Objective: To identify whether there is a relationship between solar elastosis and the development of wrinkles in human skin.

Design: Wrinkle depth was measured on the forehead and lateral canthus of human cadavers using image analysis. The thickness of the dermis was measured in skin sections obtained around wrinkles and stained with Elastica–van Gieson.

Setting: Gross Anatomy Section, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan.

Subjects: Fifty-eight male and female cadavers (age range at death, 29-93 years).

Main Outcome Measures: The ratio of solar elastosis dermal thickness to full dermal thickness (elastosis ratio) was calculated and compared between the deepest point of a wrinkle (wrinkle point) and a point within 1 mm where no wrinkle existed (nonwrinkle point). The relationship between elastosis ratios and wrinkle depths was investigated.

Results: Advanced solar elastosis was present at nonwrinkle points but was present a little bit at wrinkle points. On the forehead, a positive correlation between elastosis ratios and wrinkle depths was observed at nonwrinkle points but not at wrinkle points. On the lateral canthus, a positive correlation between elastosis ratios and wrinkle depths was observed at nonwrinkle points, as well as at wrinkle points until the wrinkle became deeper than one-half of the original dermal thickness (0.6 mm). Solar elastosis on the lateral canthus ceased developing at this point, but the wrinkle developed further.

Conclusions: Solar elastosis tends to commence with the development of a wrinkle until the wrinkle becomes deeper than 0.6 mm. This tendency is less evident at wrinkle points than at nonwrinkle points.

Arch Dermatol. 2012;148(8):913-917

WHEN CONSTANTLY exposed to the sun, facial skin shows the following 2 characteristic histological alterations: (1) wrinkles that form on its surface and (2) denaturation of elastic fibers (solar elastosis) that accumulate in its upper and middle layers.1,2 In 2011, it was reported that the dermis becomes thinner with the progression of wrinkles.3 This dermal thinning was assumed to be related to solar elastosis. However, dermal thinning was found to be more advanced at the deepest point of a wrinkle (wrinkle point) than at other points surrounding the wrinkle (nonwrinkle points).3 That finding led us to hypothesize that solar elastosis develops differently at wrinkle points vs nonwrinkle points. Three studies4-6 reported that no difference exists in the degree of solar elastosis between wrinkle points and nonwrinkle points, while 2 studies7,8 reported that the degree of solar elastosis is weaker at wrinkle points than at nonwrinkle points.

In the present study, we evaluated skin tissue on the forehead and lateral canthus of cadavers and measured the degree of solar elastosis at wrinkle points and at nonwrinkle points. Data for wrinkle points vs nonwrinkle points were compared and analyzed relative to the development of wrinkles.

METHODS

SUBJECTS

Fifty-eight cadavers (age range at death, 29-93 years) donated for medical education that had been embalmed (with a solution of formalin, phenol, alcohol, and thymol) and stored in the repository of the Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan, were used. Consent had been obtained from all donors or their relatives for the use of the skin tissue.

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body (including organs, tissues, and cells) for medical research (including anatomic examination, dissection, and other similar purposes) and for education. All methods used in this study complied with tenets of postmortem examination and corpse preservation in Japan.

Two regions of facial skin on each cadaver were investigated. These regions were approximately 10 mm above the superior margin of the right or left eyebrow (hereafter referred to as the forehead region) and 5 mm lateral from the right or left lateral canthus (hereafter referred to as the lateral canthus region).

**MARKING AND PHOTOGRAPHY**

In the forehead region of each cadaver, a reference wrinkle was chosen. A 20 × 10-mm rectangle was drawn on the surface, with its longer sides crossing perpendicular to the wrinkle. In the lateral canthus region of each cadaver, a wrinkle that originated from the lateral canthus was chosen. A point on a line 5 mm from the temporal side of the lateral canthus was marked; a rectangle was drawn by aligning its inner long side at the marked point. A close view of each rectangle was obtained with a digital camera (model D200; Nikon Corporation).

**CONSTRUCTION OF REPLICAS AND IMAGE ANALYSIS**

Using a hydrophilic vinyl silicone impression material (Exafine; GC Co Ltd), a replica was obtained of each rectangle, with a 3-mm surrounding margin. The surface image of each replica was evaluated using a linear analyzing function of a 3-dimensional image system (PRIMOS; GF Messtechnik GmbH) as detailed previously. Among multiple roughness variables defined, maximum roughness (the vertical distance between the highest peak and the lowest valley of the surface) was chosen to indicate the maximum depth of a wrinkle.

**HISTOLOGICAL INVESTIGATIONS**

As previously described, a 10 × 20-mm block of cadaveric tissue, including the skin and facial muscle, was obtained from each marked area. Each block was embedded in paraffin, which was then cut into 6-µm sections in the sagittal plane. The sections were stained with Elastica–van Gieson. Images were obtained using a light microscope (BX50; Olympus Corporation) and an image analyzer (Image-Pro Plus version 5; Media Cybernetics).

**CALCULATION OF THE RATIO OF SOLAR ELASTOSIS DERMAL THICKNESS TO FULL DERMAL THICKNESS**

The ratio of solar elastosis dermal thickness to full dermal thickness was calculated. First, the deepest point of a wrinkle (wrinkle point) was identified in each histological image by comparison with its surface photograph obtained before collection of the specimen.

Second, the area of measurement was set within 10 mm from the wrinkle point. The dermal layer was defined as the layer between the epidermis and the subcutaneous layers, including follicles and sebaceous and sweat glands but excluding any components away from the layer. Solar elastosis was defined as a thick serpentine band of curly or clumped elastic fibers that stained dark purple with Elastica–van Gieson stain.

Third, the degree of solar elastosis was evaluated by measuring the thickness of the dermis in solar elastosis. The dermal thicknesses of solar elastosis and full dermis were measured at 1 wrinkle point and at 3 other points within 1 mm where no wrinkle existed (nonwrinkle points). The dermal thicknesses were measured perpendicular to the outmost layer using the imaging analyzer. Subsequently, the dermal thicknesses at 3 nonwrinkle points were averaged. The ratio of solar elastosis dermal thickness to full dermal thickness (elastosis ratio) was calculated (Figure 1).

**DATA ANALYSIS**

The regression curve with the highest coefficient of determination was selected among the linear, logarithmic, involution, and exponential approximations using a statistical function of commercially available software (Excel; Microsoft). A paired t test was used to determine the significance of differences between groups. Statistical significance was set at P < .05.

**SAMPLE**

Fifty-eight cadavers were initially examined. Because of the existence of moles, wounds, or scars, 6 cadavers were excluded from the forehead region examination, and 8 cadavers were excluded from the lateral canthus region examination. Therefore, 52 cadavers (31 male and 21 female) were included in the forehead region examination, and 50 cadavers (31 males and 19 females) were included in the lateral canthus region. The age distribution of these cadavers is given in the **Table**.

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**Table. Age and Sex Distribution of the Study Cadavers**

<table>
<thead>
<tr>
<th>Age at Death, y</th>
<th>No. of Cadavers</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-29</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>30-39</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>40-49</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>50-59</td>
<td>7</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>60-69</td>
<td>13</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>70-79</td>
<td>17</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>80-89</td>
<td>7</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>90-99</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>52</td>
<td>31</td>
<td>21</td>
</tr>
</tbody>
</table>

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**Table. Age and Sex Distribution of the Study Cadavers**

<table>
<thead>
<tr>
<th>Forehead Region</th>
<th>Lateral Canthus Region</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at Death, y</td>
<td>No. of Cadavers</td>
</tr>
<tr>
<td>20-29</td>
<td>1</td>
</tr>
<tr>
<td>30-39</td>
<td>1</td>
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<td>80-89</td>
<td>7</td>
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<tr>
<td>90-99</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>52</td>
</tr>
</tbody>
</table>
OBSERVATION OF SOLAR ELASTOSIS IN THE DERMIS

Solar elastosis was commonly observed in the dermis of forehead and lateral canthus skin specimens obtained from older subjects. The degree of solar elastosis tended to be less in specimens with shallow wrinkles and greater in specimens with deeper wrinkles (Figure 2 and Figure 3). The degree of solar elastosis was more advanced at nonwrinkle points than at wrinkle points.

ELASTOSIS RATIOS AT WRINKLE POINTS AND NONWRINKLE POINTS

In forehead regions and lateral canthus regions, elastosis ratios were significantly greater at nonwrinkle points than at wrinkle points. The mean (SD) elastosis ratios at wrinkle points were 0.12 (0.14) in forehead regions and 0.46 (0.30) in lateral canthus regions, while those at nonwrinkle points were 0.28 (0.18) in forehead regions (P < .001 vs wrinkle points) and 0.58 (0.23) in lateral canthus regions (P < .01 vs wrinkle points).

CORRELATION BETWEEN ELASTOSIS RATIOS AND WRINKLE DEPTHS

At wrinkle points, a positive correlation between elastosis ratios and wrinkle depths was not found in forehead regions but was found in lateral canthus regions; at nonwrinkle points, a positive correlation was found in both regions. Data for lateral canthus regions were dichotomized and analyzed in a shallow wrinkle group (< 0.6 mm) vs a deep wrinkle group (≥ 0.6 mm). In forehead regions and lateral canthus regions, elastosis ratios increased with the deepening of a wrinkle to 0.6 mm, after which solar elastosis ceased developing in lateral canthus regions (Figure 4 and Figure 5).

COMMENT

Advanced solar elastosis was present at nonwrinkle points but was present a little bit at the wrinkle points. Elastosis ratios increased with the deepening of a shallow wrinkle to 0.6 mm, after which elastosis ratios remained less than 1. These findings indicate that solar elastosis likely contributes to the development of shallow wrinkles but not deeper wrinkles.

Figure 2. Representative images of forehead wrinkles (from the left, external appearance, 3-dimensional replica images, and tissue specimens [insets are enlarged on the far right]). A, Shallow wrinkle in an 80-year-old man (maximum depth, 0.17 mm; elastosis ratios of 0.16 at wrinkle point and 0.17 at nonwrinkle point). B, Mid-depth wrinkle in an 80-year-old woman (maximum depth, 0.22 mm; elastosis ratios of 0.05 at wrinkle point and 0.34 at nonwrinkle point). C, Deep wrinkle in a 72-year-old woman (maximum depth, 0.59 mm; elastosis ratios of 0.26 at wrinkle point and 0.51 at nonwrinkle point). Microscopic observations of the external appearance were made along the blue line. Three-dimensional replica images were obtained and analyzed at the same area as the external appearance. Color bar indicates the level of the surface. D indicates dermis; E, epidermis; FM, facial muscle; I, inferior direction; S, superior direction; SC, subcutaneous tissue; SE, solar elastosis; and *, wrinkle point. Elastica–van Gieson stain, original magnification ×40. The external appearance and 3-dimensional replica images in C are reproduced from our previous article.9
The present study confirmed the finding by Tsuji et al.\textsuperscript{7} that the degree of solar elastosis is weaker at wrinkle points than at nonwrinkle points but did not confirm what they believed was the cause of the variation. According to Tsuji et al., solar elastosis decreases under wrinkles because those areas are subject to less sun damage. How-

Figure 3. Representative images of lateral canthus wrinkles (from the left, external appearance, 3-dimensional replica images, and tissue specimens [insets are enlarged on the far right]). A, Shallow wrinkle in a 54-year-old woman (maximum depth, 0.12 mm; elastosis ratios of 0.02 at wrinkle point and 0.06 at nonwrinkle point). B, Mid-depth wrinkle in a 66-year-old man (maximum depth, 0.54 mm; elastosis ratios of 0.58 at wrinkle point and 0.80 at nonwrinkle point). C, Deep wrinkle in a 72-year-old woman (maximum depth, 1.4 mm; elastosis ratios of 0.68 at wrinkle point and 0.83 at nonwrinkle point). Microscopic observations of the external appearance were made along the blue line. Three-dimensional replica images were obtained and analyzed at the same area as the external appearance. Color bar indicates the level of the surface. D indicates dermis; E, epidermis; FM, facial muscle; I, inferior direction; S, superior direction; SC, subcutaneous tissue; SE, solar elastosis; and *, wrinkle point. Elastica–van Gieson stain, original magnification ×40. The external appearance and 3-dimensional replica images in C are reproduced from our previous article.\textsuperscript{9}

Figure 4. Relationships between elastosis ratios and wrinkle depths at wrinkle points. A, In forehead regions ($y=0.0001x+0.083$, $R^2=0.02$). B, In lateral canthus regions (for shallow wrinkles <0.6 mm, $y=0.0006x^{0.26}$, $R^2=0.22$; for deep wrinkles ≥0.6 mm, $y=2.27x^{0.29}$, $R^2=0.007$). NS indicates not significant.

Figure 5. Relationships between elastosis ratios and wrinkle depths at nonwrinkle points. A, In forehead regions ($y=0.0006x+0.14$, $R^2=0.17$). B, In lateral canthus regions (for shallow wrinkles <0.6 mm, $y=0.0032x^{0.18}$, $R^2=0.21$; for deep wrinkles ≥0.6 mm, $y=3.46x^{0.23}$, $R^2=0.05$). NS indicates not significant.
ever, Contet-Andonneau et al\(^8\) reported that atrophy of elastic tissues, oxytalan fibers, collagens, chondroitin sulfates, and other extracellular matrices is more pronounced at the bottom of a wrinkle than at the flanks of the same wrinkle. A possible hypothesis is that the raised rigidity caused by advanced solar elastosis at non-wrinkle points may minimize deformation of the dermis by repeated facial muscle movements and that the stress from facial muscle movements may be concentrated at wrinkle points because of less advanced solar elastosis and thinning of the dermis.\(^3\) Consequently, the stress concentrated at wrinkle points may increase the magnitude of a wrinkle. This hypothesis needs further investigation, but if structural differences between wrinkle points and nonwrinkle points increase the magnitude of a wrinkle, these points need to be treated separately using different methods to prevent wrinkle development.

This study has several limitations. First, formalin-fixed cadavers were used, so wrinkle depths may differ from the actual depths in vivo. However this difference is considered negligible because the mean wrinkle depth and the value distribution in this study are not different from those of age-matched living individuals.\(^12\) Second, the thicknesses of the dermis and solar elastosis in paraffin-embedded specimens differs from those in vivo. However, the effects of embalming and fixation were minimized by using elastosis ratios. Third, dynamic (temporal and reducible) and static (permanent) wrinkles in cadaver skin cannot be distinguished from each other. However, because muscles in a cadaver are in a relaxed or almost relaxed state, the observed wrinkles are considered static. Fourth, because all donors herein were Japanese (with skin phototypes II-IV), it remains unknown whether lighter and darker types of skin show similar tendencies as those observed herein. Because solar elastosis is commonly present in the dermis of all skin phototypes,\(^13\) it is assumed that the relationship between solar elastosis and the development of wrinkles would be the same in all skin phototypes.

In conclusion, solar elastosis tends to commence with the progression of facial wrinkles. This is less evident at wrinkle points than at nonwrinkle points. The seemingly contradictory findings about the relationship between solar elastosis and the development of wrinkles may be attributed to other factors, such as stress from facial muscle movements. The findings of this study provide new insights for the development of antiwrinkle treatments.

Accepted for Publication: December 29, 2011.

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Author Contributions: Drs Tsukahara, Tamatsu, and Shimada had full access to all the data in the study and take responsibility for the integrity of the data and accuracy of the data analysis.

Study concept and design: Tsukahara, Tamatsu, and Shimada. Acquisition of data: Tsukahara and Tamatsu. Analysis and interpretation of data: Tsukahara, Tamatsu, and Sugawara. Drafting of the manuscript: Tsukahara and Tamatsu. Critical revision of the manuscript for important intellectual content: Sugawara and Shimada. Statistical analysis: Tsukahara. Administrative, technical, and material support: Tamatsu. Study supervision: Tamatsu, Sugawara, and Shimada.

Financial Disclosure: None reported.

Funding/Support: This study was supported by Kagoshima University Graduate School of Medical and Dental Sciences.

Role of the Sponsors: The sponsors had no role in the design or conduct of the study; in the collection, analysis, or interpretation of the data; or in the review or approval of the manuscript.

REFERENCES