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Age-related Changes in Human Skin: Histological, Morphometric and Immuncytochemical Study Using S100

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Abstract

Background Aging has many effects on a person's skin, from wrinkles and sags to increased risk of certain skin conditions, such as skin cancer. As people age, their skin begins to change due to environmental, genetic, nutrition and other factors.

Objectives Understand some of the changes that occur in aging skin including changes in the general morphological, histological and architectural arrangement, epidermal thickness, basement membrane and histochemical changes in melanocytes.

Methods Skin specimens were taken from the anterior abdominal wall of 30 human males at different ages. General histological preparation for paraffin blocks was performed and the blocks were sectioned at (5-6µ) and stained with H&E. S100 protein was used to demonstrate immunohistochemistry labeled melanocytes changes with age. Histometric measurement of epidermal thickness and basement membrane thickness, using eyepiece graticule was performed on these groups.

Results The young age group showed a uniform arrangement of cells in all stratums of the epidermis while the old age group showed diminished thickness of the epidermis. A significant difference between young- adult age group (A and B groups) and the old age group (C group) was recorded. The epithelial basement membrane thickness was increased with age significantly (P value ≤ 0.001). Melanocytes demonstration using S100 showed that these cells tend to be situated at the tips of rete pages, there number are generally low and didn't varied a lot between young and adult age groups. There was yet marked decline in the number of melanocytes in old age group.

Conclusion Aging as a process have a marked influences on skin morphology, thickness, cellularity and basement membrane.

Key Words Aging, skin, S100, morphometry and basement membrane

Introduction

The skin is one of the largest and widest organs of the body, accounting for about 16% of the body weight [(1)]. Skin performs many important functions; it protects the body organism from impact and friction injuries. It is a wide sensitive organ to receive special stimulus by touch, pressure [(2)]. Regulation of body temperature, because of skin elasticity, it can expand to cover large areas in conditions associated with swelling, such as edema and pregnancy, and formation of vitamin D which is necessary for metabolism of phosphor and calcium [(3)].

Aging can be defined as a progressive, generalized impairment of function resulting in a loss of adaptive response to a stress or as the 'latter part of animate life. Gerontologists use the term senescence to describe aging as a progressive deterioration of many body functions over time, marked by a decrease in fertility and an increased risk for diseases,

culminating in multi-organ failure and death [4].

There are two main processes that induce skin aging: intrinsic and extrinsic aging. Intrinsic aging reflects the genetic background and depends on time. Extrinsic aging is caused by environmental factors such as sun exposure, air pollution, smoking, alcohol abuse, and poor nutrition [5].

Skin aging is particularly important because of its social impact. It is visible and also represents an ideal model organ for investigating the aging process [6]. "Biological clock" affects both the skin and the internal organs in a similar way, causing irreversible degeneration [7].

We aimed in this study to follow up changes of the skin with aging on the following aspects:

- General epidermal changes and its architecture
- Epidermal thickness changes.
- Basement membrane changes.
- Immunolabelled melanocytes changes.

Methods

Skin specimens were taken from the anterior abdominal wall of 30 human males at different ages, in the operating theater in Al-Kadhimiya teaching hospital. Approval from all individuals was taken prior to the operation. Samples were grouped into three age groups each consisted of (10) individuals:

Group A (1-9) years,
Group B (12-30) years and
Group C (40) years and above.

All individuals selected at different age group, was disease free in regard to hypertension and diabetes mellitus. They were stained with:

Haematoxylin & Eosin (H&E): used with paraffin sections to demonstrate the morphological changes in the skin with age including epidermal and basement membrane.

Periodic Acid-Schiff Technique (PAS): used with paraffin sections to demonstrate the changes of basement membrane thickness with age.

S100 protein was use to demonstrate melanocytes changes with age.

Nuclear differentiation special stain (NDS)[8]: composed of two solutions:

Solution A: Basic fuchsine 0.4gm in 100ml of (2.5%) methanol.

Solution B: Prepared by mixing equal volumes of: Azure II, Methylene blue, Na_2Co_3 in ethanol alcohol

Histometricale measurement of basement membrane and epidermal thickness using eye-piece graticule was perform on these groups, for each age groups (10) sections were selected randomly for each age group, and for each section (10) readings was divided (5) for rete pages and (5) for area with distinct deep dermal papilla. Data were collected and the mean for epidermal thickness was calculated, standard deviation (SD) and ANOVA test were performed on these data.

Results

General histological study

Skin at different age groups showed that young group (A) had a uniform arrangement of cells in all strata of the epidermis. All five layers of the epidermis were easily identified with decrease in the thickness of stratum cornium. The dermis showed many areas of disorganised collagen fibres especially in papillary layer with high cellularity and diminished skin appendages as seen in (Figure 1).

Adult group (B) showed a thick epidermal layer with distinct five strata and a well recognized dermal papillae which have shown an increase in number and depth as seen in (Figure 2).

The thickness of the dermis was strikingly increased with uniformed thick parallel arranged collagen fibres with distinct skin appendages (Figure 3).

Diminished thickness of the epidermis was a sticking feature in old age group (C).

Distinction among the five strata of the epidermis was not easy yet they remained present in a uniform way, although they were diminished to some extent. Dermal papillae showed decrease in both; there number and shallowness (Figure 4 & 5).

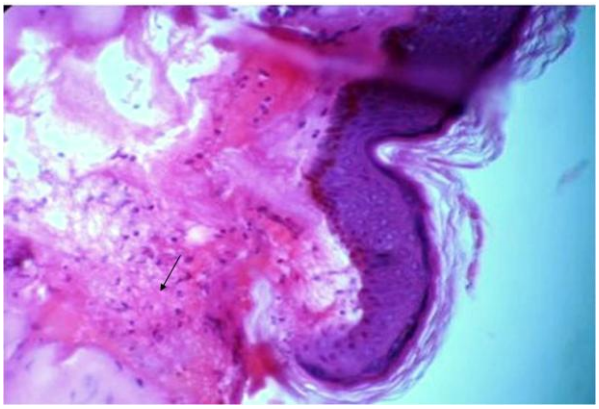

Figure 1. Section in the skin of young individual showing epidermis, the disarrangement & disorganized collagen (arrow) fibers with high cellularity of the dermis. Young age group (A), H&E, 400X

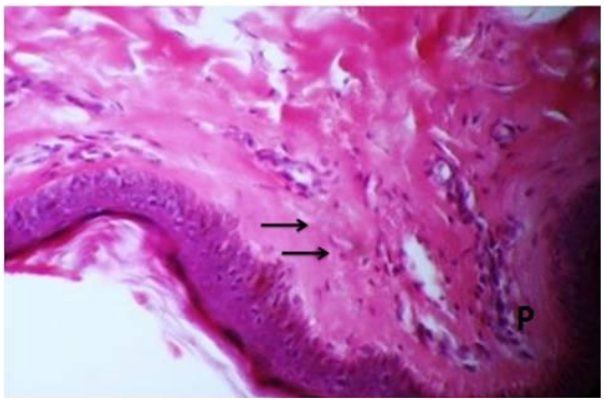

Figure 2. Section in adult skin showing the dermal papilla with well homogenized dermal fibers (arrow) and high cellularity of papillary layer (P). Adult age group (B), H&E, 400X

Old age group (group C) showed frequent appearance of well demarcated area of highly pigmented cell patches inside the epidermal layer (Figure 6).

Histometric measurement of epidermal thickness:

Histometric measurement of epidermal thickness using eyepiece graticule was perform on these groups, for each age groups (10) sections were selected randomly and for each section (10) readings was divided (5) for rete pages and (5) for area with distinct deep dermal papilla.

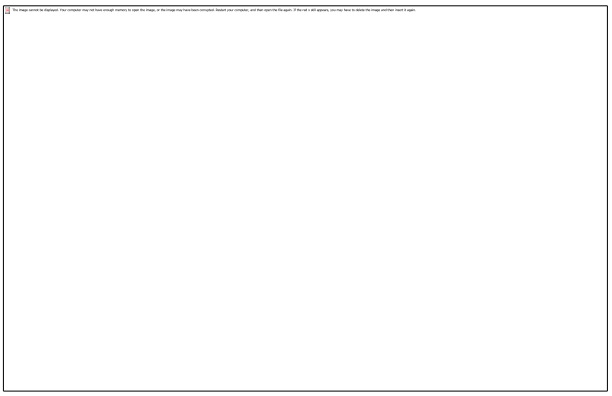

Figure 3. Section in adult Skin showing both epidermis and dermis with hair follicles (H) and sebaceous glands (S). Notice the thick dermis (D). Adult age group(B), H&E, 400X

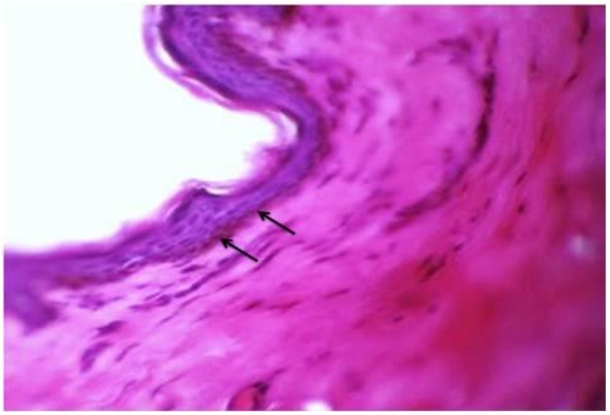

Figure 4. Decrease epidermal thickness with flatting epidermal-dermal ridges (arrow). Old age group(C), H&E, 400X

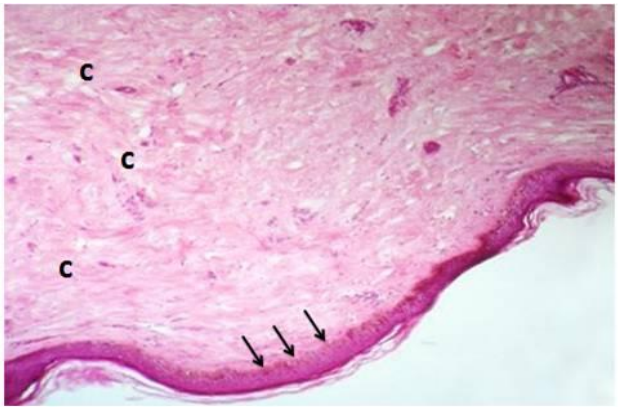

Figure 5. Decrease numbers of dermal papilla and flatting of the dermal-epidermal ridges (arrow) with diminished skin appendages and separation of collagen fibers (C). Old age group(C), H&E, 100X

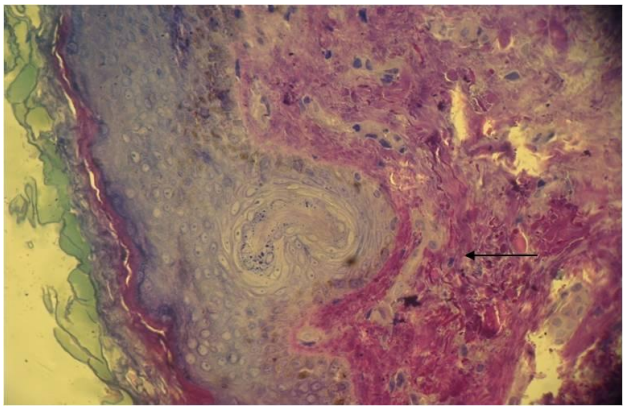

Figure 6. Epidermal patches, with disorganized collagen fibers below it (arrow). Old age group(C), NDS stain, 400X (Medium magnification)

Mean for each age group was calculate as show in (Table 1).

Table 1. Showing mean of epidermal thickness of each age groups.

Age groups	Mean ± SD of epidermal thickness
A (young age)	69.39 nm ± 2.85
B (adult age)	67.78 nm ± 3.07
Group C	10.90 nm ± 0. 40

Statistical analysis of the epidermal thickness was done using (Independent-sample t-test), there was a significant statistical difference between young and adult age group with old age group. The mean thickness of young age group (69.39 ± 2.85 nm), and adult age group (67.78 ± 3.07nm), while the old age group show a marked decrease in thickness of the epidermis with mean of (46.49 ± 2.33nm).
Independent-sample t-test show significant changes in thickness of the epidermis between each of group A & B with group C, with a (P value ≤ 0.001) as shown in (Table 2).

Changes and histometrical measurement of basement membrane:

Periodic acid Schiff's reagent was used to demonstrate and evaluate basement membrane changes. In all three age groups a well demarcated distinct basement membrane with clear boundaries was seen, thickness of basement membrane seem to changes as aging advance seen in (figure 7 and 8).

Table 2. The mean ±SD of epidermal thickness of the three age groups.

Age groups	Mean ± SD
Group A Group B	69.390 ± 2.858 (ns) 67.781 ± 3.076
Group A Group C	69.390 ± 2.858 * 46.491 ± 2.337
Group B Group C	67.781 ± 3.076 * 46.491 ± 2.337

* = P<0.001, ns= non significant

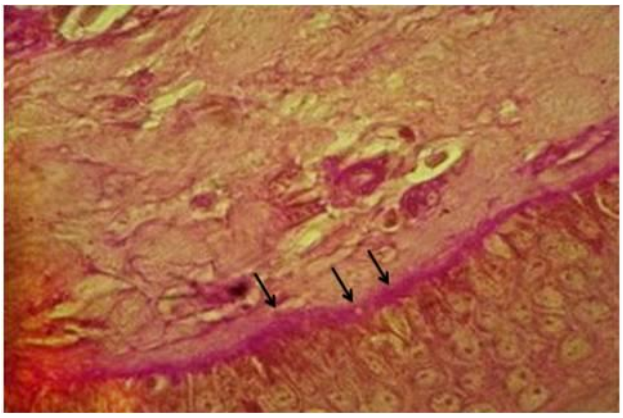

Figure 7. Young age group skin showing a distinct basement membrane (arrow) underlying the epidermis. Young age group, PAS, 400X

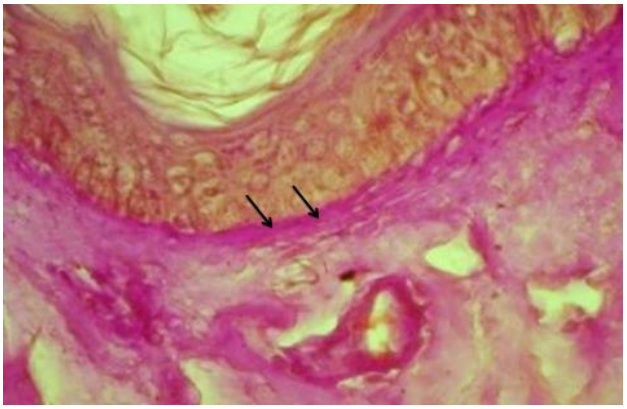

Figure 8. Old age individual skin showing the basement membrane (arrow) in PAS stain. Old age group, PAS, 400X

The mean thickness of basement membrane showed gradual increase in thickness as seen in (Table 3).

Table 3. Showing mean of basement membrane thickness of each age groups.

Age groups	Mean ± SD of basement membrane thickness
A	5.41 nm ± 0.3
B	8.30 nm ± 0.380
C	10.90 nm ± 0.40

Statistical analysis showed that there was significant statistical difference between the three age groups. The mean thickness of basement membrane of young age group (5.41± 0.31 nm) and adult age group (8.30± 0.380 nm), while the old age group show a marked increase in thickness of basement membrane with mean of (10.90 ± 0.40) as show in (Table 4). There was a gradual increase in thickness of the basement membrane starting from young age group, reaching the maximum thickness in old age group.

Statistical analysis showed significant changes in thickness of basement membrane between all age groups (A, B & C) with P value of (P≤0.001) as shown in (Table 4).

Table 4. The mean ±SD of basement membrane thickness in three age groups.

Age groups	Mean ± SD
Group A Group B	5.416 ± 0.31* 8.300 ± 0.38
Group A Group C	5.416 ± 0.31* 10.900 ± 0.40
Group B Group C	8.300 ± 0.38* 10.900 ± 0.40

* = P<0.001

Immunohistochemical study:

Studied section of S100 showed a number of melanocytes characterized by elongated or ovoid nuclei surrounded by clear space, they are usually smaller than neighboring basal keratinocytes, and appear associated between keratinocytes of stratum basale with darkly stain nuclei and obvious cytoplasmic granules (Figure 9). Melanocytes tend to be situated at the tips of rete pages, their numbers are generally low and did not vary a lot between young and adult age group, no significant differences was found between young and adult age groups (A and B) (figure 9).

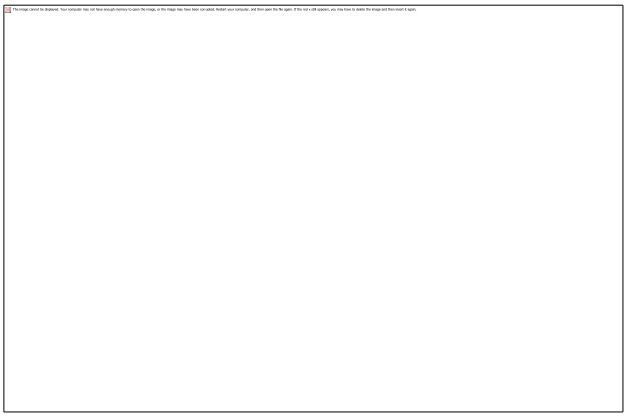

Figure 9. Melanocytes cells (M) at the tips of rete page in young age group. S100, 1000X (High magnification)

There was a marked decline in the number of melanocytes in old age group; they were difficult to be identified in the stratum basale as it was seen in (Figure 10).

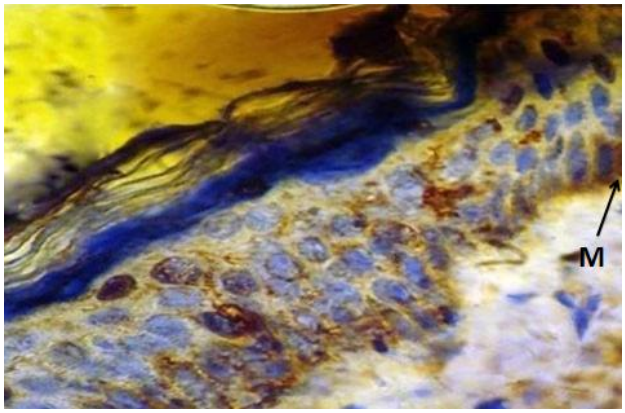

Figure 10. Melanocytes cells (M) in the stratum basale with darkly stain nuclei and obvious cytoplasm granule. S100, 1000X (High magnification)

Discussion

The skin covers the entire external surface of the human body; it is the principle sit of interaction with surrounding world. It serves as a protective barrier that prevent internal tissue from trauma, UV radiation,

temperature extreme, toxins and bacteria. Skin changes are among the most visible signs of aging. Evidence of increasing age includes wrinkles and sagging, along with the obvious graying of the hair, and with aged skin, histological and biochemical as well as function changes occur [9].

Collagen density in skin decrease annually with an average of 2% but Raine-fenning et al [10] calculated the number of collagen fibers in the skin diminished by (30-35%) after first (5years) of menopause.

The mean fractional volume of collagen fibers determined from stereological data is reported to be between (66 and 69%) for both papillary and reticular dermis for all age groups studied by [11,12].

In this study, it was found that aging Aging have influence the dermo-epidermal junction profoundly, dermal papillae flatten with the loss of the rete pegs, resulting in increased slippage between the epidermis and dermis.

The dermo-epidermal junction seem to be affected by an inter reaction of anchoring fibers, during the entire period of hormonal activity which they well affected collagen type IV and collagen type VII, the lack of hormonal stimulation causes decrease in production of these fibers, by the fibroblast of the corium, the shortening of collagen type IV in many structural protein may cause flatting of central epidermal border, which result in disturbance in the exchanges of nutritional component and also will caused thinning of the skin [13].

Epidermal thickness and its relation to age was investigated in this study as it formed the common study in dermatology research nowaday, The mechanical properties of the skin are due to the thickness and qualitative properties of epidermis and dermis. During age qualitative and quantitative change occur in the skin, lack elasticity and collagen content, increase wrinkling and aging lesions [14].

Epidermal thickness showed wide changes with aging in this study, a sticking diminished epidermal thickness was features of the old age group. The mean epidermal thickness measurement showed no significant differences between epidermal thickness in young and adult age group, with mean of (67-69 nm), while old age group showed significant decrease in epidermal thickness with mean of (45nm).

Many authors had reported the relation of epidermis and its interaction to variance factors, including aging without drowning a firm conclusion. Light microscopy may still be considered the ‘‘gold standard’’ for measurement of epidermal thickness, and by which other methods are compared [15]. Others have found difference in the epidermal thickness using different sites in the body [16]. Sun light exposure has been proved to induce thickening of skin corniume ([17] found thinner skin corniume in sun protected body sites compared to exposure body sits. In the current study a significant difference in epidermal thickness was found between (young- adult age group) and between the old age group but, no convincing difference in the epidermal thickness between young and adult age group.

Basement membranes are directly involved in the important biological process; it represents an extracellular scaffold that is necessary for morphological differentiation of thickness [18]. No previous study regarding measurement of basement membrane thickness and its changing during aging was found.

To determine the relationship between skin basement membrane and skin changes during age, hisometrical measurement of the basement membrane was performed, the outcome yields a significant difference between the three age groups.

Epithelial basement membrane thickness increased with age significantly with a (P value ≤ 0.001). We did not find previous study that has dealt with measurement of basement membrane thickness and its changing during aging.

Although changes of basement membrane in the other body parts were investigate by many authors, researcher seems to be more concerned with the structure, functional and architecture of basement membrane, due to the direct relationship between basement membrane and much significant biological process in the body [18].

Type IV Collagen fibres being one of the constituents of basement membrane of the skin had been study by (Vazquez et al [13], who evaluated type IV collagen fibres by immune labelling and morphometrical study. It was found that the thickness of Type IV fibres, plotted against age, showing a highly significant positive correlation. The increment in thickness was found to be significant in old age group 50 years and above.

Melanocytes are commonly distributed along the basal zone of the epidermis. It is one of the cells that cannot be identified by routine histological stain. Using S100 protein marker we were able to observe melanocytes changes.

Immunohistochemical labelling of melanocytes using S100 was performed in this study to demonstrate them. Melanocytes are commonly distributed along the basal zone of the epidermis, they represents type of cells that are difficult to be identified by routine haematoxylin and eosin stain.

S100 is a protein marker that has the ability to localize melanocyte cells and their changes in different conditions, and it is considered as the most frequently used marker in clinical practices. Monoclonal antibody labelled to S100 is a calcium binding portion that is usually originated by isolated from the brain and its sensitive marker that reacts with abroad rang of benign and malignant neoplasm as well as normal melanocytes, therefore is considered as a highly specific melanocytes marker [19].

Melanocytes tend to be situated at the tips of rete pages, there number are generally low and didn't vary a lot between young and adult age groups. Yet there was a marked decline in the number of melanocytes in old age group.

Examining melanocytes using S100 we did not depended on the density of the reaction but we depend upon the presence of cytoplasmic granules in the melanocytes cytoplasm, a features that is approved by all histopathologist.

Melanocytes number and its capability of synthesising melanosomes pigment is a highly affected by the process of aging, melanocytes had shown decrease in the life span as well as a decrease in the response to growth factor [20].

Aging seemed to reduce the immune functions in a naturally aged skin a function that can be referred to UV radiation in non aged skin. The numbers of melanocytes are proved to be highly effected in long standing sun exposure [21].

Conclusion

In the light of these findings of the present study, it was concluded that:

a) Many morphological and histological changes may be observed on skin with age advance.
b) The epidermal layer showed decreased on its thickness with age. This decreasing was non significant between young and adult age groups, but was highly significant between adult and old age groups.
c) The basement membrane of skin showed significant increasing in its thickness in the three age groups.
d) Immunolabelled melanocytes appeared to be affected with skin aging.

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