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Research Article

The occurrence of cutaneous aging according to age of subjects examined

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Abstract. Aim: This study aims to observe the changes in the structural components of the skin according to patient age. Material and methods: The study included 53 patients. In all patients, skin biopsy was taken from the surplus area from routine excisions, further performing histopathology. Patients were selected from those admitted to the surgery ward of the Municipal Clinical Hospital of Cluj-Napoca between January 2013 and May 2013 for various surgeries. The average age of patients was 51.7 ± 18.5 years. The following data were collected from the histological examination of biopsy samples: epidermal thickness, presence of the "honeycomb" pattern, aspect of keratinocytes, percentage of dotted pigmentation, aspect of dermal papillae, aspect of dermal capillary network, presence of different collagen fibers. Results: Mean values for epidermal thickness were 123.4 ± 47.8 µm. There was an average statistically significant negative correlation between patient age and epidermal thickness (r=-0.410, p=0.002). Patients with regular "honeycomb" pattern were younger (40± 11.6 years) than those with slightly irregular architecture (71±9.1 years) (p<0.001). The age of patients with capillary network disorganization was of greater statistical significance in older subjects (74.5±9.4) than in those without capillary network disorganization (46.4±15.9) (p<0.001). Thin reticular collagen was described in 17 (32.1%) patients, coarse collagen in 30 (56.6%) patients and huddled collagen in 6 (11.3%) patients. There were statistically significant differences in patient age depending on the type of collagen (p<0.001). Conclusions: Aging skin was marked by reduced epidermal thickness, the more frequent presence of the slightly irregular "honeycomb" pattern, of a disorganized capillary network, and of huddled collagen.

Key Words: age, skin, biopsy.

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Introduction

The technological advances of the twentieth century allowed for the establishment of a complex foundation for advanced medicine based mainly on primary and secondary prevention. With rising living standards and the development of preventive medicine, life expectancy has also increased. It is estimated that over the next 25 years, the number of people aged over 65 will double. By 2030, about 20% of the U.S. population will be represented by the elderly (Census 2010). In the past century, there has been a radical change in the main cause of death due to infectious diseases, like tuberculosis, or chronic degenerative diseases. This change affects the elderly, of which more than 60% suffer from chronic diseases (Nolte & McKee 2003). Skin conditions are common in the elderly population. Due to the fact that about 90% of the elderly have skin diseases, it is extremely difficult to differentiate between physiological skin changes that occur with age and those due to illness (Minaker 2011).

This study aims to observe the changes in the structural components of the skin according to patient age.

Materials and methods

This is an observational, prospective, analytical, longitudinal cohort study.

The study included 53 patients. In all patients, skin biopsy was taken from the surplus area from routine excisions, further performing histopathology. Patients were selected from those admitted to the surgery ward of the Municipal Clinical Hospital of Cluj-Napoca between January 2013 and May 2013 for various surgeries. The average age of patients was 51.7 ± 18.5 years.

Subjects were included in the study after signing a consent form for inclusion in the study and for performing genetic determinations.

The study protocol was approved by the Ethics Committee of "Iuliu Hatieganu" University of Medicine and Pharmacy Cluj-Napoca.

Inclusion criteria

Patients older than 18, who have signed the informed consent form and who were about to undergo abdominal surgery, were enrolled in the study.

Exclusion criteria

The following were not included in the study: patients under 18 years of age, patients who did not sign the informed consent form, patients diagnosed with abdominal or systemic skin diseases, cancer, and autoimmune diseases.

Variables

The following demographics were recorded for each patient: age, gender, area of origin (urban or rural).

The following data were collected from the histological examination of biopsy samples: epidermal thickness, presence/absence of the "honeycomb" pattern, aspect of keratinocytes, percentage of dotted pigmentation, aspect of dermal papillae, presence/absence of sebaceous glands, aspect of dermal capillary network, presence/absence of thin reticular collagen, presence/absence of coarse collagen, presence/absence of huddled collagen, presence/absence of corrugated flexible structures.

Statistical analysis

Statistical analysis was performed using MedCalc software version 13.3.1.0. Data were classified as quantitative and ordinal variables. Ordinal variables were described by calculating frequency and percentage. Quantitative variables were calculated using mean and standard deviation, after testing for normality of the distribution using the Kolmogorov-Smirnov test.

Univariate analysis of normally distributed continuous variables, based on a dichotomous variable, was performed using the independent-samples t-test. The correlation between two normally distributed quantitative variables was done using Pearson's correlation. ANOVA was used to calculate the differences between three or more categories within a quantitative variable.

The chi-squared test was used for univariate analysis of ordinal variables. The p value <0.05 was considered statistically significant.

Results

Minimum patient age was 19 years and maximum patient age was 88 years. Age values were normally distributed. The study included 25 (47.2%) women and 28 (52.8%) men.

Most patients were aged over 65 (14), while the least of them were part of the age group 56-65 years.

Mean values for epidermal thickness were 123.4±47.8 µm. Minimum thickness was 24 µm and maximum 268.3 µm. Epidermal thickness values were normally distributed.

There was an average statistically significant negative correlation between patient age and epidermal thickness (r=-0.410, p=0.002).

There were statistically significant differences between age groups in terms of epidermal thickness (p=0.03).

Regarding the precise differences in epidermis thickness between different age groups, there were statistically significant differences only in patients aged over 65 years, compared to those aged between 19 and 35 years (p=0.01, table 1).

There were no differences in epidermal thickness between women and men (p=0.7). A slightly irregular "honeycomb" pattern was described in 20 (37.7%) patients. Patients with regular "honeycomb" pattern were younger (40±11.6 years) than those with slightly irregular architecture (71±9.1 years) (p<0.001). The frequency of the slightly irregular "honeycomb" pattern was higher in the age groups 55-65 years and >65 years than in other age groups (p<0.001, table 2). Slightly irregular-shaped keratinocytes and minor atypia were described in 20 (37.7%) patients. Patients with polygonal keratinocytes, without atypia, were younger (40±11.6 years) than those with slightly irregular-shaped keratinocytes (71±9.1 years) (p<0.001).

Table 1. Comparison between age groups in terms of epidermal thickness

(I) Group	(J) Group	Mean difference (I-J)	P	95% CI	
				Min.	Max.
19-35 years	36-45 years	23.4	0.7	-33.5	80.5
	46-55 years	37	0.3	-17	91.2
	56-65 years	21.6	0.8	-37.3	80.5
	>65 years	58.4	0.01	7.2	109.5
36-45 years	19-35 years	-23.4	0.7	-80.5	33.5
	46-55 years	13.5	0.9	-43.4	70.6
	56-65 years	-1.8	1	-63.5	59.8
	> 65 years	34.9	0.3	-19.3	89.1
46-55 years	19-35 years	-37	0.3	-91.2	17
	36-45 years	-13.5	0.9	-70.6	43.4
	56-65 years	-15.4	0.9	-74.4	43.5
	> 65 years	21.3	0.7	-29.8	72.4
56-65 years	19-35 years	-21.6	0.8	-80.5	37.3
	36-45 years	1.8	1	-59.8	63.5
	46-55 years	15.4	0.9	-43.5	74.4
	> 65 years	36.808	0.3	-19.4	93
> 65 years	19-35 years	-58.4	0.01	-109.5	-7.2
	36-45 years	-34.9	0.3	-89.1	19.3
	46-55 years	-21.3	0.7	-72.4	29.8
	56-65 years	-36.8	0.3	-93	19.4

Table 2. The "honeycomb" pattern by age

		Group				
		19-35 years	36-45 years	46-55 years	56-65 years	>65 years
"Honeycomb" pattern	Regular	11	9	11	2	0
		33.3%	27.3%	33.3%	6.1%	0.0%
	Slightly irregular	0	0	0	6	14
		0.0%	0.0%	0.0%	30.0%	70.0 %

The frequency of slightly irregular-shaped keratinocytes was higher in the age groups 55-65 years and >65 years compared than in other age groups (p<0.001).

There were significant age differences depending on the percentage of visible dotted pigmentation (p=0.01). Differences were more obvious within the group with 20% dotted pigmentation (p=0.007). There were differences between age groups in terms of percentage of visible dotted pigmentation (p=0.01).

The presence of flattened dermal papillae was observed in 10 (18.9%) patients. Patients with flattened dermal papillae were older (68.5 ± 3.7) than those with normal dermal papillae (47.8 ± 17.7) (p=0.001). Flattened dermal papillae were more common in patients enrolled in advanced age groups (p=0.008 (tabel 3).

Atrophic sebaceous glands were only described in 2 (3.8%) subjects. Capillary network disorganization was observed in 10 (18.9%) patients. The age of patients with capillary network disorganization was of greater statistical significance in older

subjects (74.5±9.4) than in those without capillary network disorganization (46.4±15.9) ($p<0.001$, table 4).

Table 3. The presence of normal/flattened dermal papillae by age

		Group				
		19-35 years	**36-45 years**	**46-55 years**	**56-65 years**	**>65 years**
Dermal papillae	Flattened	0	0	2	1	7
		0.0%	0.0%	20.0%	10.0%	70.0%
	Normal	11	9	9	7	7
		25.6%	20.9%	20.9%	16.3%	16.3%

Table 4. The presence of normal/disorganized capillary network by age

		Group				
		19-35 years	**36-45 years**	**46-55 years**	**56-65 years**	**> 65 years**
Dermal capillary network	Normal	11	9	11	6	6
		25.6%	20.9%	25.6%	14.0%	14.0%
	Disorganized	0	0	0	2	8
		0.0%	0.0%	0.0%	20.0%	80.0%

Thin reticular collagen was described in 17 (32.1%) patients, coarse collagen in 30 (56.6%) patients and huddled collagen in 6 (11.3%) patients. There were statistically significant differences in patient age depending on the type of collagen ($p<0.001$). Age differences were obvious for all collagen types (table 5). The frequency of collagen types differed by age.

Table 5. Collagen types by age

		Group				
		19-35 years	**36-45 years**	**46-55 years**	**56-65 years**	**> 65 years**
Collagen	thin	11	3	3	0	0
	reticular	64.7%	17.6%	17.6%	0.0%	0.0%
	coarse	0	6	7	8	9
		0.0%	20.0%	23.3%	26.7%	30.0%
	huddled	0	0	1	0	5
		0.0%	0.0%	16.7%	0.0%	83.3%

The presence of flexible wavy skin structures was observed in 15 (28.3%) patients. The age of patients with corrugated flexible skin structures was of greater statistical significance in older subjects (69.5±12.7) than in those without capillary network disorganization (44.7±15.5) ($p<0.001$).

Discussions

The skin is the largest organ of the human body and it accounts for approximately 15-20% of its body weight (Yannas 2001). Even if the primary function of the skin is to protect the muscles, bones and internal organs, it has many other functions. Thus, the skin has an important role in maintaining homeostasis, preventing excessive percutaneous loss of electrolytes and proteins, in tactile and thermal sensitivity, and in immunity (Farage et al 2007).

The skin is the most obvious indicator of aging. By the use of skin biopsy samples taken from areas not exposed to the sun, Branchet et al. showed a progressive loss of skin components of approximately 7% per decade of age. However, this skin loss reveals big differences between individuals (Branchet 1990). Cutaneous aging is a complex process involving both intrinsic and extrinsic factors. Skin-tissue reduction is mainly due to the loss of cells and extracellular matrix (Oender et al 2008). Aging skin also leads to the emergence of specific pathologies. Thus, about 7% of elderly outpatients suffered from dermatological diseases. Moreover, approximately 50% of older people suffer from treatable skin diseases (Beauregard et al 1987).

This study investigated the changes observed in certain skin components for areas of skin not exposed to the sun, in patients with different ages.

We obtained an average negative correlation between patient age and epidermal thickness. The relationship between age and epidermal thickness was a relatively linear one, with a deviation in data linearity in patients aged between 56 and 65 years. The greatest difference in thickness was observed when comparing younger patients aged between 19 and 36 years with those older than 65 years (58 μm). Using optical coherence tomography (OCT), Gambichler et al. measured epidermal thickness and showed significant differences based on age, regardless of the anatomical region examined. One of the reasons for decreased epidermal thickness is the decrease in villous cytoplasmic projections into the dermis with age (Gambichler et al 2006).

The presence of the slightly irregular "honeycomb" pattern, of slightly irregularly-shaped keratinocytes and of minor atypia was more frequent in the elderly patient group. Other studies have shown the same findings, in both areas quasi-permanently exposed to the sun and those less frequently exposed to the sun (Longo et al 2001; Busam et al 2001; Wurm et al 2012; Liao et al 2013). With age, keratinocytes start to have an irregular shape and lose their organized structure.

In this study, flattened dermal papillae were more frequently described in elderly patients. The average age difference between patients with flattened dermal papillae and those with normal papillae was approximately 20 years. Flattening of dermal papillae could be part of the aging process, with cell loss in the papillary dermal compartment. The reduced potential of fibroblast growth in the dermis with age was determined 40 years ago, but their papillary location has only recently been demonstrated (Smith & Hayflick 1974; Mine et al 2008). As the body ages, dermal papillae tend to flatten and can increase in number. In the latter case, each papilla seems to turn into a group of cells, remaining at the same level, but with much smaller individual size. Flattening or even disappearance of papillae in the dermoepidermal junction has also been found in other studies (Montagna & Carlisle 1989; Sauermann et al 2004).

The disorganized capillary network was more frequently described in elderly patients in our study (80%), compared to young patients (0%). Patients with a disorganized capillary network were approximately 30 years older than those with a normal capillary network. Aging skin is associated with the reduction and disorganization of capillaries and small vessels, a regression of vascular density (Grove 1989; Waller & Maibach

2005). The presence of a disorganized capillary network was also more common in the elderly in other studies (Li et al 2006). Collagen makes up 70–80% of the dry weight of the dermis (Makrantonaki et al 2007). Thin reticular collagen fibers form a delicate network around the apertures of hair follicles. In our study, this type of collagen was detected in younger patients (34.6 years). Adhesion is a common tendency observed in coarse collagen fibers, however, these fibers also have a more irregular network and are larger in size. In our study, coarse collagen fibers were found in adults (58.1 years). Huddled collagen (huddled) is an amorphous block of collagen fibers for which differentiation is still not possible (Longo et al 2013). This type of collagen was described in elderly patients (78 years) in our study.

Conclusions

Aging skin was marked by reduced epidermal thickness, the more frequent presence of the slightly irregular "honeycomb" pattern, of slightly irregularly-shaped keratinocytes, of a disorganized capillary network, and of huddled collagen.

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