Int Ophthalmol (2020) 40:1987–1992
https://doi.org/10.1007/s10792-020-01373-w

ORIGINAL PAPER

Assessment of COL1A1 and MMP9 expression in patients with dermatochalasis

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Received: 12 December 2019 / Accepted: 10 April 2020 / Published online: 22 April 2020

Abstract

Purpose Dermatochalasis is a clinical condition characterized by loss of elasticity of eyelid skin and soft tissue, which typically affects the elderly population. The aim of this study is to investigate the mRNA expression levels of collagen type 1 alpha 1 (COL1A1) and matrix metalloproteinase-9 (MMP9) genes in dermatochalasis tissue.

Methods The study group consisted of 15 patients who underwent upper eyelid blepharoplasty and were above 40 years old. The patients in our control group were divided into two subgroups according to their ages. Fourteen patients who were under 40 years old and had anterior blepharoptosis surgery for blepharoptosis were designed as the young control group. Sixteen patients who were older than 40 years old and had anterior blepharoptosis surgery for blepharoptosis were designed as the old control group. The patients in the dermatochalasis group were also evaluated according to their smoking status. Surgical tissue specimens were analyzed for COL1A1 and MMP9 mRNA gene expression levels by using real-time polymerase chain reaction.

Results COL1A1 and MMP9 mRNA gene expression levels were not statistically different between the groups ($p = 0.247$; $p = 0.052$, respectively). When compared in means of the smoking habit, smokers in the dermatochalasis group exhibited higher COL1A1

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mRNA expression levels when compared to nonsmokers ($p = 0.008$). MMP9 gene expression levels of smokers exhibited almost statistically higher levels but at the limit when compared to nonsmokers ($p = 0.05$).

Conclusions This study represents a preliminary study to detect the tissue changes at a molecular level in dermatochalasis, which is known to be related to connective tissue pathology. Collagen and MMPs are essential components of the extracellular matrix, and smoking might affect their gene expression. Further prospective studies on these regulatory genes and encoded protein levels with a larger group of patients may provide particular contribution to explaining the pathophysiology of dermatochalasis.

Keywords Dermatochalasis · Sagging eyelid · COL1A1 · Gene expression · MMP9

Introduction

Dermatochalasis is a condition of upper or lower eyelids in which the elasticity of connective tissue is excessively loosened [1]. Although encountered mainly in elderly, it may occur as early as in the age of 40. In addition to its cosmetic aspect, particularly the loosened skin of the upper eyelid leads to pseudoptosis and upper visual field loss. The only medical treatment as either cosmetic or functional problem for dermatochalasis is surgical intervention [2].

The pathophysiology of dermatochalasis is concurrent with the changes which occur during the normal process of skin aging. Intrinsic factors like genetic inheritance, hormonal alterations, and metabolic oxidant stress are implicated in the normal aging process, as well as the extrinsic factors like exposure to the ultraviolet light beam, alcohol consumption, smoking, and psychological stress [3–5]. Jacobs et al. reported the concomitant contribution of both intrinsic and extrinsic factors in dermatochalasis [6].

Skin aging is a process that involves dehydration, loss of elasticity, and increased oxidative stress [7, 8]. Collagen and elastin are critical structural components of the extracellular matrix. The synthesis and degradation of both fiber types are found to be lowered during the process of intrinsic aging. Matrix metalloproteinases (MMPs) represent a subfamily of zinc-dependent endopeptidases and are responsible for the degradation of collagen and elastin in the extracellular matrix during physiological conditions and various diseases [9]. Naval et al. investigated a set of 13 different single nucleotide polymorphisms in genes encoding for proteins that play a role in antioxidant capacity, hydration, and elasticity of the skin and reported a genetic predisposition to elasticity loss due to genetic polymorphisms in MMP3 and MMP9 encoding genes [10]. The same study has also revealed polymorphism-based tendency to oxidative stress-related variations in genes that encode NADPH dehydrogenase, erythroid 3-related factor, glutathione peroxidase I and catalase.

To date, there are no data on biochemical alterations and gene expression levels of the essential components of the extracellular matrix. In this study, we aimed to evaluate the levels of collagen type 1 alpha 1 (COL1A1) and MMP9 gene expression (mRNA) in excised dermatochalasis tissue specimens by using real-time polymerase chain reaction. These biomarkers were chosen because of their potential involvement in connective tissue alterations.

Materials and methods

Subjects and procedure

This study was approved by the Ethics Committee for Clinical Studies and Research of the affiliated university (No. 2015/15) and was performed in accordance with the guidelines in the Declaration of Helsinki. The study was conducted at Ankara Ataturk Training and Research Hospital Ophthalmology Department between June 2015 and June 2016.

Although there is no well-defined grading system for the severity of dermatochalasis, we used the 4-level Photonumerical Severity Scale, which was described by Jacobs et al. previously [6]. In the dermatochalasis group, 15 patients with moderate and severe sagging eyelids were planned for upper eyelid blepharoplasty. Right upper eyelid specimens were obtained during upper eyelid blepharoplasty.

The patients in our control group were divided into two subgroups according to their ages. Fourteen patients who were under 40 years old and had anterior blepharoptosis surgery for blepharoptosis were

designed as the young control group. Sixteen patients who were older than 40 years old and had anterior blepharoptosis surgery for blepharoptosis were designed as the old control group. Upper eyelid specimens were obtained during anterior blepharoptosis surgery for blepharoptosis.

Patients with chronic diseases (diabetes mellitus, thyroid dysfunction, rheumatological disease, renal disease, chronic dermatitis), regular systemic drug intake, and with history of previous eyelid surgery or eyelid trauma were excluded from the study.

All patients were questioned about their smoking habits. Patients who smoke ≥ 5 per day cigarettes at least for 1 year were accepted as smoker, and patients who do not smoke at least for 1 year were accepted as nonsmoker. Patients with indoor working activity were selected and included in our study to standardize exposure to UV as much as possible. After written informed consent was obtained from all subjects, tissue samples were immediately collected and stored at − 70 °C until analysis.

RNA isolation and real-time polymerase chain reaction (RT-PCR)

RNA was isolated from frozen eyelid specimens using the PureLink® RNA mini kit for purification of total RNA isolation (Catalog numbers: 12183018A, 12183025). Total RNA from each sample was quantified using the NanoDrop ND-1000. cDNA was obtained from the samples using a high-capacity cDNA reverse transcription kit (Applied Biosystem Part Number 4375575). cDNA was generated with high-capacity RNA-to-cDNA kit (Applied Biosystems, EUA) from 200 ng of total RNA in RNAse-free water 9 µl mixed with 10 µl of 2X RT buffer and 1 µl Enzyme Mix. The mix reaction was heated to the first cycle 50 °C 2 min., 40 cycles with 95 °C 15 s. 60 °C 1 min. The samples were stored at − 20 °C after the completed reaction. PCR products were electrophoresed on a 2% agarose gel stained with ethidium bromide to detect the specific PCR products.

Expression levels of MMP9, COL1A1, beta-actin genes were analyzed using Step One plus Applied Biosystems real-time PCR. MMP9, COL1A1, Beta-actin gene expression analyses were performed via TaqMan Gene Expression Assays. We used the MMP9 gene expression assay (Thermo Fisher, Hs00957562_m1) to determine the MMP9 mRNA level. Also, COL1A1 gene expression levels were determined with the COL1A1 gene expression assay (Thermo Fisher, Hs00164004_m1). For each sample, the CT (threshold cycle) amount for the RNA of interest was normalized to the amount of beta-actin. The relative quantification of gene expression was analyzed by the $2^{-\Delta\Delta CT}$ method. The method is a relative quantification which calculates the fold change in the gene expression by comparing normalized CT values ($\Delta\Delta CT$) of treated samples to those of untreated samples for three independent experiments. All amplification reactions were performed in triplicate.

Statistical analysis

The gene expressions were calculated by the $\Delta\Delta CT$ method. In comparison, the $2^{-\Delta\Delta CT}$ values of the patient group, and the $2^{-\Delta\Delta CT}$ values of the control groups (young and old) were compared. The Kruskal–Wallis test was used for triple comparisons, and the Wilcoxon–Mann–Whitney test was used for binary comparisons. p value < 0.05 was considered statistically significant in analyses. p value of 0.017 was considered to be statistically significant based on the Bonferroni correction. The summary statistics were expressed as mean ± standard deviation, median (minimum–maximum). All statistical analyses were performed using the R program.

Results

The demographical characteristics of dermatochalasis and control groups are shown in Table 1. There were 10 female and 5 male patients in the dermatochalasis group, 7 female and 7 male subjects in the young control group, and 6 female and 10 male subjects in the old control group ($p = 0.266$). The mean age was 47.87 ± 6.12 (43–65 years) in the dermatochalasis group, 29.71 ± 7.14 (18–38 years) in the young control group, and 55.50 ± 10.16 (41–67 years) in the old control group ($p < 0.001$). The percentage of smokers in dermatochalasis group was 60%, and 50% in both young and old control groups ($p = 0.121$).

The median value for COL1A1 gene expression levels was 1.15 (0.08–37.69) in the dermatochalasis group; 0.89 (0.02–13.50) in the old control group; and 1.42 (0.81–13.14) in the young control group

Table 1 Demographical characteristics of dermatochalasis and control groups

	Dermatochalasis (n = 15)	Control		p value
		Young (n = 14)	Old (n = 16)	
Age	47.87 ± 6.12	29.71 ± 7.14	55.50 ± 10.16	< 0.001
Sex (Female, %)	10 (0.67)	7 (0.50)	6 (0.38)	0.266
Weight	66.53 ± 2.37	64.33 ± 2.33	72.92 ± 2.04	0.062
BMI (kg/m^2)	24.22 ± 2.93	23.15 ± 2.39	26.03 ± 1.38	0.005
Cigarette smoking (Yes, %)	9 (0.60)	7 (0.50)	8 (0.50)	0.121

BMI Body mass index

(p = 0.247). The median value for MMP9 gene expression levels was 0.46 (0.03–4.23) in the dermatochalasis group; 1.20 (0.12–11.91) in the old control group; and 1.65 (0.24–9.49) in the young control group (p = 0.052).

Statistically different expression for COL1A1 gene mRNA levels was found between the smokers (n = 9) (3.30 (0.74–37.69)) and nonsmokers (n = 6) (0.89 (0.08–1.15)) in the dermatochalasis group (p = 0.008). MMP9 gene expression levels of smokers (0.66 (0.23–4.23)) were on the boundary of the significance when compared to nonsmokers (0.13 (0.03–1.27)) (p = 0.05).

Discussion

Dermatochalasis is an eye condition in middle-aged and older adults, which is characterized by sagging eyelids. At first glance, it seems to be a cosmetic problem, but it also leads to loss of superior visual field, headaches caused by attempting to keep the eyelids up by brow elevation, dry eye, and alterations in corneal biomechanics [11–13].

The Rotterdam Study and the Twins UK Study are the two main epidemiological studies that were designed to determine the risk factors for sagging eyelids [6]. The most common cause of dermatochalasis is the normal skin aging phenomenon. Skin aging is one of the most popular research topics as people live longer. Intrinsic (race, sex, and genetics) and extrinsic factors (sun exposure, alcohol use, smoking, and nutritional deficits) are held responsible in the skin aging process. Collagen and elastin are the major structural components of the skin. Therefore, studies for understanding aging are focused on the expression and metabolism of these proteins. Ultrastructural and enzymatic abnormalities in elastin and MMPs related to eyelid aging were summarized in a review by Damasceno et al. [14].

In the literature, there are specific histological studies to understand the pathophysiology of dermatochalasis. DeAngels et al. showed affected elastic fibers and collagen degeneration histologically in dermatochalasis [15]. Nagi et al. [16] and Karnaz et al. [17] also described particular histopathological findings in dermatochalasis. Both studies reported significantly higher lymphangiectasis density, lymphatic vessel diameters, and a higher number of macrophages in the tissues of the dermatochalasis group compared to their controls.

Agliano et al. [18] investigated lymphatic morphology in eyelids of patients with dermatochalasis and reported numerous eyelid lymphatic vessels with a wider lumen and more tortuous endothelial profile when compared to patients with chalazia. All these findings indicate a subclinical inflammation in tissues with dermatochalasis.

Collagens represent a fibrous protein family and are consisted of three helical alpha chains. There are 46 different genes encoding collagens throughout the human genome. The products of these 46 genes are proteins that join together to form over 28 different collagen fibers. The most common type is Type I collagen, and this type stands for 90% of the collagen in the human body [19].

Gene expression for the abundant Type 1 collagen in the skin was evaluated in the present study. COL1A1 gene expression levels were detected to be highest in the young control group, even though there

was no statistically significant difference between the groups. This finding could be explained by the excess of collagen synthesis in healthy young individuals. The higher levels of COL1A1 gene expression in the dermatochalasis group, compared to their age-matched control group, might be related to subclinical inflammation in dermatochalasis, stimulating collagen synthesis by inducing fibroblasts [20].

The matrix metalloproteinases are defined as a zinc-dependent endopeptidase enzyme family involved in tissue reconstruction in both physiological and pathological circumstances. These proteolytic enzymes have wide substrate specificity and are responsible for the hydrolysis of extracellular matrix components. Keratinocytes and dermal fibroblasts produce MMPs in response to stimulation by oxidative stress, ultraviolet irradiation, and cytokines. The matrix metalloproteinases could be subclassified according to their substrate specificity and structural configurations as well [21]. They play an important role in skin aging biochemistry by the degradation of elastin and collagen. The balance between the synthesis and degradation of collagen is essential for remodeling of the skin.

Naval et al. [10] have reported a genetic predisposition for elasticity loss, based on polymorphisms in MMP3 and MMP9 encoding genes. The MMP9 enzyme gene expression levels were investigated in the present study since the proteolytic effects of MMP enzymes in dermal tissue are related to skin aging. We found the highest levels of MMP9 in the young control group in this study, while there was no statistically significant difference. It might be associated with active connective tissue metabolism in young, healthy individuals, although it is not an exact explanation for the given results. We found lower levels of MMP9 gene expression in the dermatochalasis group than in the similar age-matched control group. Previous studies have revealed that macrophages enhance the synthesis of regulatory molecules such as peroxisome proliferator-activated receptors (PPARs), and these molecules have inhibitory effects on the expression of MMPs [22–24]. Macrophages that were shown to be up-regulated in dermatochalasis tissue may also cause reducing MMP expression by a similar mechanism.

Smoking habits and the effects of tobacco are associated with impaired dermatological conditions like poor wound recovery, skin aging, squamous cell carcinoma, melanoma, oral cancers, hair loss, acne, and psoriasis in addition to the strong relationship with numerous systemic disorders [25]. In vitro studies with the tobacco extract showed that collagen production was impaired by exposure, and beside this, the productions of MMPs and tropoelastin were found to be increased. Resultant findings were summarized as the production of abnormal elastosis material [26]. In this study, COL1A1 gene expression levels were compared between smokers and nonsmokers in the dermatochalasis group, and smokers exhibited significantly higher levels than those of nonsmokers. MMP9 mRNA levels were found on the border of significance although the amount in smokers was apparently higher when compared to nonsmokers. Previous in vitro and in vivo studies have revealed evidence for increased expression of MMPs in dermal tissue based on exposure to tobacco or cigarette smoking [26, 27].

The most important limiting factor of this study is the small number of patients. The significant age differences between the control groups (old and young) and the patient group might cause a possible bias. Also, the determination of protein levels, together with gene expression levels of COL1A1 and MMP9, could have provided better interpretation. Another vital component of connective tissue, elastin, could also be investigated.

To the best of our knowledge, this is the first study evaluating collagen metabolism in dermatochalasis. Understanding of dermatochalasis pathophysiology may provide some new options in the treatment of this common clinical condition, which occurs as a functional and cosmetic issue in affected individuals. For this reason, further studies with larger numbers of cases and involving more specific molecules could help for a better understanding of the complex connective tissue metabolism in physiological and pathological conditions.

Acknowledgements This study was supported by the Research Fund of the University Mugla Sitki Kocman (No: 15/147).

Compliance with ethical standards

Conflict of interest The authors report that there is no conflict of interests.

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