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HISTOMORPHOMETRICAL CHARACTERISTICS OF HUMAN SKIN FROM CAPILLITIUM IN SUBJECTS OF DIFFERENT AGE

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A b s t r a c t: The aim of this paper is to present the dynamics of changes in the structural components of the skin of the capillitium throughout the entire life by use of morphometric analysis. For this purpose skin biopsy specimens were collected from patients from Plastic and Reconstructive Surgery Clinic and cadavers from the Pathology Institute and the Forensic Medicine Institute. The subjects of this research were patients, i.e. cadavers of different age, separated into five different subgroups according to this criterion (newborns to elderly). The results obtained with this morphometric analysis have shown an increase in total skin thickness of the capillitium (epidermis and dermis) by 1.7 mm (during the first year), 3.8 mm (puberty and adolescence) and 1.8 mm (beyond 55 years). There is no significant change in the case of the epidermis, only from 0.1 mm to the value of 0.2 mm. The thickness of the dermis from a morphological point of view, starts from 1.5 mm (the first year), goes to 3.7 mm (puberty and adolescence) and finally goes down to the value of 1.6 mm (elderly). The volume of conglomerates of sweat glands for the first group is 0.008 mm^3 to 0.09 mm^3 for the second. The volume of sebaceous glands is 0.01 mm^3 for the first, 0.1 mm^3 for the second and 0.4 mm^3 for the third group. The diameter of follicles of fibres starts from 0.1 mm for the first group, and rises up to the value of 0.4 mm during puberty. The length of follicles of fibers is 1.4 mm for the first group and it reaches 3.1 mm in adolescence and old age.

The results show that the morphology of the skin of the capillitium undergoes changes during human life as a result of changes that occur in the connective tissue of the dermis. The increase of thickness in the dermis is the result of the increase of thickness in the reticularae stratum, where the greatest changes are obvious. These changes occur due to quantitative and structural repartition of collagen fibres.

Key words: human healthy skin, different body regions, age-related variations, epidermis, dermal-epidermal junction, corium, skin appendages.

Introduction

Located between the internal and external environment, the skin is a structural complex, a multifunctional and sophisticated vital organ that is specialized in carrying out very crucial human system functions. The skin's features such as flexibility, strength, continual and invisible desquamation, the vinegar PH on the surface, the fat protection layer together with its sensory and immunobiology properties, make the skin a morphological and functional barrier for the protection of the entire human body against many external influences. The epidermis along with its architectonics, the dermis along with its collagen and flexible fibre and the subcuticus as an isolator, provide the skin with defense mechanisms against many external mechanical influences. Every single zone of the human body skin has its own particular structural and functional variations which are identical or equal but on the opposite side of the body.

Taking this fact into consideration, we made a histomorphological analysis of the skin components from 15 different regions of the human body in order to create a database of qualitative and quantitative properties of normal human skin from different body regions and their age-related variations. This paper presents a part of that comprehensive research.

Materials and methods

The subject of the analysis were skin biopsies of the capillitium of a size of 0.5 cm which included the overall skin thickness and part of the subcutaneous fat tissue. They were collected from: patients from the Skopje University Plastic and Reconstructive Surgery Clinic, autopsy procedures done at the Pathology Institute and autopsy procedures done at the Forensic Medicine Institute. In order to examine the skin properties in the different periods during a lifetime, the biopsy specimens were classified into five different subgroups depending on the patient (cadaver) age: neonatal period (full-term infants up to the age of one year); childhood (from the age of 1 up to the age of 12 years); puberty and adolescence (from the age of 13 up to the age of 22); adult subjects (from the age of 23 up to the age of 55); elderly subjects (from the age of 56 up to the age of 73).

In addition, we took into consideration the proportional representation of both sexes. Each subgroup based on patient age contained at least 12 samples, and the overall process of research consisted of 60 skin biopsy specimens.

Paraffin slides were stained according the generally accepted methods (H.E., Azan – Mallory, PAS, Floranten.). This morphological analysis covered the epidermis along with all its layers, the corium or the dermis along with the two dermal segments: the papillary and reticular layers, the disposition of the collagens, elastic fibres, reticular fibres, cells elements, etc. A quantitative morphometric analysis was made by using the Pathology Institute's computer system for image processing and analysis (Lucia M, Version 3, System for Image Processing and Analysis). The morphometric analyses provided us with quantitative dimensions of the structural skin components. More precisely we were able to determine the following: the total skin thickness of each skin region and each age; the total thickness of the epidermis; the dimensions of the attitude of *Str. basalae*, *Str. spinosum*, *Str. granulosum* and *Str. corneu*; the total thickness of the corium; the thickness of the papillary and reticular layers of the corium. The obtained numerical data were mathematically and statistically elaborated, and this enabled more precise understanding of the differences in the structure of a healthy skin and its specifics based on sex and age as well.

Results

The average values of the total skin thickness of capillitium (epidermis and corium) in different age groups (shown in Figure 1) show that the maximal value is achieved at the age of 22 years. After that it slowly decreases, and finally after the age of 56, we have almost the same value as in the beginning (childhood).

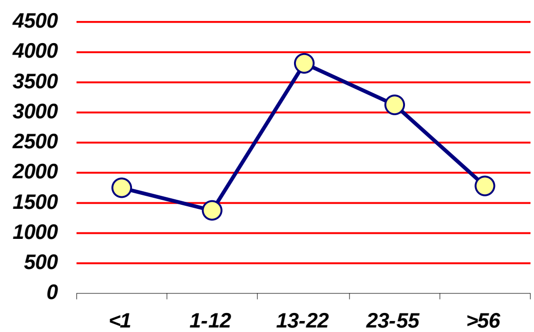


Figure 1 – Changes in total thickness of skin of capillitium during human life

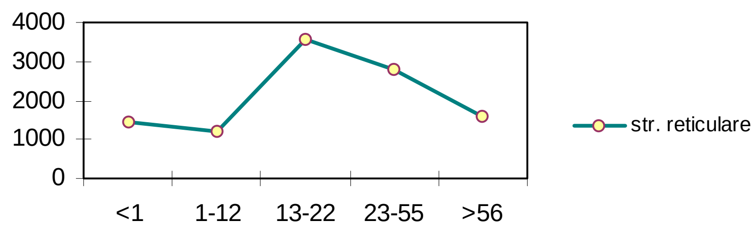
Thicknesses of particular skin segments

Statistically, the thickness of each of the three capillitium skin segments has significant changes during a life (Table 1). The numerical data show the dominance of the thickness of the *stratum reticularae* over the rest. Its thickness is more expressed around the age of 22 years, then it decreases gradually (until the age of 55), and afterwards goes down more quickly and achieves almost the same value as it had during childhood (Fig. 2). The same variations apply to the *stratum papillary* too, but what differentiates this from the previous one is that the maximal value is achieved at an age between 23 and 25 years. The values we have gathered by measuring the epidermis thickness show some fluctuations that follow the same line of increase as in the case of the corium, but here they are significantly lower.

Table 1

Average values of total skin thickness of skin of capillitium and average values of thickness of particular layers of skin of capillicium during human life

Ages	**Thickness (micrometers µm)**			
	cutis	**epidermis**	**str. papillare**	**str. reticulare**
< 1	**1714.6**	**160.8**	**105.6**	**1448.2**
1–12	**1401.9**	**98.3**	**85.5**	**1218.1**
13–22	**3879.3**	**158.7**	**141.6**	**3579**
23–55	**3211.1**	**174.6**	**256.7**	**2779.8**
56	**1782.8**	**112**	**72.2**	**1598.6**
F/t		**32.40**	**44.27**	**49.17**
p		$P < 0.01$	$P < 0.01$	$P < 0.01$



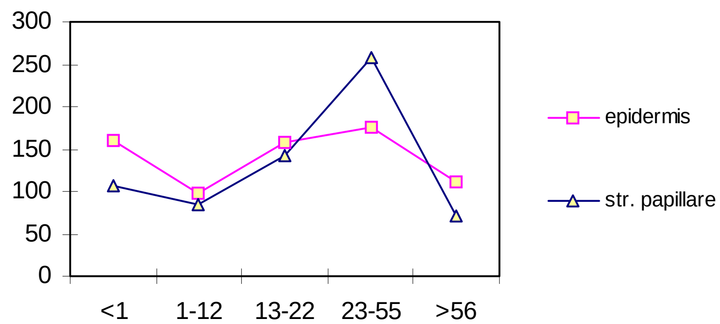


Figure 2 – Dynamic of changes related to thickness of layers of skin of capillitium

Table 2

Average thickness of layers of epidermis of capillicium during human life

Ages	Thickness (micrometers μm)			
	str. basale	str. spinosum	str. granulosum	str. corneum
< 1	**10.9**	**19.9**	**9.3**	**120.7**
1–12	**13.9**	**23.7**	**7.3**	**53.4**
13–22	**17.8**	**52.8**	**14.7**	**73.4**
23–55	**15.5**	**50.4**	**11.2**	**97.5**
> 56	**12.4**	**31.2**	**10.2**	**58.2**
F/t	**509.9**	**65.2**	**27.6**	**58.9**
p	p < 0.05	p < 0.05	p < 0.05	p < 0.05

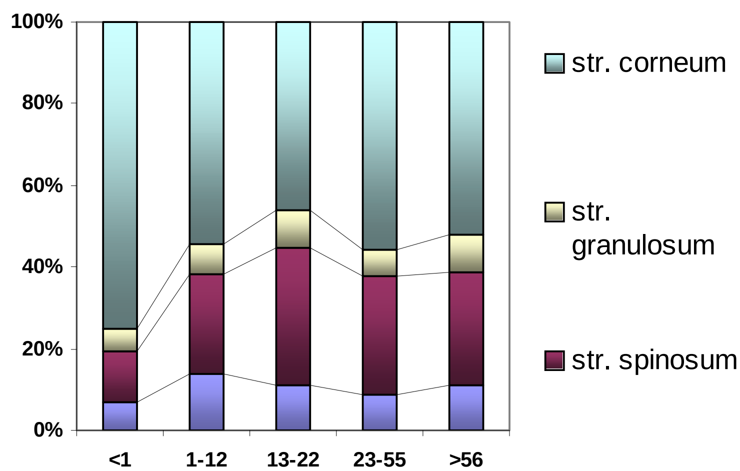


Figure 3 – Participation of all layers of epidermis of capillitium in percents

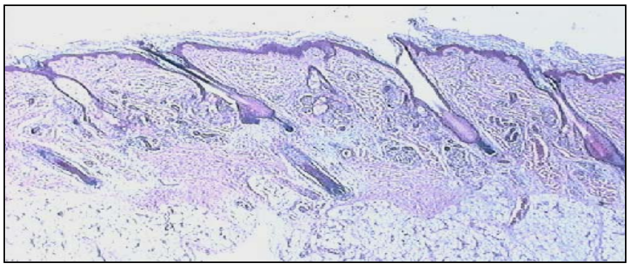
Follicles of hair, sebaceous and sweat glands

To analyse the follicles of hair and sebaceous and sweat glands, a morphometric quantitative method was introduced. The results in Table 3 show the following: the length and width of follicles of fibres increase over a lifetime. The diameter of the follicles increases from the value of 0.130 mm (at the beginning) up to 0.558 in the elderly. The length of the longitudinal section of the follicles of hair for newborns is only 1.113 and during the lifetime can increase and reach a value of 4.5 mm in the case of adolescents. The dimensions of the sebaceous and sweat glands show a tendency to increase until adolescence and then a slow decrease in dimensions occurs.

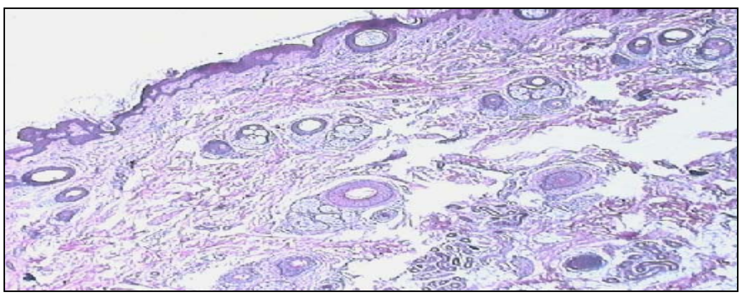
Table 3

Dimensions of follicles of hair, sebaceous and sweat glands in skin of capillitium

Ages	*Sebaceous glandsvalues of tranverse section mm*	*Sweat glandesvalues of tranverse section mm*	*Follicles of hair*	
			Tranverse section mm	*Longitudinal section mm*
0–1	0.256 × 0.480	0.034 × 0.056	0.130 × 0.150	0.140 × 1.133
1–12	0.236 × 0.370	0.035 × 0.043	0.200 × 0.200	0.180 × 1.890
13–22	0.425 × 0.500	0.045 × 0.060	0.170 × 0.250	0.250 × 2.375
22–55	0.278 × 0.391	0.044 × 0.052	0.200 × 0.100	0.250 × 4.500
55 >	0.279 × 0.463	0.051 × 0.058	0.300 × 0.558	0.237 × 2.406

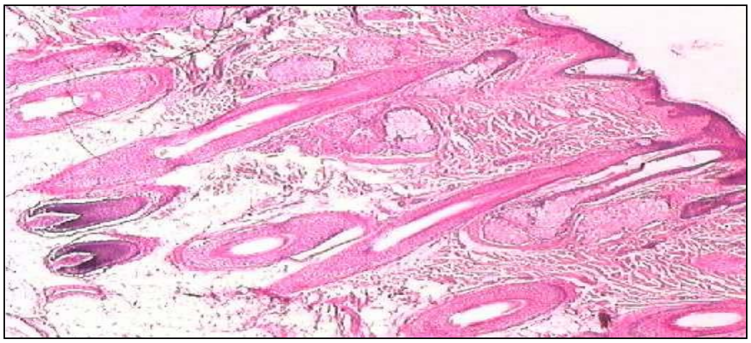


Picture 1 – H&E section of skin of capillitium for first age group



Picture 2 – H&E section of skin of capillitium for second age group

Picture 3 – H&E section of skin of capillitium for third age group



Picture 4 – Elastica Van-Gieson section of skin of capillitium for fourth age group

Picture 5 – H&E section of skin of capillitium for fifth age group

Discussion

The epidermis in the case of full-term newborns (in contrast with the corium) shows a substantially greater ripeness and functionality in terms of its barrier function. Its total thickness in the case of newborns is noticeable and greater compared to the second age group and almost the same compared to the values of the epidermis in some regions in the case of the third group.

Acknowledgement of functionality of the epidermis in the case of newborns was done during researching the loss of water through the surface of the skin, and the results showed that in the case of full-term newborns the leakage was minimal [9]. The barrier function of the epidermis reflects on the lower penetration of substances which are applied to the skin. A subject of interest for many authors has been percutaneous absorption in the case of newborns, applying substances which are part of certain pharmacological medicaments (antiseptics, corticosteroids, boric acid, alcohol, antibiotics, salicylic acid). As a result of this, they found that the specific structure and ripeness of the epidermis in newborns are not an ultimate barrier for most of the substances, but that the barrier function of the epidermis in the case of full-term newborns is much more advanced compared to that of pre-term newborns. In the case of pre-term newborns the absorption efficacy of the skin is not proportional to the gestational age. The explanation of the existence of percutaneous absorption although there is an existence of the striking barrier functions of an epidermis is because the absorption takes place deep in fibre follicles (where a keratin layer of follicles does not exist) and whose number in the case of the newborn is much larger and can be found throughout the body.

A comparison was made between the components of the corium in the case of a foetus and a newborn and as a result of this it was found that the elastic fibres are undeveloped, thin and make up only 3–4% of the entire corium, while the collagen fibres are still very thin and of low order [8]. The fact that cellular elements lose their dominances after the baby is born (from 20% in the case of the foetus descreases to the value of 4% in case of a newborn) shows that their metabolic activities result in rapid production of febrile elements (24% in the skin of a foetus, to the end of the first year they make up approximately 60% of the volume of the corium).

During childhood the surface of the skin grows very rapidly, but as in the other organic systems, there are processes of maturation and putting into final shape.

While the epidermis is responsible for the barrier function of the skin, the corium is a segment dedicated to the mechanical characteristics of the skin. This is because of the presence of fibroblasts that have more than one role: to secrete growth factors, to create a high concentration of inhibitors of enzymes that are responsible for the separation of macromolecules of matrix, to synthesize molecules for creating connective fibrillars, etc. [10]. During childhood, the fibroblasts are the most active and as a result of this there follows the creation of a dense network of collagen fibres that start to organize themselves and to create a specific rhomboid shape in the active matrix.

Also the second fibrillar component of the corium (elastic fibres) progresses during childhood and starts to locate itself between collagen fibres, positioning itself vertically in order to reach the dermal-epidermal boundary zone.

Puberty is a period when, under the influence of sex hormones, the structure of the skin takes its final shape as a precondition for the establishment of its optimal functions. Adolescence is a period of hormone stabilisation and final reaching of genetically predetermined qualities of the skin. After this period, all further changes that the skin will suffer from are the result of external factors.

For the life period between 13 and 22 years, the establishing of optimal equilibrium among structural components of the corium is typical as a result of reaching a ripeness of components. It was found that in the second decade of life the cell component is substantially reduced and participates with only 1.5% in the corium [8]. The collagen is dominant and participates with 70% of the total volume of the corium, while the volume and diameter of the elastic fibres increase only in the reticular layer and reach 5% of the corium volume. Into the papillary layer, they are substantially thin.

The measuring of the dimensions of the collagen fibres in this period of life proved that they have the same appearance, ripeness and diameter as that reached the maximal value (110–115 nm) [8].

There are no great differences between the qualitative and quantitative characteristics of the skin for people who are between 23 and 25 years old, and the characteristics that were previously confirmed. This period of life is characterized by the existence of a huge number of soft variations of the parameters. Some of them continue to show a tendency for growth and organization, some keep the same values and others start to reduce gently. These variations are related to certain particular layers of the skin as well as to some particular regions.

During the examination of the healing of people's injures at different ages it was discovered that the turnover of keratocytes decreases twice more from the third to the seventh decade of life [4]. The results show that slow recovery and slow epidermal reparation are due to a slow-down of epidermopoesis. Through the examination of the ageing of keratocytes, it was discovered that after the third decade of life a substantial decrease in mitotic processes starts [9].

During the third decade of life the process of ageing begins (in the beginning tenuously and invisibly).

As a part of the body, the skin with the whole of its surface is the organ most exposed to external factors. But through the compact structure of the corium (as a dominant skin layer) these changes during the third and fourth decade are still relatively well hidden.

In the last group, that covers people who are between 56 and 73 years old, the regressive processes clearly reflect the qualitative and quantitative characteristics of the skin of all regions. The epidermis is significantly spindly due to a decrease in a number of rows of cells in every particular layer, especially emphasized in the *stratum spinosum*. This happens because of a strong reduction in mitotic separations (whose first products are spinous cells) and the decreased number of turnovers of keratocytes (which leads to a reduction in their vitality and volume) [2].

In vitro researches were done (on isolated epidermis) and showedthat it is almost identical compared to the case of young individuals. *In vivo* researches have shown that percutaneous absorption is slower in the case of old people. The substances cross faster through the spindly epidermis, but the process slows down at the level of the papillary corium, where reduced vascularization exists. In any case, we should present that there also exist opposite results of certain authors' researches who found that the spindly *stratum corneum* has thickening keratinocytes which prolong the time that substances need to pass through the epidermis.

Also the connective component of the skin of the elderly suffers from substantial structural changes. Our analyses show that the most noticeable changes occur to collagen bundles after the fifth decade of life. They suffer from fragmentation and disorientation, creating zones of dense collagen mass separated from spaces where a fibrillar component does not exist. They are very obvious in the reticular layer of the corium and enable the cell elements to become more visible, although their total number is reduced. These changes affect not only the size of the corium, but also its elasticity.

Through certain research it was shown that in the second decade of life the collagen, collagen fibres and bundles participate with 70% of a total dermis and after the 60th year of the life they go down to the value of 40% [8].

The dermal-epidermal boundary zone starts to level slowly due to fragmentation of the vertically situated elastic fibres that even start to move into a horizontal position. Due to fragmentation, contact with the basal lamina becomes weaker (as well as the strong connection between the corium and epidermis).

In the same study there is presented one interesting fact that showed that the concentration of elastic fibres in the case of the dermis is 3–4% in the first year of life, 5% in the fifth decade of life and 7–8% of the total volume of corium in older people. Their explanation, which we fully agree with, is that because of the decreased percentage of the collagen component, there is a greater percentage of elastic fibres. The opposite would mean that the elderly skin becomes more elastic, and this is something that it is not possible to confirm taking into consideration the previously-mentioned parameters.

Going even deeper (researching on a molecular level) with the purpose of examining the point of damage, it was discovered that actually the fibrillian component in the peripheral layers of the elastic fibres goes down and suffers from degradation, and after that abnormal grouping and the accumulation of abnormal elastic deposits occurs. It was also discovered that the accumulation of abnormal elastic deposits was most obvious in the papillary layer of the corium in which the soft microfibillar network slowly starts to unplait and is replaced by deposits. According to this study, lysosomes are in charge of discarding these deposits but in the case of old skin this activity is limited. The impact of UV rays is considered to be a primary factor for the occurrence of these changes, because the naked parts of the body have suffered more than others [12].

In some studies has been determined that one of the reasons for the changes in the extracellular matrix is a dislocation of balance between the enzymes and their inhibitors [10]. In the case of the skin of young individuals the activity of inhibitors is maximal, so that the enzymes (MMPs) metalloproteinases are in charge of the digestion of matrix macromolecules and tearing of their connections is inhibited. When a human becomes older, especially under the impact of UV rays, this balance starts to break down, and the enzymes lead to separation of the matrix and loss of the three-dimensional structure. The old fybrocytes secrete more degradation enzymes which modify the balance in the extracellular matrix.

Changes in the extracellular matrix are as a result of elderly changes that primarily occur at cell level [10]. Free radicals are created in the fybrocytes, and antioxidants are in charge of their elimination, but when cells become older the antioxidant system becomes less efficient, so that the oxidants start to damage almost all the structures of the cell. The damaged mitochondrials lead to energetic crises in the cell. In the mitochondrial theory of ageing, it was determined that over the years the damage to mitochondrial DNA increases up to one thousand times and leads to oxidant stress [10]. Human fibroblast, exposed to the impact of oxidant stress, reacts in different ways. The mitotic answer for the factors that support their rises goes down, and ATP production and the synthesis of polypeptides and procollagens goes down.

The abnormal connection of amino acid to extracellular space leads to the creation of a completely new molecule (AGEs – advanced glucose and products). The existence of AGEs leads to changes in the architecture and function of the amorphous matrix and fibrillar components. Instead of a parallel one, collagen fibrils all together form a structure that looks like a flower. This state changes the physical and mechanical properties of the extracellular matrix

[11]. Many authors in their own scientific researches emphasize that there are no significant differences in the structure of the skin between the two sexes. Regardless of this fact, at the beginning of our research, through the analysis of variance in which sex was included as a variable, we also found that numerical values of the examined parameters did not show any significant differences relating to sex. This is the reason why this variable was excluded in the further analysis.

It can be concluded that skin of the capillitium changes in the course of a life, which is due to the changes of the morphological structure of the dermis and adnexes.

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Р е з и м е

ХИСТОМОРФОМЕТРИСКИ КАРАКТЕРИСТИКИ НА ХУМАНА КОЖА НА КАПИЛИЦИУМ КАЈ РАЗЛИЧНИ ВОЗРАСНИ ГРУПИ

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Целта на овој труд беше со помош на морфометриска анализа да се утврди динамиката на промени на структурните компоненти на кожата на капилициумот во текот на животот. Материјал за анализа беа кожни биопсии од пациенти од Клиниката за пластична и реконструктивна хирургија и биопсии земени при обдукција на кадавери од Институтот за патолошка анатомија и Институтот за судска медицина при Медицинскиот факултет во Скопје. Пациентите беа на различна возраст, поделени во пет старосни групи – од новороденче до лица во длабока старост. Резултатите од морфометриската анализа покажаа раст на целосната дебелина на кожата на капилициум (епидермис и дермис) од 1,7 мм (прва година од животот до 3,8 мм пубертет и адолесценција и 1,8 мм кај лица постари од 55 години. Епидермисот не се менува сигнификантно во текот на животот од 0,1 мм на 0,2 мм. Дебелината на дермисот од морфолошка гледна точка се менува од 1,5 мм во првата година од животот на 3,7 мм во пубертетот за да опадне во старост на вредност од 1,6 мм. Волуменот на конгломератите од потни жлезди кај првата возрасна група изнесува 0,008 мм^3. Кај возрасната група расте до 0,09 мм^3. Волуменот на лојните жлезди се менува од 0.01 мм^3 кај првата возрасна група на 0,1 мм^3 во пубертетот до 0,4 мм^3 кај старата возрасна група. Дијаметарот на фоликулите на влакната од 0,1 мм кај првата возрасна група расте до 0,4 мм во пубертетот. Должината на фоликулите на влакната од 1,4 мм кај првата возрасна група расте до 3,1 мм кај адолесцентите и постарите лица. Во однос на полот не постојат сигнификантни разлики.

Резултатите покажуваат дека морфологијата на кожата на капилициумот се менува во текот на животот како резултат на промените кои се случуваат во сврзното ткиво на дермисот. Порастот на дебелината на дермисот е резултат на порастот на дебелината на stratum retikulare каде што се видливи најголемите промени. Тие промени се должат на квантитативното и структурното прераспределување на колагените влакна.

Клучни зборови: хумана кожа, регионални карактеристики, различни возрасти, епидермис, дермо-епидермална граница, кориум, кожни аднекси.

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