	15	10	110
Total	26	44	49	45	23	28	25	240

mal skin in each specimen. Nonexposed skin was obtained from the buttocks and lower back. Facial and the extensor part of the forearm was selected as exopsed skin. We have excluded patients who had various skin disease affecting the elastic fiber, for example, cutis laxa, pseudoxanthoma elasticum, Marfans syndrome and Busche-011endorffs syndrome.[8] The 240 specimens were divided by vaious age groups and site(Table 1). All specimens were fixed in formalin solution and embedded in paraffin. Five micron sections were stained by the H&E and Verhoeff stain for elastic fiber.

Computerized digital image analysis

The equipment for computerized digital image analysis system is present in figure 1. The sections for image analysis were stained by Verhoeff stains which were examined by 01ympus light microscope using a 40X objective. The image were continuously monitored by video camera(Pulnix, IM440) and the images were displayed on a RGB monitor(Sony Inc. PVM1342Q). The analog video signal was digitized and analyzed with an IBM PC 386-based image processing system.(AIC Inc. Roswell, GA)

Rectangular areas measuring 155×116μm were digitized. Three rectangles were selected as a vertical unit. Two vertical units were studied for each slide. Densest method was employed for the selection of the microscopic fields for analysis.[9]) That method consisted of choosing three vertical units

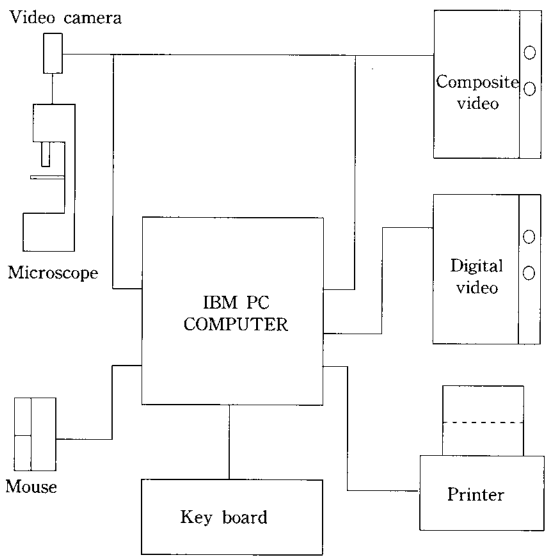

Fig. 1. Schematic of the computerized digital image analysis system.

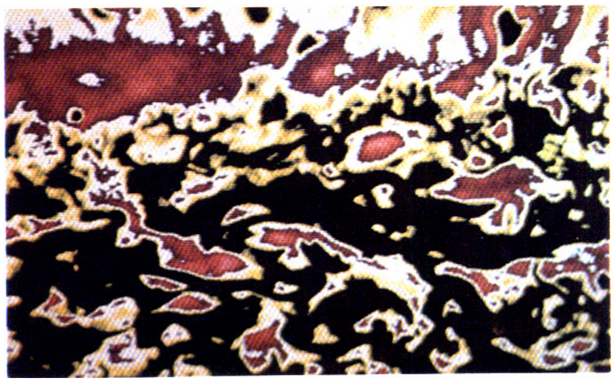

Fig. 2. Photograph of monitor showing the thresholded image where the green areas are elastic fiber.

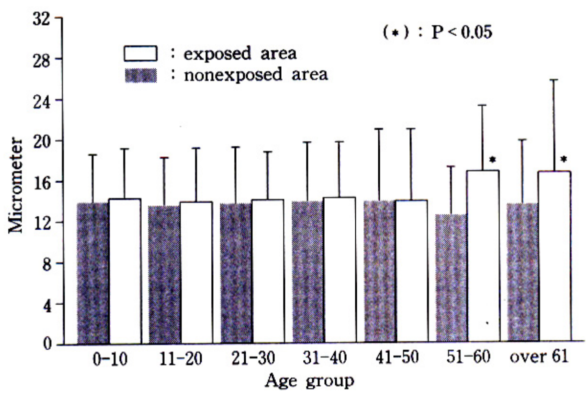

Fig. 3. Comparisons of length between exposed and nonexposed area (Mean ±SD).

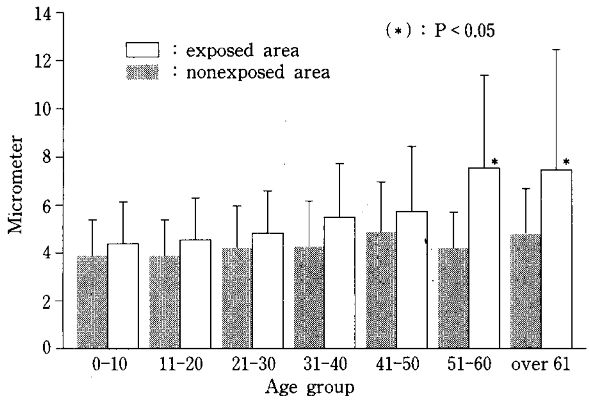

Fig. 4. Comparisons of breadth between exposed and nonexpoed area (Mean ±SD).

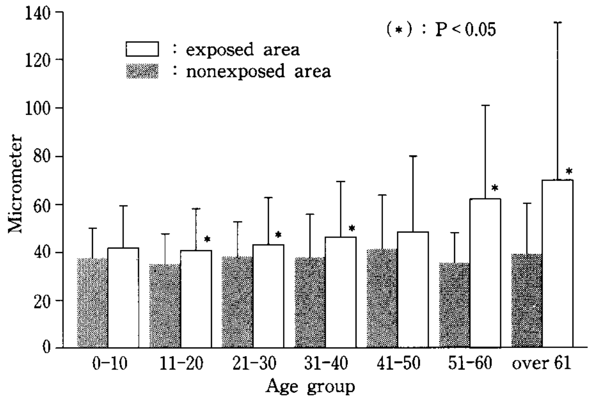

Fig. 5. Comparisons of perimeter between exposed and nonexposed area (Mean ±SD).

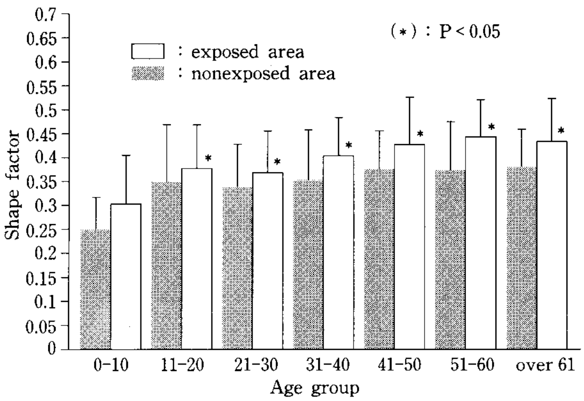

Fig. 6. Comparisons of shape factor between exposed and nonexposed area (Mean ±SD).

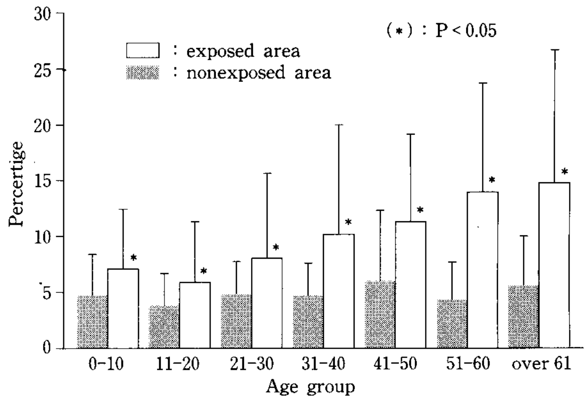

Fig. 7. Comparisons of area between exposed and nonexpoed area (Mean ±SD).

that had the highest total positive pixels. Locations of these vertical units were chosen by viewing the entire section at low power and subjectively choosing the area to quantify. The vertical units were always located in interfollicular areas. We tried to compare the various morphological factor such as length, breadth, shape factor and perimeter of each elastic fiber and summed the area of elastic fibers in a field.

RESULTS

The black and white images sensing various gray levels of the microscopic fields were converted to binary images by adjusting the threshold to a level that provided a binary image of the fiber corresponding as closely as possible to the black and white images of elastic fiber(Fig. 2). The results were analysed by Student t-test between the exposed and nonexposed area.

The length measurement calculates the largest diameter between any two points on the boundary of the each elastic fiber. The result from a parameter of the length are displayed in figure 3. There were significant differences in the length between the exposed and nonexposed area after the the age of fifty($p<0.05$).

The breadth measurement is caliper diameter at 90 degrees to length. The breadth of each elastic fiber of the exposed area was thicker than that of the nonexposed area in all age groups(Fig. 4).

The perimeter measurement is the distance around the boundary of the elastic fiber. There were significant differences in perimeter of each fiber except for the first and fifths decades(Fig. 5, $p<0.05$). The units of the above three morphological factors are micrometer.

The shape factor is the breadth/length ratio. A value of 1 would be a perfect circle where as a value of 0 would be a line. In the shape factor, there are significant differences between the exposed and nonexposed area after the second decade(Fig. 6, $p<0.05$).

The percentage unit of area is summed the area of each fiber in a field. The differences of the means were statistically significant in all age groups between the exposed and nonexposed area (Fig. 7, $p<0.05$).

DISCUSSION

Elastic fibers have been recognized by dermatologists to be one of the principal constituents of the connective tissues, particulary those tissues that contain elastomeric properties in the skin. Cumulative repeated exposure to natural sunlight contributes to the development of degenerative alterations of the connective tissue of the skin, especially elastic fiber.[10,11] The experimental production of elastosis in animals using artificial ultraviolet sources have been reported.[12,13,14] Our study compared two types of elastic fiber degeneration; the first, age associated, is found in the skin from a nonexposed area; the second, related to sun exposure, is found in an exposed area. The turnover of mature elastic fiber in normal undamaged tissue is very slow, but the ageing process is much accelerated when skin is frequently exposed to sunlight including ultraviolet radiation.[15,16,17] We believe that actinic elastosis and chronological ageing are two distinct phenomenon, the two varieties of modification can be identified together in the exposed area, and only age-related modification occur in the nonexposed area.[18,19] Although the literature contains many references to subjective observations in the state of the elastic fiber in dermis in various pathologic conditions, actual quantitation of elastic fiber have to our knowledge been recorded in a few selected conditions.[2,9,20,21] But there are several reports about the ultrastructural alterations in exposed and unexposed aged skin.[6,22,23,24]

The author compared the various morphological factors of elastic fibers using a computerized digital image analysis system between exposed and nonexposed areas in the same age groups.

In our present work, we found increased length of each elastic fiber after fifties in the skin of exposed area, but length decreased after fifties in the nonexposed area. After fifties, there were significant differences in length between exposed area and nonexposed area. But the breadth showed a significant difference between the two areas in all age groups. That result indicated that breadth is more sensitive than length after sun exposure. The amount of elastic fiber, the percentage area, showed a significant difference in all age groups between exposed area and nonexposed area. The amount of elastic fiber was variable in the nonexposed area, but increased steadily according to age in the exposed area. Lavker reported an increase of elasic fiber in papilary dermis,[6] but the other investigators observed marked age-associated decrease of elastic fibers.[25]

Elastotic matrial appears very early in the exposed areas(under 10 years in our observation). Its progressive development begins with hyperplasia and degeneration of the elastic fibers of the reticular dermis. In fair skinned people, elastic hyperplasia began as early as the first decade and was clearly evident in a majority of young adults before the age of 30.[7]

The cause of elastotic degeneration has not been clarified. Some investigators have suggessted that elastotic matrial is primarily derived from elastic fibers and not from preexisting or newly syntesized collagen. [26,27,28]

It is hoped that our results could be use as basic data in reseach for quantitation of elastic fiber. Quantitative study of elastic fiber using digital image analysis system is a very useful tool to assess the photoageing and chronological ageing process of human skin. The ability to objectively assess the severities of solar elastosis is of particular interest, as the implications of ultraviolet light and ozone depletion are being studied with increasing frequency.

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