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Quantitative changes of the capillary bed in aging human skin

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Summary. The present study focuses on the quantitative changes of the capillary bed in aging human skin. Forty-five skin samples were excised from the anterior thoracic region of cadavers of caucasian origin in the age range 33-82 years. The immunohistochemical method with anti-human CD34 was used for the detection of the capillary endothelium. Morphometric analysis was done by Vision Assistant software. The capillary bed was quantified by two parameters: capillary area (CA) and intercapillary distance (ID) in 6 age groups. Results revealed no quantitative changes of the capillary bed up to the age of 60 years. In the papillary dermis a significant reduction of the capillary area was seen in the 7th, 8th and 9th decennium. A considerable decrease, by 33%, was determined in the 7th decennium. During the 8th and 9th decennium the capillary area was reduced by a further 19% and 13%. In total from the 4th till the 9th decennium, the capillary bed in the papillary dermis was diminished by 65%. The intercapillary distance in the papillary dermis singnificantly increased during the 8th decennium. On the basis of the mutual evaluation of both the observed parameters, CA and ICD, the authors supposed that the reduction of the capillary bed in the papillary dermis during the 7th decennium was probably caused only by the shortening of the capillary loops, which copied flattened dermal papillae, and during the 8th decennium also by the decreased number of the capillary loops. In the reticular dermis the capillary bed remained unchanged.

Key words: Skin, Aging, Intercapillary distance, Capillary area

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Introduction

The skin is a large heterogenous organ consisting of two main structural layers: epidermis and dermis. Each of these layers has a typical morphology, physiology and function. The outer layer, the epidermis serves as protection against mechanical, chemical, osmotic and ultraviolet ray damage and also as an anatomical barrier against microbial invasion with immunological surveillance. The deeper layer, the dermis provides the structural support, preserves sensory function of the skin and contains the blood vessels, which are responsible for the oxygenation and nutrition of the skin and for control of body temperature (Agache and Humbert, 2004; Standring, 2008).

The blood supply for the skin comes from musculocutaneous and fasciocutaneous perforators and from direct cutaneous vessels, all arising from deeply located vessels and crossing the superficial fascia (Standring, 2008). Skin microcirculation is arranged in two plexuses: a deep reticular plexus and a superficial subpapillary plexus (Yen and Braverman, 1976; Braverman and Yen, 1977; Braverman, 1989). The deep reticular plexus is formed from perforating vessels at the dermal-subcutaneous interface. Its lateral branches supply the upper part of the hypodermis and the lower reticular dermis. Vertically ascending vessels fill the superficial subpapillary plexus situated 1 - 1.5mm below the skin surface. The subpapillary plexus supplies the upper part of the reticular dermis, the papillary dermis and also the epidermis from the capillary loops within the dermal papillae (Braverman, 1989; Braverman, 2000; Agache and Humbert, 2004). Each dermal papilla has its own single capillary loop, which arises from a terminal arteriole of the superficial plexus. The capillary loop consists of an ascending limb, an intrapapillary loop and a descending limb connected with a

postcapillary venule (Braverman and Yen, 1977). The superficial and deep plexus differ in their structure. The vessels of the deep reticular plexus are of larger caliber in all segments of the microcirculation (Braverman, 1989, 2000) and their smooth muscle cells specifically express smoothelin in the cytoplasm, which is not detected in the superficial subpapillary plexus (Aneiros-Fernandez et al., 2011).

The cutaneous microcirculation is altered not only during pathological conditions, but also during aging, which comprises intrinsic aging and photoaging. These processes influence much more the structure and the function of the superficial subpapillary plexus, while the reticular plexus is affected to a lesser extent (Gilchrest et al., 1982; Braverman, 1989; Montagna and Carlisle, 1990; Chung and Eun, 2007). Ultrastructural studies showed the appearance of thin-walled vessels in the superficial plexus caused by intrinsic aging (Braverman, 1989, 2000; Braverman and Sibley, 1990; Montagna and Carlisle, 1990) and thickening of the basal lamina of the capillaries in the dermal papillae caused by photoaging (Toyoda et al., 2001; Chung and Eun, 2007).

During life the capillary bed is permanently preserved and remodeled by the process of angiogenesis, which is delayed in aging due to impaired growth factor expression (Sadoun and Reed, 2003; Ryan, 2004). However, quantitative age-dependent changes are usually assumed and exact investigations of the diminution of the cutaneous microvasculature are rare. Therefore, the aim of this study was the quantification of the capillary area within the dermis in different age groups.

Material and methods

Tissue samples

Forty-five skin samples were excised from the anterior thoracic region of cadavers of caucasian origin, which were donated for dissection at the Institute of Anatomy and Pathological Anatomy of the Jessenius Faculty of Medicine in Martin Comenius University in Bratislava. Donors of both genders (25 women, 20 men) were in the age range 33-82 years. All persons were without skin disease in anamnesis and nine persons suffered from systemic diseases (hypertension, diabetes mellitus and metabolic syndrome). The study was approved by the local Ethics Committee. Specimens were fixed in 10% neutral buffered formalin, and embedded into paraffin blocks. Perpendicular and parallel 4 μm thick sections from each specimen were mounted on silane coated slides.

Immunohistochemical staining

Immunohistochemical staining was performed with anti-human CD34 (Monoclonal Mouse Anti -Human CD34 Class II clone QBEnd-10 M 7165, Dako-Cytomation Denmark A/S) for the detection of the capillary endothelium (Ramani et al., 1990). Sections were dewaxed by xylene and rehydrated in a series of graded alcohols. Endogenous peroxidase was blocked by 0.3% H_2O_2. Heat-induced epitope retrieval was done after the immersion of the slides in the retrieval solution (Target Retrieval Solution High pH, Code No. S3308, DakoCytomation Denmark A/S). Afterwards the

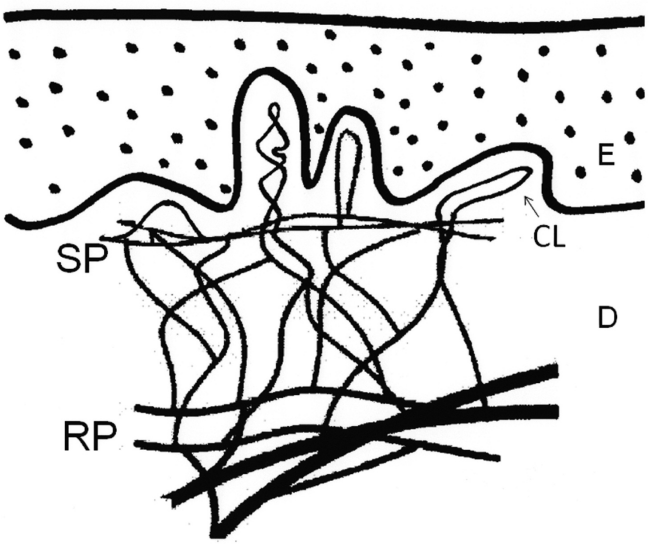

Fig. 1. Organization of cutaneous microcirculation. SP: subpapillary plexus; RP: reticular plexus; CL: capillary loop; E: epidermis; D: dermis.

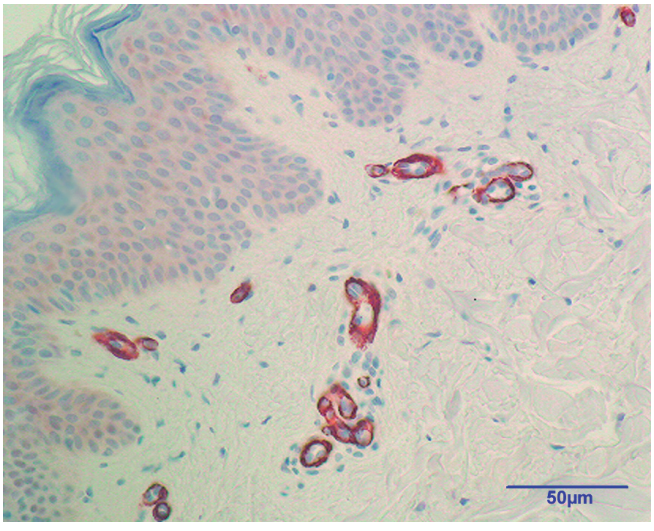

Fig. 2. Immunohistochemical detection of the capillary endothelium. Skin of the anterior thoracic region, woman 36 years, perpendicular section. x 400

specimens were incubated with a diluted primary monoclonal antibody at room temperature. The primary antibody was detected by biotinylated immunoglobulins and horseradish peroxidase streptavidin-biotin complex (LSAB™ +/ HRP kit, code No. K0679, DakoCytomation Denmark A/S). Staining was completed with 3-amino-9-ethylcarbazole as a chromogen for the visualization of the peroxidase reaction. Finally the sections were counterstained with Mayer's hematoxylin. All steps of the immunohistochemical staining were managed as per the manufacturer's recommendations.

Sections were screened and digital pictures of microscopic views at magnification of x100, x200 and x400 were taken with an Olympus Evolt E-420 installed in an Olympus BX41N microscope.

Computer-assisted morphometric analysis

Morphometric analysis of the digital images was done by a LabView-based programme - Vision Assistant version 7.1.1 (National Instruments, Austin TX, USA). The threshold values for background intensity were determined in the control tissue without a primary antibody. The capillary bed was quantified by two parameters: capillary area and intercapillary distance in 6 age groups.

The capillary area was defined as the average percentage of CD34-stained area in the papillary and reticular dermis divided into standard fields in 10 serial slides from each skin sample to depict, to a certain extent, the partial volume of the papillae. Fields with the capillary networks around hair follicules and glands were eliminated.

The intercapillary distances between the capillary loops in the papillary dermis were determined in the horizontal - parallel sections directly above the imaginary line connecting the deepest points of adjacent rete ridges (Fig. 3). Digital images of the microscopic views were processed by Vision Assistant software into binary maps with cross-sections of the capillary loops - of their ascending and descending limbs. The Delaunay triangulation was used to establish the nearest neighbouring capillary loops, between which the intercapillary distances were measured (Fig. 4). This method is usually accepted as a suitable representation of Krogh's cylinder model (Zhong et al., 2000; Sainthillier et al., 2003). This geometrical tissue model explains the theory of oxygen delivery to tissues defined by August Krogh. He supposed that the rate of oxygen transport is also dependent on the number and distribution of the capillaries (Krogh, 1919).

Statistical analysis

Morphometric data were expressed as the mean±standard deviation. Statistical analysis was performed using the analysis of variance (ANOVA) and unpaired t-test. A p value less than 0.05 was considered the minimum for statistical significance.

Results

The capillary area and intercapillary distance were determined in 6 different age groups. The individual variability of both parameters in the specimens inside each age group was rejected on the basis of ANOVA tests ($p>0.05$), despite the fact that 3 donors suffered from hypertension, 2 from diabetes, 3 from both hypertension and diabetes and 1 from both hypertension and metabolic syndrome.

Capillary area in the papillary dermis

Table 1 demonstrates the average values of the capillary area in the papillary dermis with significant age-dependent variability ($p<0.001$). During the 4th, 5th and 6th decennium the values of the capillary area varied insignificantly from 1.41% to 1.43% ($p>0.05$). A statistically significant decrease of the capillary area was detected in the 7^{th}, 8^{th} and 9^{th} decennium ($p<0.001$). A considerable reduction of the capillary bed, by 33%, was determined in the 7^{th} decennium. During the 8^{th} decennium the capillary bed was diminished by a further 19% and during the 9^{th} decennium by 13%. In total from the 4^{th} till the 9^{th} decennium, the capillary area in the papillary dermis was decreased by 65% (Fig. 5).

Capillary area in the reticular dermis

Results from the reticular dermis (Table 1, Fig. 3) revealed no age-dependent changes in the capillary area ($p>0.05$). In comparison with the values of the capillary area in the papillary dermis, the average values in the reticular dermis were significantly lower in all age

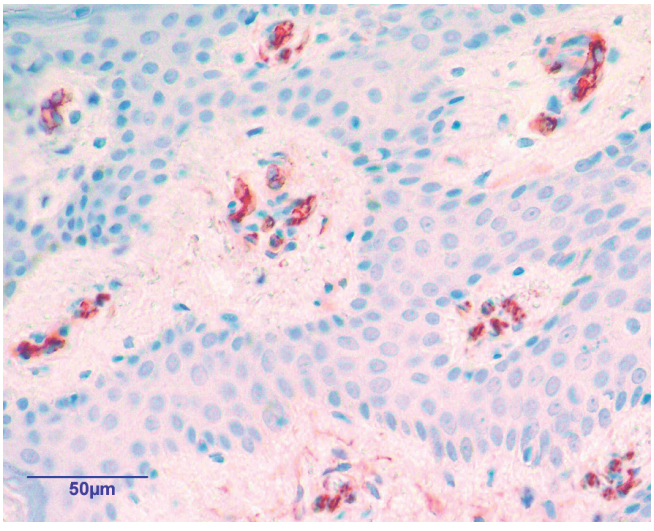

Fig. 3. Immunohistochemical detection of the capillary endothelium. Skin of the anterior thoracic region, woman 45 years, parallel section. x 400

groups (p<0.001).

Intercapillary distance between the capillary loops in the papillary dermis

The capillary loops of the dermal papillae showed a variability in their height and course. There were higher and wavy capillary loops in the skin of younger persons. With aging, the capillary loops became lower and copied a flattened dermo-epidermal junction. The average intercapillary distances between the capillary loops in the papillary dermis are demonstrated in Table 2. The average intercapillary distance in the 4th decennium was 116.23±27.83 μm, and till the 8th decennium increased to 143.96±34.07 μm (by 23%). A comparison of the intercapillary distances between age groups confirmed the statistically insignificant differences up to the age of 70 years (p>0.05), but a significant increase of the intercapillary distance in the papillary dermis in the 8th and 9th decennium (p<0.001).

These results indicate that a decreased capillary area

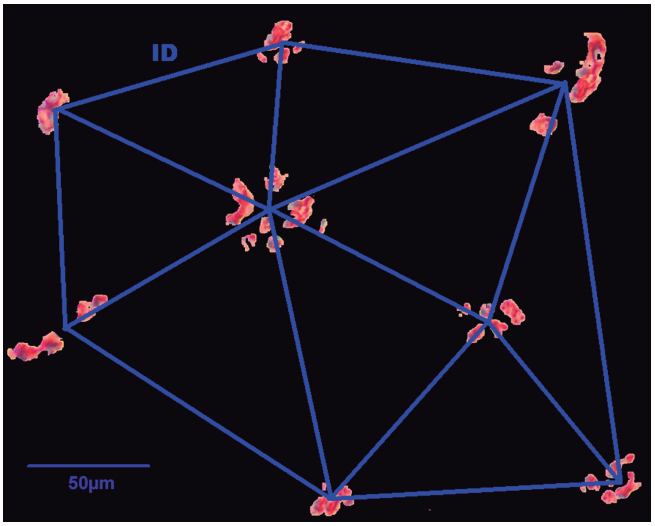

Fig. 4. Binary maps with Delaunay triangulation for the evaluation of intercapillary distances in the papillary dermis. Skin of the anterior thoracic region, woman 45 years, parallel section. ID: intercapillary distance in the papillary dermis. x 400

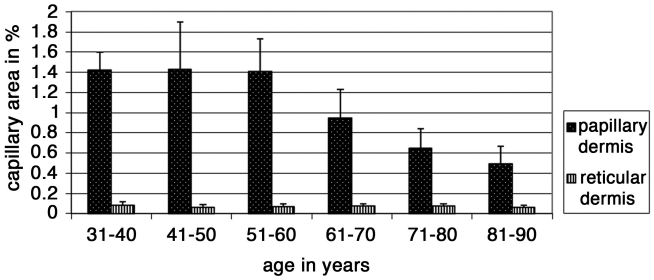

Fig. 5. Capillary area in the papillary and reticular dermis depending on age.

Table 1. Capillary area in the papillary and reticular dermis depending on age.

age in years (n=45)	papillary dermis (PD)					reticular dermis (RD)	PD vs RD
	capillary area in %	individual variability ANOVA p value	T-test p value			capillary area in %	T-test p value
			comparison to 31-40	comparison to 61-70	comparison to 71-80		
31-40	1.42±0.18	0.825				0.084±0.034	9.3E-10
41-50	1.43±0.47	0.904	0.932			0.064±0.025	1.1E-08
51-60	1.41±0.32	0.899	0.951			0.076±0.020	3.8E-16
61-70	0.95±0.28	0.731	7.9E-06			0.078±0.020	1.6E-12
71-80	0.65±0.19	0.932	2.4E-09	0.0002		0.079±0.016	1.0E-10
81-90	0.50±0.17	0.851	3.6E-09	1.80E-05	0.059	0.065±0.019	6.5E-05
age-dependent variability ANOVA			4.4E-07			0.992	

Table 2. Intercapillary distance in the papillary dermis depending on age.

age in years (n=45)	intercapillary distance in μm	individual variability ANOVA p value	T-test p value	
			comparison to 31-40	comparison to 71-80
31-40	116.23±27.83	0.942		
41-50	121.98±33.22	0.589	0.438	
51-60	120.33±30.77	0.985	0.548	
61-70	125.54±36.27	0.653	0.234	
71-80	143.96±34.07	0.257	0.0003	
81-90	144.86±24.02	0.322	1.9E-05	0.892
age-dependent variability ANOVA		0.039		

before the 8th decennium was probably caused by the shortening of the capillary loops in the papillae, because the intercapillary distance was not significantly changed but a critical increase of the intercapillary distance in the 8th decennium could imply a decreased number of the capillary loops in the papillary dermis.

Discussion

The microcirculation of the skin performs several different functions: nutritional support, homeostasis, thermoregulation, regulation of blood pressure, immune surveillance and wound healing. Tissue nutrition is carried out mainly by the capillaries and it represents only 15% of the total blood flow in the skin. The thermoregulatory function is performed predominatly by the vessels of the reticular plexus through the regulation of the remaining 85% of the blood flow (Agache and Humbert, 2004).

The aging process noticably affects the skin microcirculation and its functions. The present study was focused on the quantitative changes of the capillary bed in the dermis and revealed that while in the reticular dermis the capillary bed remained unchanged, in the papillary dermis the capillary bed diminished almost to one third from the 4th to the 9th decennium. No quantitative changes of the capillary bed were demonstrated up to the age of 60 years. A significant reduction of the capillary bed in the papillary dermis during the 7th decennium was probably caused by a shortening of the capillary loops in flattening dermal papillae, but the number of the capillaries did not change. During the 8th decennium, the microcirculation seemed to be remodeled because of an increased intercapillary distance, which means a decreased number of the capillary loops in the papillary dermis.

Only a small number of quantitative data are available for comparison. Videocapillaroscopic studies found significantly reduced dermal papillary loops by 37-40% in old skin compared to younger skin in the forearm, back of the hand and the forehead (Kelly et al., 1995; Humbert et al., 2005; Li et al., 2006a,b). Our study highlighted the decrease by 65% in the papillary dermis of the skin from the anterior thoracic region. Differences can be caused in the evaluation by different methods and in different topographical regions. Results of the immunohistochemical study by Helmbold et al. (2006) showed that the mean value of the skin capillary density decreased from birth untill the age of 70 years totally by 77%. Their investigations also confirmed a significant age-dependent decrease of the density of pericytes, which could have, in their opinion, a key role in microvascular aging due to their contractile function, matrix protein synthesis and angiogenesis control (Helmbold et al., 2004, 2006). It is suggested that pericytes, pluripotent perivascular cells, are generated by in situ differentiation of mesenchymal precursors at the time of endothelial sprouting (Benjamin et al., 1998). Some in vitro studies uncovered that pericytes and smooth muscle cells influence endothelial cell proliferation and migration (Orlidge and Amore, 1987; Sato and Rifkin, 1989; Nehls et al., 1992), stabilize newly formed vessels and their acquisition means the end of a plasticity window for blood vessel remodelling (Benjamin et al., 1998). A disruption of the endothelial - pericyte association can lead to the excessive regression of vascular loops and abnormal remodelling (Sato and Rifkin, 1989).

An age-dependent reduction of the capillary bed in the papillary dermis is accompanied by thinning of the epidermis caused by the retraction of rete ridges (Waller and Maibach, 2005), a significant 30-50% decrease of epidermal turnover rate (Yaar andt Gilchrest, 2001; Gilhar et al., 2004) and reduced numbers and heights of epidermal cells (Marks, 1981). During the 7th decennium the dermo-epidermal junction flattens (approximately by 35%), as a result of a decreasing number and size of the dermal papillae (Montagna and Carlisle, 1990; Huzaira et al., 2001; Sauermann et al., 2002; Neerken et al., 2004). Consequently it means a smaller contiguous surface between the dermis and epidermis and diminishing of the nutrition to the epidermis (Farage et al., 2010).

Restricted nutritional support in aging skin substantially influences wound healing. Non-healing wounds in persons above 65 years and older form 85% of all non-healing wounds (Menke et al., 2007; Guo and DiPietro, 2010). The effect of aging causes a temporal delay in wound healing, but it does not affect the quality of healing (Gosain and DiPietro, 2004). Available studies of the age-related changes in healing capacity demonstrate delayed immune responce, decreased secretion of growth factors, delayed reepithelialization, reduced collagen turnover and delayed angiogenesis in the elderly (Gerstain et al., 1993; Gosain and DiPietro, 2004; Bishop, 2008; Rodriguez et al., 2008). Increased intercapillary distances and a diminution of the capillary bed influence wound healing as a local factor, which can determine oxygen delivery to the wounds (Rollins et al., 2006; Guo and DiPietro, 2010). Because of the extended intercapillary distance, pO_2 is often nearly zero in the central devascularized region of a wound. However, oxygen delivery to wounds is a complex process and it depends on other factors like blood perfusion, arterial pO_2, oxyhemoglobin dissociation conditions, mass transfer resistances, and the local oxygen consumption rate (Rollins et al., 2006).

Altered skin microcirculation was also observed in some systemic diseases. In essential hypertension a functional and structural rarefaction of the skin capillaries was observed both in older and in younger patients in the skin of the fingers and toes (Prasad et al., 1995; Antonios et al., 1999; Serne et al., 2001a,b; Debbabi et al., 2006). It is not yet clear whether the reduction in skin capillary density is primary or secondary to the hypertension. It is supposed that structural rarefaction of capillaries is caused by reduced angiogenesis and diminished microvascular growth in

primary hypertension (Noon et al., 1997). Increased skin capillary density with a loss of autoregulatory capacity was found in diabetic patients (Tibirica et al., 2007). Surprisingly unchanged capillary density in fingers and increased capillary density in toes was observed in diabetic patients without late complications, but increased capillary density in both fingers and toes was seen in diabetic patients with late complications (Jorneskog et al., 1995; Jorneskog and Fagrell, 1996; Tibirica et al., 2009). In our study 9 donors suffered from these systemic diseases, but the values of the capillary area did not differ from the other values of healthy subjects in the same age group. This discrepancy is probably caused by the regional variability of the capillary density, which was found also in cited studies.

The immune response of the skin - recruitment of inflammatory cells, recognition and effective elimination of foreign agents followed by repair, functional impairment to stimuli such as ischemia, and thermoregulation are other functions negatively influenced by age-dependent changes of the skin microcirculation (Swift et al., 1999, 2001; Vollmar et al., 2000; Ryan, 2004).

Since it is known that tumour angiogenesis is also altered by underlying age-dependent changes, other investigations of impaired and delayed angiogenesis in the aging process could be a useful and potential target for halting and reducing of a tumour growth and progression (Pili et al., 1994).

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