Influence of aging on the quality of the skin of white women. The role of collagen[1]

Influência do envelhecimento na qualidade da pele de mulheres brancas. O papel do colágeno

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ABSTRACT

PURPOSE: Evaluate the influence of aging on the quality of the skin of white women, analyzing the dermal collagen.

METHODS: Pre-auricular flaps were collected for histological and morphometric analysis of 218 white women who underwent spontaneous facial aesthetic plastic surgery. Picrosirius ultrared stain was used for analysis and quantification of collagen in five age groups (<40 years, 40 to 49 years, 50 to 59 years, 60 to 69 years and 70 to 79 years) .

RESULTS: Histological analysis showed changes suggestive of skin aging (fragmentation and disorganization of collagen fibers), especially in patients over 60 years. There were no significant changes in the relationship of age with the thickness of the dermis and epidermis, but there was with the percentage of the collagen I, III and total (p<0.001), which decreased with increasing aging.

CONCLUSION: There is reduction in collagen with increasing age, and an increase in its degradation, leading to fragmentation of the fibers.

Key words: Skin. Collagen. Skin Aging.

RESUMO

OBJETIVO: Avaliar a influência do envelhecimento na qualidade da pele de mulheres brancas analisando o colágeno dérmico.

MÉTODOS: Realizou-se análise histológica e morfométrica de 218 retalhos pré-auriculares de mulheres brancas que se submeteram espontaneamente à cirurgia estética facial. Foi usada a coloração de Picrosirius Ultrared para analisar e quantificar os colágenos I, III e total em cinco grupos etários (<40 anos, 40 a 49 anos, 50 a 59 anos, 60 a 69 anos e 70 a 79 anos).

RESULTADOS: A análise histológica mostrou alterações sugestivas de envelhecimento cutâneo (fragmentação e desorganização das fibras de colágeno), especialmente em pacientes acima de 60 anos. Não houve diferenças significantes entre a idade e a espessura da derme e da epiderme, mas houve diferenças significantes entre as percentagens de colágeno I, III e total (p<0,001) com o aumento da idade.

CONCLUSÃO: Existe redução do colágeno com o aumento da idade e um aumento na sua degradação, levando à fragmentação das fibras.

Descritores: Pele. Colágeno. Envelhecimento da Pele.

Introduction

The average of life expectancy in Brazil moved from 69.3 years to 72.7 years in a decade (1997-2007). Preliminary results from the 2010 census showed that in Brazil there was an enlargement on the top of the age pyramid, since the population $\geq$ 65 years increased from 4.8% in 1991 to 5.9% in 2000 and 7.4% in 2010[1].

Aging is a natural and unavoidable process for all organs of the body, and the skin is the ideal marker of chronological age. However, being an exposed organ it is subject to environmental damage such as Ultraviolet Radiations (UVR). So, the skin aging can be classified into cutaneous intrinsic aging or chronological aging and cutaneous extrinsic aging or photo-aging[2].

In order to review the influence of aging on the quality of the skin of white women, were evaluated pre-auricular retails of 218 patients, who underwent spontaneous facial aesthetic plastic surgery.

Methods

The project was approved by the Ethics Committee on Human Research at PUC University of Parana and developed after its approval.

In order of being included in the study, patients had to present themselves in perfect physical and mental health and with full understanding of the process to which they were submitted, besides signing an informative consent form.

The patients answered a questionnaire with data on comorbidities, previous diseases and lifestyle habits. Those ones that had any elements that could influence on the quality of the skin to be evaluated, such as smoking, tanning, topical treatments, use of botulinum toxin and previous surgeries were excluded from the study.

To assess the quality of the skin were collected pre-auricular retails at the period between june 2009 and may 2011 .The flaps of skin were fixed in 10% formalin and sent to the Laboratory of Experimental Pathology - PUCPR.

The central part of the fragments was prepared for histological processing. From each block were made cuts with five micrometers thick.

Hematoxylin-eosin staining (HE) was used for general study and organization of the skin as well as measure of the thickness of the epidermis and dermis.

The density and organization of collagen was evaluated by Ultrared Picrosirius staining. Five fields of each histological section were analyzed and the average of the readings determined the density of collagen in each piece.

Images were captured with a Sony camera, CCD101 and sent to a Sony Trinitron color monitor, frozen and scanned by an oculus TCX board. Finally the images were analyzed by the Image Plus® 4.5 for Windows® Media Cybernetics micro-line Pentium computer.

After evaluating all the pieces, the data received statistical analysis using parametric tests as needed for results, and ANOVA methodology. For all tests it was used values of $p \leq 0.05$ or 5%.

Results

In a universe of 250 women evaluated during the study period, it was possible to select 218 patients who constituted the sample. The age ranged between 33 and 77 years, mean 54.9 ± 8.68 years (Table 1).

TABLE 1 - Age of women.

Age Range	Frequency	Percentage
<40	9	4.13
40 to 49	50	22.93
50 to 59	96	44.05
60 to 69	49	22.47
$\geq$70	14	6.42
Total	218	100.0

Microscopic analysis of histological sections stained with HE in the age groups showed no significant changes between them. The thickness of the epidermis measured in micrometers (µm) showed no significant differences between age groups ($p = 0.152$) and there were no significant differences in the thickness of the dermis (in µm) in relation to age ($p = 0.506$). There were no significant differences in the correlation between age and thickness of the epidermis ($p = 0.546$) and age and thickness of the dermis ($p = 0.343$).

At Ultrared Picrosirius staining, there was slight disruption and fragmentation of collagen fibers till the sixth decade, and became more evident from this age.

Histological sections of skin from the group of women aged below 50 years old showed large amounts of type I collagen fibers arranged neatly (Figure 1).
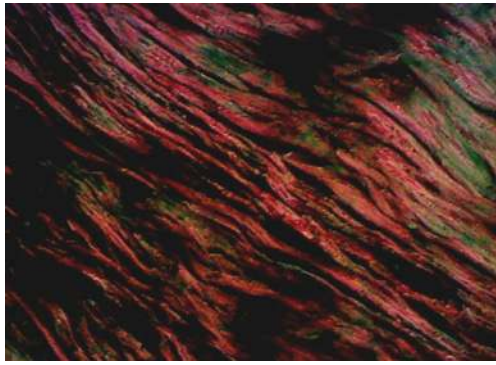
FIGURE 1 - Microphotograph of histological section of skin of women of age <50 years (PS – 400x) Collagen type I = red, Collagen type III = green.

Histological sections of skin from the group of women aged 50 to 59 years showed mild fragmentation of collagen type I (Figure 2).
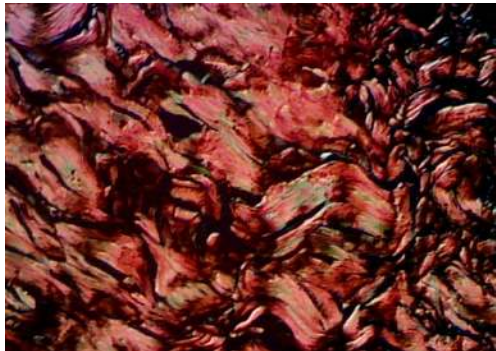

FIGURE 2 - Microphotograph of histological section of skin of women aged 50 to 59 (PS – 400x) Collagen type I = red, Collagen type III = green.

The histological sections of skin from the group of women aged 60 to 69 years showed marked reduction of type I collagen fibers and the presence of type III collagen fibers (Figure 3).
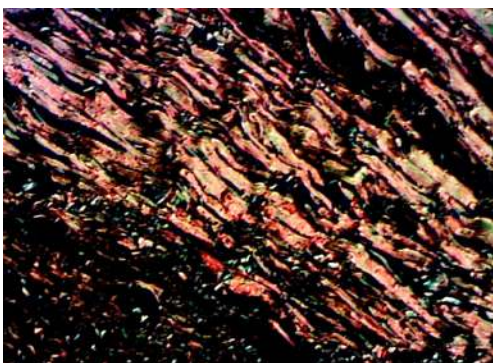

FIGURE 3 - Microphotograph of histological section of skin of women aged 60 to 69 years (PS – 400x) Collagen type I = red, Collagen type III = green.

Histological sections of skin from the group of women

aged 70 to 79 years showed marked disorganization and fragmentation of fibers of collagen type I and presence of type III collagen fibers (Figure 4).
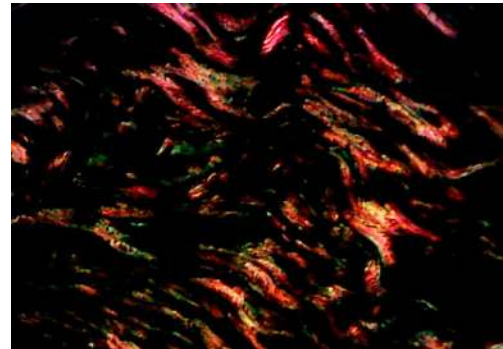

FIGURE 4 - Microphotograph of histological section of skin of women aged 70 to 79 (PS 400x) Collagen type I = red, Collagen type III = green.

Significant differences were found between the age groups and the percentage of collagens I, III and total ($p < 0.001$) (Table 2).

TABLE 2 - Descriptive statistics and p values of collagen I, III and total by age group.

Variable	Age	n	Mean	Median	Mínimum	Máximum	Standard Deviation	Value of p*
Col I	<40	9	22.08	22.47	16.22	25.87	2.88	
	40 to 49	61	16.24	16.00	10.31	29.52	3.62	
	50 to 59	83	13.23	13.68	6.37	18.89	2.80	
	60 to 69	39	9.91	9.75	3.58	18.74	3.06	
	≥70	12	7.54	7.68	5.54	10.00	1.36	<0.001
Col III	<40	9	17.96	17.93	13.26	23.21	3.38	
	40 to 49	61	13.03	12.47	3.33	23.52	3.52	
	50 to 59	83	10.87	10.72	5.33	17.33	2.68	
	60 to 69	39	8.08	8.22	2.32	14.50	3.19	
	≥70	12	6.42	7.25	2.73	9.48	2.05	<0.001
Total	<40	9	40.05	39.92	29.58	49.07	5.56	
	40 to 49	61	29.27	28.11	19.19	44.79	5.81	
	50 to 59	83	24.10	24.49	11.70	36.22	5.23	
	60 to 69	39	18.00	17.10	7.35	31.39	5.80	
	≥70	12	13.96	15.17	9.12	18.42	3.14	<0.001

One-factor ANOVA, $p<0.05$
14/218 excluded for failed reading

Comparing the age groups, two by two in relation to collagens I, III and total, differences were significant ($p<0.001$ for all comparisons except that between 60 to 69 x ≥ 70 ($p = 0.007$ for collagen I; $p = 0.076$ for collagen III and $p = 0.012$ for total collagen).

The comparison between age and percentage of collagen I, III and total showed a decrease of total collagen with increasing
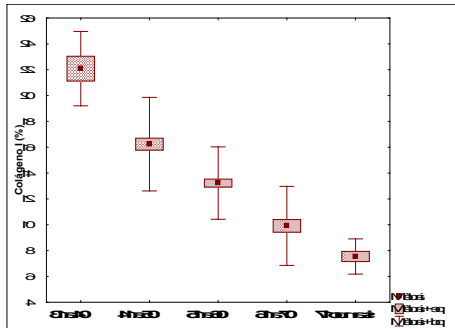
age (Figures 5, 6 and 7).

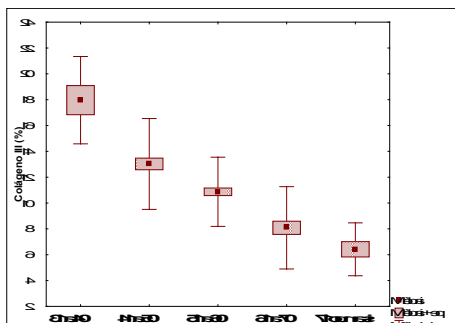**FIGURE 5** - Relationship between percentage of collagen I and age groups.

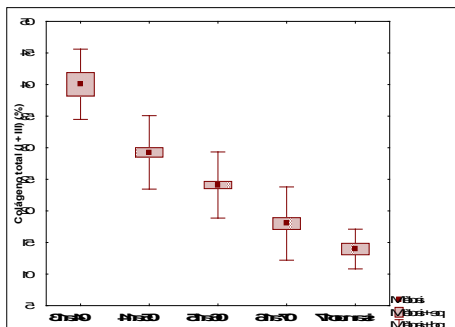**FIGURE 6** - Relationship between percentage of collagen III and age groups.

FIGURE 7 - Relationship between percentage of total collagen and age groups.

There were significant differences (p <0.001) between age and the percentage of collagens I, III and total (Table 3).

TABLE 3 - Correlation between age and percentage of collagen I, III and total.

Variables	Pearson's Correlation Coefficient	Value of p
Age x col. I	-0.709	<0.001
Age x col. III	-0.614	<0.001
Age x total col	-0.706	<0.001

Discussion

Aging is a natural and unavoidable process for all organs of the body, and the skin is the ideal marker of chronological age. However, being an exposed organ, it is subject to environmental damage such as that caused by UVR. So, the skin aging can be classified into cutaneous intrinsic aging or chronological aging and cutaneous extrinsic aging or photo-aging, given the relevance of UVR[3-5].

According to Landau, the most important aspect of aging would be the intrinsic flattening of the dermal-epidermal junction. However, while the thickness of the epidermis remains constant over the years, the thickness of the dermis reduces from the eighth decade[6]. In this sample there was no significant difference in the thickness of the epidermis and dermis among age groups assessed, going against the observations of this author.

Oriya *et al*.[7] collected the abdominal skin of 18 cadavers between 20 and over 60 years, and found a significant reduction of the epidermis and dermis in individuals over 60 years of age, suggesting that these parameters are not fallen continuously, but it is more pronounced in advanced decades. The authors did not specify the causes of death and the general conditions of the research subjects. The patients who formed the present study were in perfect health, which could have contributed to a better quality of skin, especially among younger age groups.

Structural and functional alterations of dermo epidermal junction were cited by Le Varlet *et al*.[8] after analysis of 14 samples of retro-auricular skin of women between nine to 79 years old. These changes were more homogeneous in young and older patients, and heterogeneous in intermediate group. The fact that retro-auricular skin has been examined is conflicting with the current study, because pre-auricular skin is more susceptible to UVR, being more exposed.

Rabe *et al*.[9] noticed elongated and flattened fibroblasts in the photo aging skin, but still able to produce collagen, indicating that changes in collagen synthesis did not seem to be the cause of the decrease in collagen I and III.

The changes found in aged skin are: volume loss, reduction cell (fibroblasts and mast cells), reduction of blood vessels, shortening of capillary loops and abnormal nerve-endings[10].

Moragas *et al*.[11] showed changes of the dermal collagen in a study involving 86 patients from 3.5 months to 86 years, but they were more pronounced from the seventh decade. These authors obtained results similar to those of the present study, although the group of Moragas *et al*.[11] presented extremes of age

more pronounced and also had fewer cases.

In this study, the decrease in the percentage of collagen I, III and total across age groups was evident, and structural changes in organization of collagen fibers were more pronounced in the range 60 to 69 and ≥ 70 unlike Varani *et al.*[12], who concluded that the decrease in collagen, as well as the number of fibroblasts was more pronounced from 80 years.

In the study by El-Domyati *et al.*[13] was evaluated exposed and unexposed skin of 38 donors between six to 84 years old. The changes started from the fifth decade, but they were only significant in the eighth and ninth decades, whith only traces of collagen were noticed. Perhaps the number of cases (218) in our sample may have contributed to the difference in results.

The changes that lead to decreased collagen with increasing age in this group and in the general population are not yet fully understood. Aging can vary from person to person according to lifestyle, current and previous diseases, genetic and environmental influences[14].

In the elderly, the fibroblasts continue to produce collagen (even *in vitro*), which could suggest that the decrease of collagen would be subject to several factors, including increased degradation since the synthesis of matrix metalloproteinases (MMPs), enzymes responsible for degradation of collagen, increases with aging without an equivalent increase in their inhibitors. The beneficial effect of retinoids on skin aging due to the inhibitory effect on MMPs may be an indicator of this phenomenon[15].

There are so many questions about skin aging and many studies are being made to clarify them. Only with the perfect understanding of this process will settle the ways to establish effective methods to prevent and to treat aging skin and perhaps to extend the benefits to other organs.

Conclusions

In white women, aging leads to: qualitative and degenerative changes in the dermis; decrease in total collagen and its fractions I and III; fragmentation and disorganization of collagen fibers especially above 60 years of age.

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