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Article

Exploring the effects of intrinsic aging on human skin: a histologic perspective

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Abstract: Aging remains an inevitable and irreversible phenomenon affecting all living beings. In humans, skin aging carries unique importance due to the critical role appearance holds in contemporary common dynamic. Understanding the structural transformations that occur with aging is essential for comprehending its underlying mechanisms, which is a critical step toward managing, addressing, and potentially reversing these changes. This research underscores the significance of skin aging in healthcare and society by examining the microscopic, morphological, histological, and architectural changes associated with intrinsic aging. Through a histological study of a protected body area, it isolates internal aging effects, focusing on alterations in epidermal thickness and basement membrane structure. During numerous surgical interventions at Al-Hussein Teaching Hospital in Dhi Qar Governorate, skin samples were composed from the anterior abdominal region of 24 male participants spanning different age ranges. Consent for participation was obtained beforehand from all research specimens. The participants were categorized into four separate age categories: group one (ages 0–11), group two (ages 12–24), group three (ages 25–50; n = 6), and group four (ages 51 and above), with 6 participants included in each group. All contributors had to be selected carefully to ensure they did not have previous experiences of hypertensive or hyperglycemia. In the younger age group, the epidermis displayed a consistent and organized cell arrangement across all its layers. In contrast, the older age group exhibited a noticeable reduction in epidermal thickness. A statistically significant difference was identified between the younger and adult groups (Groups 1 and 2) compared to the older age group (Group 3). Furthermore, the thickness of the epithelial basement membrane exhibited a marked increase with advancing age. The examination exposed a decline in epidermal thickness and a reduction in the degree of interdigitation between layers as age progressed. Meanwhile, the papillary dermis verified a considerable increase in thickness, unlike the reticular dermis, which remained relatively unchanged. Specifically, the papillary layer underwent significant expansion, whereas the reticular dermis exhibited a reduction in thickness. Aging was also associated with a decrease in cellular density and vascularization within both dermal layers. This morphometric evaluation of photo-protected skin highlights the substantial impact of endogenous aging concerning with structural integrity of cutaneous layer. Aging significantly

impacts the cutaneous composition, basement membrane architecture, besides overall cellular composition.

Keywords: Cutaneous aging. Intrinsic aging. Morphometric assessment

Introduction

Cutaneous aging is a complicated phenomenon influenced by both intrinsic (internal) and extrinsic (external) influences. The aforementioned combined elements lead to progressive structural and functional alterations in all layers of the skin (Arnal-Forné et al., 2024). The skin acts as a vital barrier, shielding the body from mechanical impacts such as friction and trauma, while also functioning as a sensitive receptor for stimuli like touch and pressure (Karim and Aryani, 2021). Additional roles include regulating body temperature, adapting to changes such as swelling or pregnancy due to its elasticity, and aiding in the production of vitamin D, which is essential for calcium and phosphorus metabolism (Bocheva et al., 2012). Aging, in general, is characterized as a progressive decline in physiological functions, resulting in diminished adaptability to stress and culminating in the later stages of life. Gerontologists often describe this process, known as senescence, as the gradual deterioration of multiple bodily functions, a reduction in reproductive capacity, and an elevated risk of diseases, ultimately leading to organ failure and death (Papaccio et al., 2022).

Two primary mechanisms contribute to skin aging: intrinsic and extrinsic. Intrinsic aging driven via genetics besides the natural passage of time. Whereas in other type, aging is influenced by external conservational externalities for example UV radiation, contamination, smoldering, excessive drinking consumption, and inadequate dietary habits. The aging of the skin is predominantly significant due to the situation visibility and societal influence, making it a prime model for studying the aging process (Sifaki et al., 2025). The "biological clock" influences the layer of skin and the inner organs are the same means, leading to unstoppable deterioration as time progresses (Keaney, 2016).

This study aims to examine the histopathological changes associated with intrinsic aging through a detailed histological assessment of skin from a photo-protected region of the body.

Materials and Methods

Specimens of skin were procured from the anterior abdominal wall of 24 male subjects of varying ages, during surgical procedures at Al-Hussein Teaching Hospital in Dhi Qar Governorate. Consent obtained after providing information secured from every contributors prior to sample collection. The individuals were divided into four age-based cohorts, each consisting of 6 subjects:

- **Group 1** (age 0-11 years; n = 6)
- **Group 2** (age 12-24 years; n = 6)
- **Group 3** (age 25-50 years; n = 6)
- **Group 4** (age ≥ 51 years; n = 6)

All participants were carefully selected to ensure they had no history of hypertension or diabetes mellitus.

Microscopic analysis

Hematoxylin and Eosin (H&E)

Skin samples were obtained and preserved in a 10% neutral buffered formalin solution to prepare tissue sections. The specimens were embedded in paraffin wax, sliced into sections of 5-micron thickness, and subsequently

stained using hematoxylin and eosin (H&E). These prepared sections were meticulously analyzed under a microscope to identify any histopathological alterations.

Periodic Acid-Schiff Technique (PAS)

The Periodic Acid-Schiff (PAS) technique, applied to paraffin-embedded sections, is utilized to visualize age-related changes in basement membrane thickness. This process aimed at the fluorescent periodic acid-acriflavine technique comprises the preparation of two solutions. Solution A consists of 0.5% periodic acid, prepared via dissolving 1 gram of periodic acid in 200 milliliters of distilled water. Solution B is the fluorescent Schiff reagent, created by combining 1 gram of acriflavine with 200 milliliters of distilled water, 2 grams of potassium metabisulfite, and 20 milliliters of 1N hydrochloric acid. This technique enables detailed examination of structural alterations in the basement membrane across different age groups. Dissolve the dye in water, ensuring it is fully mixed. Then, incorporate potassium metabisulfite and hydrochloric acid, stirring thoroughly. Seal the container securely and let it sit in the dark for 72 hours, after which it should be filtered.

Morphometric analysis of the images

Twenty-four abdominal skin samples were assessed. The research received approval from the local Ethics Committee. Digital images (taken with a 2X objective) were captured for each skin specimens utilizing a digital camera attached to an Olympus Bx51 microscope (Olympus Co., Shinjuku-ku, Tokyo, Japan). The thickness of both the epidermis and dermis was measured in micrometers (μm) through numerical calculations utilizing Pixera software along with the image investigation system (latest version-Compix Inc).

Statistical analysis

The collected data were analyzed to determine the average epidermal thickness, with calculations including the standard deviation (SD) and applying the ANOVA test for statistical evaluation. For comparisons, unpaired Student's t-tests were conducted, Significance was evaluated statistically with a two-tailed p-value set at less than 0.05.

Results and Discussion

General histological change

To examine the relationship between aging and alterations in skin layers, the thickness of the epidermis, dermal papillae, and epidermal ridges was assessed. Figures (1a, b) present illustrative low-magnification images representing the four examined groups.

Skin samples from various age groups revealed that the younger group 1 exhibited a consistent arrangement of cells across all epidermal stratum. The epidermis was observed to consist of all five distinct layers with clearly distinguishable, besides a noticeable reduction in the depth of the outermost stratum corneum layer. On the contrast within the dermal layer, numerous regions displayed disordered collagen fibers, particularly in the papillary layer, accompanied by increased cellularity and a reduction in skin appendages, as illustrated in (Figure 1a).

Conversely, the results from participants in group 2 revealed an extensively developed epidermis featuring distinctly identifiable five layers and prominently structured dermal papillae, where exhibited a rise in both the quantity and depth of these structures, as shown in (Figure 1a). This tissue vision comes close to what was reached by a similar study by Lee and Kim, (2021).

Histological analysis revealed that the thinning of the epidermal layer was a prominent characteristic within the older age category (group 4). While the five strata of the epidermis were still identifiable, their distinction was

more challenging, and they appeared reduced in thickness. The dermal papillae exhibited a decline in both their quantity and depth, as depicted in (Figure 1a).

The elderly group (group 4) exhibited a noticeable presence of clearly defined regions with densely pigmented cell clusters within the epidermal layer (Figure 1a). These data are in parallel with a previous study by Kazanci and alper, (2017).

Considering the histological modifications observed in relation to the basement membrane across all experimental groups, it was clear that the basement membrane underwent notable changes as aging progressed. To evaluate these changes, histometric analysis was conducted using Periodic Acid-Schiff's reagent, which is effective for highlighting alterations in basement membrane structure. Within all four age categories, a distinct and clear structure was observed besides definitely demarcated basement membrane was visible, which containing well-established limits clearly distinguishable. Notably, the thickness of the basement membrane appeared to vary with age, becoming more pronounced as the individual aged. These observations are clearly depicted in (Figure 2 a, b), where the changes in basement membrane thickness due to aging are evident across the different age groups, highlighting the progressive nature of these alterations.

Histometric measurement

A noticeable decrease in the thickness of the epidermal layers was detected in the fourth group in comparison with the first group ($p = 0.05$), second group ($p < 0.01$), and the third group ($p < 0.05$) as shown in (Figure 1b. Table 1). The epidermal ridge thickness is at its peak in group two, with a marked decrease ($p < 0.05$) observed between group two and group four (Figure 1c. Table1). A comparable pattern was identified investigating the cutaneous papillae (Laio *et al*. 2014), where the length of groups three and four was considerably shorter in compression with group two ($p < 0.05$) (Figure 1d. Table 1). Likewise, an essentially substantial decline was revealed contrasting the papillary measurements for individuals beyond 51 ages old besides those within 25 and 50 years. Ultimately, ridge and papillae measures showed a small but not significantly different rise among groups of one and two. To assess the integrity of the dermo-epidermal junction, the guide of epidermal interlocking, that reflects the interaction between the two layers of skin at their interface, was computed. When compared to younger adults (group two), the index of epidermal interlocking. showed a significant decrease ($p < 0.05$) within 25 and 50 year old category, with an even more pronounced decline observed in the individuals representing the older group ($p < 0.05$) (Figure 1e. Table 1).

Table 1: The quantitative data collected for each measured criterion in recent work.

Macroscopic Measured parameter	Mean ± standard deviation			
	Group I	Group II	Group I	Group VI
Epidermis thickness (μm)	38.84 ± 1.8	36.55 ± 3.7	37.23 ± 4.	30.22 ± 3.28
Epidermal ridges thickness (μ	88.18 ± 5.4	112.94 ± 5.4	99.27 ± 5.	71.79 ± 5.42
Dermal papillae thickness (μr	66.62 ± 11.1	112.23 ± 19.	71.18 ± 12	61.21 ± 10.17
Interdigitation index	4.41 ± 0.12	3.99 ± 0.89	3.18 ± 0.7	2.91 ± 0.98

Group 1 (0-11 years; n = 6), group 2(12-24 years; n = 6), group 3 (25-50) years; n = 6), group 4(51 years and above; n = 6)

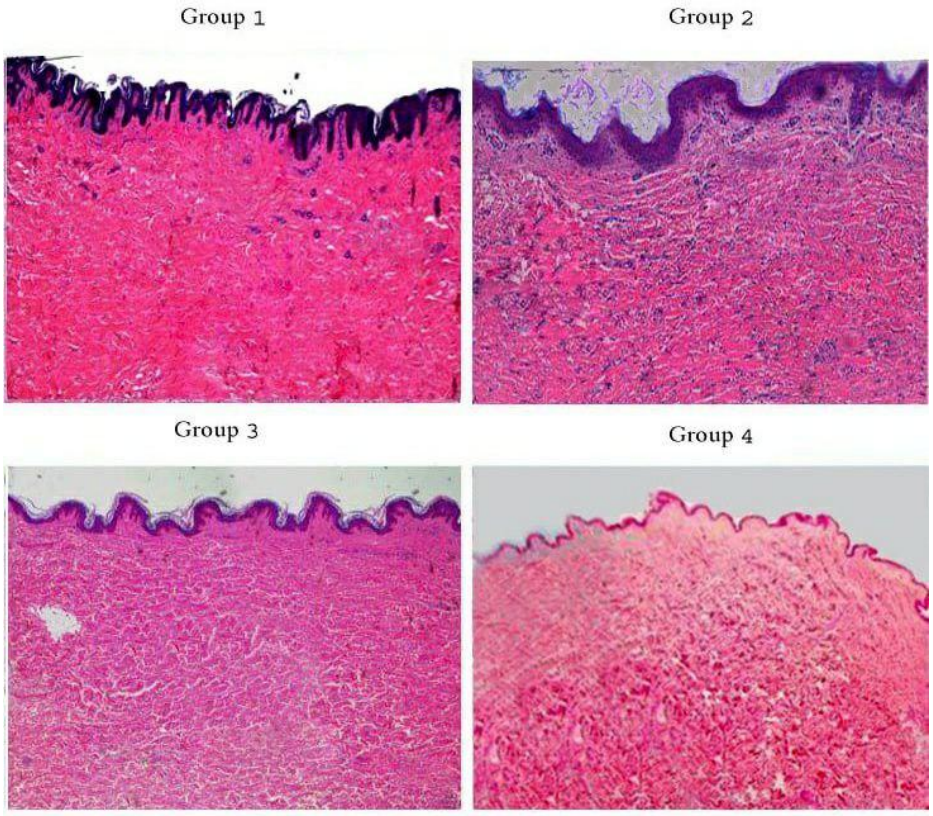
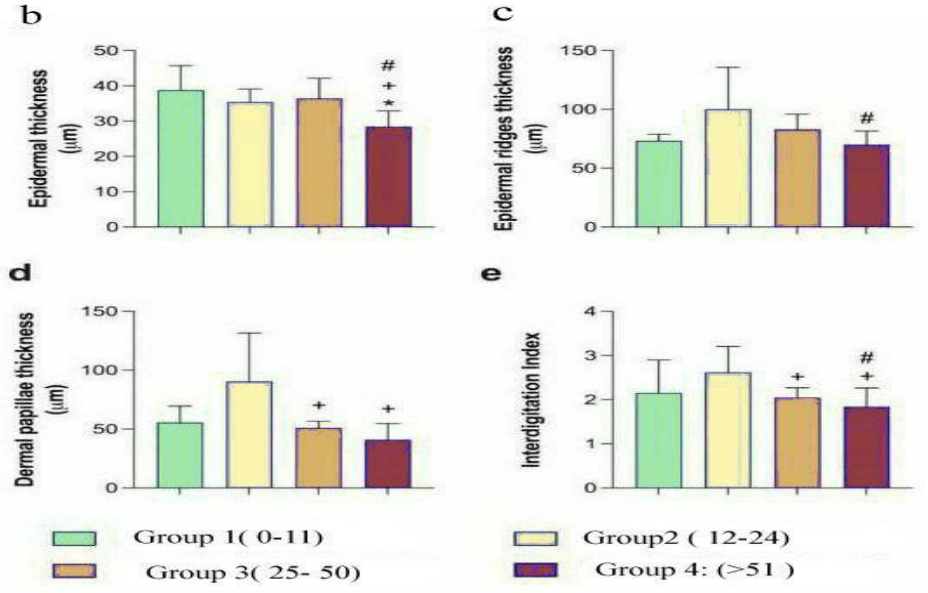


Figure 1a: Assessment of the thickness of the epidermis and dermis layers through morphometric analysis across different age ranges with hematoxylin and eosin stain.



Epidermal thickness (b), ridge thickness (c), dermal papillae (d), and interdigitation index (e) were analyzed using Image-Pro Plus software. A blinded observer scored the images, and data were analyzed via an unpaired Student's t-test. Significant differences were noted with *p < 0.05, **p < 0.01 for group comparisons. Scale bar: 2 mm.

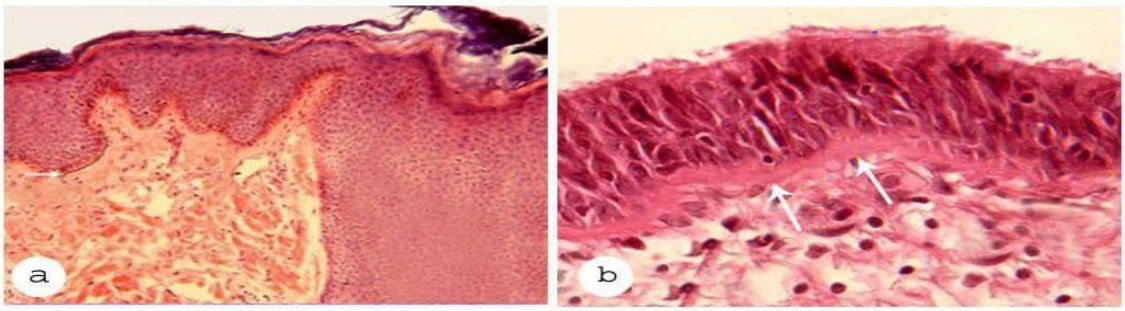


Figure 2 :(a) The skin of the young age group reveals a well-demarcated basement membrane (arrow) positioned directly below the epidermis;**(b)** Skin of an elderly individual displaying the basement membrane (arrow).

The skin, which serves as the outer service of the body, spans a significant surface area of approximately 2 to 3 square meters (Lee and Kim, 2021). The process of skin maturation and its gradual decline over time, which result in progressive variations within its composition, function, and outward morphology, is a multifaceted phenomenon influenced via internal and external influences. Beyond being a natural physiological occurrence, skin aging poses health risks, including increased fragility, delayed and impaired wound healing, a higher susceptibility to infections, and a greater likelihood of developing carcinoma. Chronological or intrinsic aging is evident within extents of the skin not exposed to sunlight, highlighting the role of genetic factors. Conversely, photoaging-an extrinsic form of aging-is primarily driven by ultraviolet (UV) radiation and predominantly affects frequently exposed areas like the face and forearms. Other contributors include air pollution and cigarette smoke (Arnal et al., 2024). In general, structural, functional, and visual changes in the skin are more pronounced in photoaged skin layer in compression with skin affected by chronological aging. However, distinguishing between these two forms of aging is challenging, especially in sun-exposed regions where their effects overlap. Both types share similar clinical features, such as alterations in the dermal matrix, which result in wrinkles, laxity, and increased fragility (Khavkin and Ellis, 2011).

Recent experimental report is dedicated to exploring chronological aging of the skin, excluding the influence of external factors like photoaging, as the skin specimens are collected from a shielded part of the body-the area surrounding the belly button region-utilizing quantitative-morphometric examination. Such method was formerly utilized in the direction of identify microscopic alterations within cutaneous features resulting from various interventions (Costello et al., 2023).

This research explored the relationship between epidermal thickness and age, a topic that has become a focal point in contemporary dermatological studies. The mechanical of the skin properties is influenced by both the thickness and the quality of its epidermal and dermal layers. With aging, the skin undergoes significant qualitative and quantitative changes, including reduced elasticity, diminished collagen levels, and the appearance of wrinkles and age-related lesions (Jin et al., 2021). In contrast, this study revealed notable variations in epidermal thickness associated with aging, with a marked reduction observed in the elderly group. The average measurements indicated no significant differences in epidermal thickness between the younger and middle-aged groups. However, a significant decline was evident in the older age group.

Numerous researchers had documented the connection between the epidermis and its response to varying factors, such as aging, remains complex without arriving at a definitive conclusion. Light microscopy is often regarded as the "gold standard" in assessing epidermal thickness, serving as the benchmark against which other techniques are evaluated (Lintzeri et al., 2022). Other researchers have discovered variations in epidermis thickness at various places throughout the human organism (Oltulu et al., 2018). Exposure to sunlight has been shown to cause thickening of the skin's stratum corneum. Kaddurah et al. (2018) detected that sun-protected areas of the body had a thinner stratum corneum in comparison with the regions frequently exposed to sunlight. In this present research, a significant variation in epidermal thickness was observed when comparing the younger adult group with the older age group, nevertheless there was not any apparent distinction among the younger and adult age

groups. The conclusions from our investigation of the dermal layer exposed a thickening of the papilliform dermis alongside a thinning of the reticular dermis as aging progresses. Our findings recommend that intrinsic aging influences the distribution of glycosaminoglycans (GAGs), demonstrated via their increased concentration within the papillary dermis besides a corresponding decline within the inner dermal layer. Certainly, these findings differ significantly from what is currently reported in the existing literature, highlighting a contrast with the general trends observed in previous studies. Certain research imply that intrinsic aging of the skin generates overexpression of GAG (Li et al., 2022). In contrast, other studies have indicated that photoaging, rather than intrinsic aging, is responsible for the elevated expression of glycosaminoglycans (GAGs) (Oh et al., 2018). Additional studies have revealed that specific glycosaminoglycan (GAG) components in the skin, such as decorin proteoglycan, undergoes a decrease in extent, resulting in a diminished dermal region containing GAG (Li et al., 2013). Additionally, the expansion of the upper dermal layer or superficial dermis

is able to be interpreted in consequence of the of the diminished quantity as well as extent of ridges and upper dermal layer, which result in the papillary dermis filling the resulting space.

Basement membranes are integral to vital biological functions, acting as an extracellular structure that facilitates the morphological development and thickness differentiation (Kanazawa et al., 2021). As far as we know, no prior research has been found that specifically measures the thickness of the basement membrane and its variations throughout the aging process. In order to explore the connection between the skin's basement membrane and age-related skin changes, histometric analysis was conducted. The results revealed a notable disparity in basement membrane thickness among the three distinct age categories. There was a marked and significant increase in the thickness of the epithelial basement membrane as age progressed. Additionally, no prior research was initiate that have clarified the dimension of basement membrane thickness and its alterations over the course of aging.

Conclusion

Based on the results of the current study, it can be concluded that significant morphological and histological changes occur in the skin as age progresses. Specifically, the thickness of the epidermal layer decreases with age. However, this reduction that represents the difference in thickness between the young and adult groups was not statistically significant, whereas a highly significant variation was observed between the adult and elderly groups.

Furthermore, such thickness of the cutaneous basement membrane showed a significant increase across all samples that were studied histologically and morphometrically.

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