The Relationship Between Wrinkle Depth and Dermal Thickness in the Forehead and Lateral Canthal Region

Kazue Tsukahara, PhD; Yuichi Tamatsu, DDS, PhD; Yasushi Sugawara, MD, PhD; Kazuyuki Shimada, DDS, PhD

Objective: To identify a relationship between dermal thinning and wrinkle formation.

Design: We assessed the wrinkle depth of the forehead and lateral canthus of 58 male and female human cadavers (range of age at death, 29-93 years) using image analysis and measured the dermal thicknesses in Azan-Mallory-stained skin sections obtained around the wrinkles.

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

Main Outcome Measures: The maximum depth of the wrinkle was obtained from the forehead and lateral canthus. The dermal thickness was measured at the deepest point of the wrinkle (wrinkle point) and at a location where no wrinkle existed within 1 mm of its surface (nonwrinkle point). The ratio of the dermal thickness at the wrinkle point to the dermal thickness at the non-wrinkle point was calculated.

Results: The dermal thickness underneath a wrinkle decreased as the depth of the wrinkle increased ($P < .001$). When the dermis became thinner than one-half of its original thickness, the dermis stopped thinning. Microscopic observations revealed that the junction between the dermis and subcutaneous layers under advanced wrinkles curved downward with invaginations of the dermis into the subcutaneous layer.

Conclusions: The dermis under a wrinkle becomes thinner in association with the progression of wrinkles until the dermis becomes thinner than one-half of its original thickness. When the dermis stops thinning, wrinkles develop further by dermal invagination into the subcutaneous layer.

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THE SURFACE MORPHOLOGIC characteristics of facial wrinkles are well described, but little is known about the morphologic changes beneath those wrinkles. Kligman et al. defined wrinkles as "a configurational change, like the grooves worn into an old glove, without specific structural alterations at the histological level." Tsuji et al. reported ultrastructural differences between wrinkled areas and the surrounding areas. Contet-Andonneau et al. reported significant decreases of several extracellular matrix components at the bottoms of wrinkles compared with the wrinkle edges. However, none of those reports addressed potential differences in dermal thickness between wrinkled and nonwrinkled areas.

The purpose of this study was to measure dermal thickness in wrinkles on skin from the forehead and from the lateral canthus and to analyze the relationship between dermal thickness at the deepest point of wrinkles and in the surrounding areas.

STUDY SUBJECTS

The 58 cadavers used in the study had been donated for medical education (age at death, range, 29-93 years). They had been embalmed with a formalin-phenol-alcohol-thymol solution and were stored in the repository of the Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan. Consent had been obtained from all donors and their relatives for the use of the body (including organs, tissues, and cells) for medical research (including anatomical examination, dissection, and other similar purposes) and for education. All methods used in this study complied with the Postmortem Examination and Corpse Preservation Act of Japan.

Two regions of facial skin were investigated. Regions approximately 10 mm above the superior margin of the right or left eyebrow (hereinafter referred to as the forehead region) and 5 mm lateral from the right or left lateral canthus.
MEASUREMENT OF DERMAL THICKNESS

By comparison with its surface image, the concavity of the reference wrinkle was identified in the histologic image. The area of measurement was set within 10 mm from the deepest point of the wrinkle. The thickness was measured at the deepest point of the wrinkle (hereinafter referred to as the wrinkle point), and at 3 other points, where no wrinkle existed within 1 mm of its surface (hereinafter referred to as the nonwrinkle points). The dermal thickness at each of the 3 nonwrinkle points was averaged. In this study, the dermal layer was defined as the layer between the epidermis and the subcutaneous layer, including follicles and sebaceous and sweat glands but excluding any components away from the layer. The thickness was measured perpendicular to the outermost layer using the Image Pro Plus analyzer (Figure 1).

DATA ANALYSIS

The dermal thickness was analyzed in relation to the $R_{\text{max}}$ value. Because dermal thickness varies according to age, sex, and anatomical site, the ratio of the dermal thickness at the wrinkle point to the dermal thickness at nonwrinkle point was calculated to mitigate the effects of these variations and to minimize the effects of embalming and paraffin embedding. Figure 1 illustrates how we obtained the $R_{\text{max}}$ and the dermal thickness ratio. The thickness ratio was then analyzed with the Rmax value.

HISTOLOGIC METHODS

As previously described, a 10 × 20-mm block of cadaveric tissue, including the skin and facial muscle, was obtained from each marked area. The block was then embedded in paraffin, which in turn was cut into 6-µm sections in the sagittal plane. The sections were stained with Azan-Mallory stain. Images were captured using a light microscope (model BX50; Olympus Corp, Tokyo, Japan) and were then analyzed using a linear analyzing function of the 3-dimensional image analyzer (PRIMOS system; GF Messetechnik GmbH, Teltow, Germany) as detailed previously. Among multiple roughness parameters defined, maximum roughness ($R_{\text{max}}$), which is the vertical distance between the highest peak and the lowest valley of the surface, was chosen to indicate the maximum depth of a wrinkle (Figure 1).

THREE-DIMENSIONAL IMAGE ANALYSIS

Using hydrophilic vinyl silicone impression material (GC Exafine; GC Co Ltd, Tokyo, Japan), a replica was obtained from each rectangle with a 5-mm surrounding margin. The surface image of each replica was captured and was then analyzed using a linear analyzing function of the 3-dimensional image analyzer (PRIMOS system; GF Messetechnik GmbH, Teltow, Germany) as detailed previously. Among multiple roughness parameters defined, maximum roughness ($R_{\text{max}}$), which is the vertical distance between the reference level and the lowest valley of the replica surface to indicate the maximum depth of the wrinkle, was obtained by calculating the vertical distance between the reference level and the lowest valley of the replica surface, with the maximum depth of the wrinkle. The dermal thickness at the nonwrinkle point was obtained by averaging the dermal thicknesses at 3 points, which were 1 mm away from a wrinkle. The dermal thickness ratio (h, c) was obtained by calculating the ratio of dermal thickness at the wrinkle point (b) to the dermal thickness at the nonwrinkle point (c).

RESULTS

SAMPLE

Of the initial 58 cadavers, 6 were excluded from each regional observation so that the forehead region of 52 cadavers (31 males and 21 females) and the lateral canthal region of 52 cadavers (33 males and 19 females) were observed. The age distribution of these cadavers is shown in Table 1.

MORPHOLOGIC RELATIONSHIP BETWEEN THE DERMIS AND WRINKLES IN SPECIMENS

Microscopic observations of the forehead skin specimens with various degrees of wrinkles revealed the following findings. The dermis had a constant thickness when the wrinkle was shallow ($R_{\text{max}}=0.11$ mm) (Figure 2A). The dermis became thinner at the deepest point of a middle-level wrinkle ($R_{\text{max}}=0.37$ mm) (Figure 2B) and became even thinner at the equivalent point of a deep wrinkle ($R_{\text{max}}=0.47$ mm) (Figure 2C). The dermis-subcutaneous junction was relatively flat in most samples.

Microscopic observations of the lateral canthus skin specimens with various degrees of wrinkles revealed the following findings. As was observed in the forehead skin specimens, the dermis had a constant thickness when the wrinkle was shallow ($R_{\text{max}}=0.20$ mm) (Figure 3A) and became thinner at the deepest point of a middle-level wrinkle ($R_{\text{max}}=0.59$ mm) (Figure 3B). With a thickness similar to that of a middle-level wrinkle, the dermis-subcutaneous junction under deep wrinkles ($R_{\text{max}}=1.16$ mm) (Figure 3C) curved downward with invaginations of the dermis into the subcutaneous layer.

<table>
<thead>
<tr>
<th>Table 1</th>
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<tr>
<td><strong>Sample</strong></td>
</tr>
<tr>
<td><strong>Forehead region</strong></td>
</tr>
<tr>
<td>Cadavers: 52</td>
</tr>
<tr>
<td>Males: 31</td>
</tr>
<tr>
<td>Females: 21</td>
</tr>
<tr>
<td><strong>Lateral canthal region</strong></td>
</tr>
<tr>
<td>Cadavers: 52</td>
</tr>
<tr>
<td>Males: 33</td>
</tr>
<tr>
<td>Females: 19</td>
</tr>
</tbody>
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No major age- or sex-dependent difference was found in these tendencies. The age dependency was examined in specimens obtained from middle-aged and elderly subjects. Specimens from young subjects were excluded owing to the lack of variation in wrinkle severity.
VALUES OF THE DERMAL THICKNESS AND THE WRINKLE DEPTH

In the forehead, the average dermal thickness was 0.95 mm at the nonwrinkle point and 0.81 mm at the wrinkle point, whereas the average value of $R_{\text{max}}$ was 0.25 mm and the maximum value was 0.60 mm. In the lateral canthus, the average dermal thickness was 0.61 mm at the nonwrinkle point and 0.41 mm at the wrinkle point, whereas the average value of $R_{\text{max}}$ was 0.62 mm and the maximum value was 1.64 mm. The deep lateral canthus wrinkles were found to be 3 times deeper than those on the forehead (Table 2).

CORRELATION BETWEEN THE DERMAL THICKNESS AND $R_{\text{max}}$

In both the forehead and canthus regions, a significant correlation was found between the dermal thickness at the wrinkle point and the $R_{\text{max}}$ ($P < .001$ for both comparisons), whereas the correlation was insignificant between the dermal thickness at the nonwrinkle point and the $R_{\text{max}}$ (Figure 4 and Figure 5) ($P = .10$ and $P = .35$ for the forehead and canthus regions, respectively), thereby suggesting a possibility that the dermis at the wrinkle point becomes thinner in association with the wrinkle’s progress ($P < .001$ for both comparisons). The regression curve with the highest coefficient of determination is shown in Figure 6.

Table 2. Dermal Thickness and $R_{\text{max}}$, Values

<table>
<thead>
<tr>
<th>Region</th>
<th>Length, Mean (SD) [Range], mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dermal thickness</td>
<td></td>
</tr>
<tr>
<td>Wrinkle point</td>
<td>0.81 (0.31) [0.12-1.74]</td>
</tr>
<tr>
<td>Nonwrinkle point</td>
<td>0.95 (0.28) [0.35-1.65]</td>
</tr>
<tr>
<td>$R_{\text{max}}$</td>
<td>0.25 (0.13) [0.05-0.60]</td>
</tr>
<tr>
<td></td>
<td>0.62 (0.40) [0.11-1.64]</td>
</tr>
</tbody>
</table>

Abbreviation: $R_{\text{max}}$, maximum roughness.

* $P < .001$ (vs forehead) for all comparisons.
The correlation was analyzed by the method described herein. Only in the lateral canthus region, however, the correlation was found between the thickness ratio and the \( R_{\text{max}} \) in both facial regions (forehead: \( y = -0.046 \ln(x) \), \( R^2 = 0.33 \), \( P < .001 \)). The circle indicates the nonwrinkle point \( y = 0.47x + 0.04 \), whereas no such correlation was found in the shallow wrinkle group (\( R_{\text{max}} < 0.60 \) mm). The mean (SD) thickness ratio in the shallow wrinkle group was 0.51 (0.15).

**CORRELATION BETWEEN THE DERMAL THICKNESS RATIO AND \( R_{\text{max}} \)**

The dermal thickness ratio tended to decrease in association with an increase of the \( R_{\text{max}} \) in the forehead and lateral canthus regions. A significant correlation was found between the thickness ratio and the \( R_{\text{max}} \) in both facial regions (forehead: \( y = -0.001x + 1.10, R^2 = 0.59, P < .001 \); lateral canthus: \( y = -0.26 \ln(x) + 2.30, R^2 = 0.62, P < .001 \)). The correlation was analyzed by the method described herein. Only in the lateral canthus region, however, the decrease of the thickness ratio became milder when the \( R_{\text{max}} \) became greater than 0.60 mm. Based on this information, the lateral canthus skin data were sorted into 2 groups: a group of shallow wrinkles (\( R_{\text{max}} < 0.60 \) mm) and a group of deep wrinkles (\( R_{\text{max}} \geq 0.60 \) mm). The possible correlation between the thickness ratio and the \( R_{\text{max}} \) was analyzed in each group, and a correlation similar to that of forehead wrinkles was found in the shallow wrinkle group; however, no such correlation was found in the deep wrinkle group (Figure 6). The mean (SD) thickness ratio in the deep wrinkle group was 0.51 (0.15).

**COMMENT**

In our study, a significant correlation between the wrinkle depth and dermal thickness at the wrinkle point was found, whereas no correlation was found between the wrinkle depth and dermal thickness at the nonwrinkle point (Figure 4 and Figure 5). Others have reported that the dermis in facial skin becomes thinner with intrinsic aging, and it becomes thicker with photoaging.10,11 The report by Tsuji et al12 suggested that the dermis underneath a wrinkle decreases its thickness in association with the wrinkle deepening until the wrinkle depth reaches 0.60 mm. Despite the further deepening of the wrinkle, the dermis stops thinning. LCS indicates a small \( R_{\text{max}} \) group: \( R_{\text{max}} < 0.60 \) mm; number of data=22, \( y = -0.046 \ln(x) + 0.82, R^2 = 0.002 (P = .69) \).

In our study, a significant correlation between the wrinkle depth and dermal thickness at the wrinkle point was found, whereas no correlation was found between the wrinkle depth and dermal thickness at the nonwrinkle point (Figure 4 and Figure 5). Others have reported that the dermis in facial skin becomes thinner with intrinsic aging, and it becomes thicker with photoaging.10,11 The report by Tsuji et al12 suggested that the dermis underneath a wrinkle looked relatively thinner compared with the dermal thickness of the surrounding area, which was thickened by solar elastosis. However, our results indicate that dermal thickness underneath a wrinkle decreases absolutely, not relatively. Contet-Andonneau et al13 reported that levels of collagens IV and VII, chondroitin sulfates, and oxytalan fibers were significantly lower in facial skin with wrinkles compared with facial skin without wrinkles or in sites that had not been exposed to the sun (eg, the abdomen). Our results support those of their study, and we conclude that facial
wrinkles are formed by morphologic changes of the dermis beneath wrinkles rather than by elastotic changes of the surrounding areas.

The dermal thickness ratio was found to decrease in association with an increase of the \( R_{\text{max}} \) in the forehead and lateral canthus. The \( R_{\text{max}} \) of the forehead wrinkles increased up to 0.60 mm, whereas the \( R_{\text{max}} \) of the lateral canthus wrinkles increased more than 0.60 mm. In the lateral canthus region, the decrease of thickness ratio became milder when the \( R_{\text{max}} \) increased more than 0.60 mm. The microscopic observation of the lateral canthus skin revealed no marked difference in dermal thickness between deeper and shallower wrinkles. These results suggest that the thickness ratio decreased in association with the wrinkle's deepening until the \( R_{\text{max}} \) increased to 0.60 mm, after which the thickness ratio remained around 0.5 despite any further increasing of the \( R_{\text{max}} \). Taken together, it seems reasonable to conclude that dermal thickness at the wrinkle point decreases during wrinkle development, but this decrease terminates at a certain point. The dermal thickness decreases during development of the wrinkle until the thickness at the wrinkle point becomes one-half of its initial thickness. Once a wrinkle develops to a point deeper than one-half of the initial dermal thickness, the decrease in dermal thickness terminates. Once this 50% dermal thinning occurs, further deepening of the wrinkle does not correspond with an additional increase in dermal thinning. We assume that wrinkles can become deeper than 0.60 mm by invaginations of the dermal layer into the subcutaneous layer in association with structural changes in the subcutaneous layer.

In about one-third of specimens of skin with deep wrinkles, we observed insertions of the dermis into the subcutaneous layer. Most of those samples were skin specimens from the lateral canthus regions. More specifically, such insertions were rarely observed in the forehead skin specimens and were found more frequently in the lateral canthus specimens, especially those with wrinkles deeper than 0.60 mm. These results lead us to believe that skin tissues involved in wrinkle development differ between the forehead and the lateral canthus. When the forehead and lateral canthus wrinkles are compared, the \( R_{\text{max}} \) in the lateral canthus is considerably greater than that in the forehead. Subsequently, at both the wrinkle and nonwrinkle points, the dermal thickness is considerably thicker in the forehead than in the lateral canthus. As a result (Table 2), the depth of the wrinkle reached 26% of the initial thickness (the thickness at the nonwrinkle point) in the forehead, whereas it reached nearly 100% in the lateral canthus. Consequently, one can assume that because the dermal layer is relatively thick in forehead skin, forehead wrinkles stop deepening at the point where morphologic changes remain within this layer. However, lateral canthus wrinkles become deeper because of the relatively thinner dermis. This deepening is assumed to cause the invagination of the dermis into the subcutaneous layer, which causes morphologic changes in the subcutaneous layer.

Questions may arise about whether the wrinkle or the dermal thinning came first and whether the wrinkle was the cause of the thinning or the thinning was the cause of the wrinkle. We did not observe any specimens with thin dermis that did not also contain wrinkles. From these data, it seems that wrinkles are a prerequisite for dermal thinning. This leads us to postulate that the wrinkle comes first. This postulation agrees with our finding in the lateral canthus specimens (Figure 3C), in which the dermis stopped thinning but the wrinkle deepened further by invaginating the dermis into the subcutaneous layer. Consequently, it seems reasonable to assume that wrinkles are the cause of dermal thinning. In the future, we will examine these assumptions in detail, considering other factors involved in wrinkle formation (eg, the loss of skin elasticity, frequent facial muscle movements).

It must be mentioned that this study has 3 limitations. First, because this study used embalmed cadavers and paraffin-embedded specimens, measured values may be different from the actual values in vivo. We considered this difference negligible in wrinkle depth because the mean wrinkle depth and the value distribution obtained in this study were not different from those of age-matched living patients. However, we considered the difference not negligible in the dermal thickness and thus calculated the dermal thickness ratio between the wrinkle and nonwrinkle points of the same sample in order to detect an association with \( R_{\text{max}} \). Second, in cadaver skin, dynamic (temporal, reducible) and static (permanent) wrinkles cannot be distinguished from one another. However, because muscles in a cadaver are in a relaxed or nearly relaxed state, it is very likely that the observed wrinkles are static ones. Third, because all of the donors are Japanese (skin phototypes II-IV), it remains unknown whether lighter or darker skins have similar tendencies.

In both the forehead and lateral canthus regions, the dermis is thinner at a wrinkle point than that at a nonwrinkle point. The dermis underneath a wrinkle becomes thinner in association with the deepening of the wrinkle until the dermis decreases to one-half of its original thickness. This suggests that when the dermal thickness becomes one-half of its original thickness, the dermis stops thinning further but invaginates into the subcutaneous layer, thereby further deepening the wrinkle. The findings of this study will contribute to the understanding of facial wrinkle development and to the development of antiwrinkle treatment.

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Correspondence: Yuichi Tamatsu, DDS, PhD, Gross Anatomy Section, Kagoshima University Graduate School of Medical and Dental Sciences, 8-35-1 Sakuragaoka, Kagoshima, 890-8544 Japan (tamatsu@dent.kagoshima-u.ac.jp).

Author Contributions: Drs Tsukahara, Tamatsu, and Shimada had full access to all of 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: Tsukahara and Tamatsu.


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