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ORIGINAL PAPER

Structural features of eyelid connective tissue in patients with primary open-angle glaucoma

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Abstract

Purpose To study the connective tissue (CT) structure of upper eyelid skin of primary open-angle glaucoma (POAG) patients.

Patients and methods Forty-seven patients aged 47–91 expecting blepharoplasty formed 3 groups: group 1 [16 subjects without POAG, median age 55 years (interquartile range 54–55.5)], group 2 [12 subjects without POAG, median age 73 (72–76.5)], and group 3 [(19 subjects with POAG, median age 74 (70–80.5)]. Age differences between groups 1 and 2 and groups 1 and 3 are significant ($p < 0.05$). Thermodynamic parameters of skin samples taken during blepharoplasty: Endothermic peak (T_d, °C) and denaturation enthalpy (ΔH_d, J/g of dry weight) were determined using differential scanning calorimetry.

Results ΔH_d and T_d in groups 1–3 were, respectively, 8.41 (7.42–10.25) and 66.55 (59.9–66.7); 7.10 (5.76–10.17) and 67.35 (67.0–68.03); 11.40 (9.0–14.9) and 67.70 (67.05–68.45). T_d differences between groups 1 and 2 are significant ($p < 0.05$), and Spearman's correlation between the age and T_d is direct, medium ($R = 0.638$) and significant. ΔH_d in group 3 is significantly higher than in group 2. ΔH_d and T_d in patients without POAG (groups 1 and 2) and those with POAG (group 3) are, respectively, 7.79 (6.9–10.17) and 66.6 (61.2–67,3); 11.40 (9.0–14.9); 67.7 (67.05–68.45); the respective differences are significant.

Conclusion Patients without POAG show a significant increase in T_d with age, while ΔH_d slightly decreases. In POAG, ΔH_d is significantly higher and T_d tends to grow, which may indicate structural changes in eyelid CT (collagen accumulation and cross-linking level rise). Since the upper lid is unaffected by increasing IOP directly, the changes may be viewed as manifestations of systemic CT pathology.

Keywords Glaucoma · Collagen · Cross-links · Connective tissue · Eyelid skin

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Introduction

Primary open-angle glaucoma (POAG) remains a very topical issue. Moreover, it has become especially important recently due to the fact that the number of relatively young subjects of working age (45–55 years) suffering from this disease is growing [1–3]. Many scientific and practical papers focus on a variety of aspects of pathogenesis, diagnosis, drug and surgical therapy of POAG, but a lot of issues have not been resolved yet [4–8].

A relatively new direction in POAG research is to investigate the state of the corneoscleral shell of the eye affected by glaucoma, in particular, its extracellular matrix (ECM) and the main connective tissue protein, collagen. The aim of this investigation is to assess a possible pathogenetic role of sclera pathology in POAG development [9–13]. So far, there has been no unambiguous answer to the question of what is primary—the altered state of ECM (i.e., collagen accumulation and increased number of cross-links) which contributes to POAG development, or, conversely, the development of the glaucomatous process which brings about a pathological change in the corneoscleral ECM which in its turn aggravates the glaucoma process. A definite answer seems to be important as it decides whether scleral pathology needs to be diagnosed in order to evaluate the risk of POAG onset and progression, and whether it is expedient to develop means and methods of targeted corrections of disorders found.

Aging processes may be playing a certain role in the change in the corneoscleral ECM. However, their contribution into POAG pathogenesis has not been definitely established. In particular, it is disputed whether pathological acceleration of natural age-related changes in the sclera and its increasing rigidity in the absence of stable IOP normalization may contribute to the onset of glaucoma or else cause its progression [14, 15].

Geraghty et al. [16] performed a series of mechanical tests of scleral strips, which demonstrated that the rigidity of the sclera is increasing with age. In patients with confirmed diagnosis of POAG, the functional activity of outflow pathways is lower than in healthy subjects of the same age due to the densification of ECM of the anterior chamber structures which has a negative effect on the permeability of aqueous humor outflow pathways [17–19]. We were able to confirm this fact by evaluating hydrodynamic parameters of POAG patients' eyes on an individual basis, taking into consideration disturbed elastic properties of the corneoscleral shell [20]. Apart from that, it was established that an increased rigidity of lamina cribrosa of the sclera in POAG, induced by ECM densification, lowers the resistance of optic nerve fibers to IOP fluctuations [21, 22].

Yet, by no means all people develop glaucoma with age. Our recent studies showed that the rigidity of the corneoscleral shell, the quantity of collagen and the level of its intra- and intermolecular cross-links are higher in POAG patients than in subjects of the same age without glaucoma [23]. The hypothesis that POAG is a consequence of accelerated natural gerontological processes of changing elasticity, distensibility and density of ECM of fibrotic eye shells is disproved by the data of detailed studies of the CT state of a glaucomatous eye [22]. Obviously, biomechanical structure disorders of the corneoscleral shell in POAG (increased cross-linking and accumulation of the scleral collagen) do not fit into the framework of natural (even accelerated) aging processes: Rather, they are probably based on specific pathological changes in CT metabolism [24].

It is not clear so far whether the revealed changes in the corneoscleral shell in POAG are isolated CT lesions of the eye or, rather, manifestations of systemic disorders of CT metabolism of the whole body, similar to the fact that patients with progressive myopia develop, in parallel with the reduction of biomechanical stability of the corneoscleral shell (target tissue), systemic biochemical and biomechanical disorders of CT and the musculoskeletal system (flat foot, scoliosis, gastroptosis, hypermobility of joints, etc.) [25, 26].

The assumption that POAG is related to systemic CT disorders is corroborated, among other things, by the fact that pseudoexfoliation syndrome (PEX) which often accompanies POAG is due to the increased activity of matrix metalloproteinases. They destroy the collagen and the elastin, which leads to a deposition of protein substance not only in eye structures, but also in other organs, e.g., the heart [27]. With a high probability, PEX is associated with cardiovascular diseases, such as cardiac ischemia and aortic aneurysm, as well as with pelvic organ prolapse in women [28]. Besides that, a retrospective analysis showed that patients with cardiovascular conditions demonstrate a higher rate of glaucoma progression [29].

Capillaroscopy did not reveal a statistically significant link between systemic sclerosis and glaucoma, yet 23% of systemic sclerosis patients were diagnosed with glaucoma [30]. Various sources find that from 2 to 12% of patients suffering from mucopolysaccharidosis have glaucoma [31].

The results of a study into collagen metabolism in POAG conducted by Yaroshevsky and Svyatkovskaya demonstrate the presence of CT metabolic disorders in the eye and in the whole body [32–34]. The researchers showed a strong dependence of collagen metabolism disorders on the stage of POAG, irrespective of the patient's age. In their opinion, this excludes the age character of the revealed changes, which should be considered as manifestations of the condition itself.

To answer the question of whether structural biomechanical changes affecting the sclera in POAG are due to a sustained effect of increased IOP or they may be a manifestation of general CT pathology and may themselves serve as a risk factor for glaucoma development, we decided to investigate the CT of the periorbital area of POAG patients, in particular the eyelids, since these tissues are not subject to a direct influence of IOP. We undertook a comparative study of the skin and cartilage of the upper eyelid of a small group of patients, which demonstrated an increasing trend of cross-linkage of collagen in these structures in POAG as compared to age-related changes [35]. Earlier, we had revealed similar changes in the sclera of POAG patients of varied age [12, 23]. The obtained data allowed us to assume the similarity of changing patterns of properties of the connective tissue in eye shells and adnexa in POAG patients. However, the results of this preliminary studies require corroboration.

As is known, ECM of the derma (including that of the eyelids), as of any other connective tissue, consists of amorphous substance, collagen and elastin fibers. On average, the collagen makes 80% of dry matter of the derma and 90% of all its proteins [36]. One of the modern methods used to determine the state of ECM is differential scanning calorimetry, or DSC, which is able to measure collagen denaturation temperature (T_d, °C)—the maximum temperature at which cross-links are destroyed and the conformation helix–coil transition of the protein occurs. DSC is also used to determine the amount of energy needed to destroy the system of hydrogen links that stabilize the triple-helix collagen—enthalpy (ΔH_d, J/g of dry weight) [37, 38]. Some papers show that denaturation temperature of human skin samples grows with age, while the enthalpy (heat) of the process remains the same [39]. Similar results were obtained in a comparative study of skin samples of young and old rats [37]. Such changes are explained by increased collagen cross-linkage and accumulation of glycation products in the connective tissue throughout the life.

In an earlier study of scleral samples conducted by DSC, we found that samples of POAG patients' sclera had higher T_d and ΔH_d than those of healthy patients of the same age, which is an evidence of its increased cross-linkage and collagen accumulation. These parameters increase with glaucoma progression: Even in stage I they are higher than in subjects of the same age without glaucoma [23].

Purpose

The objective of this work was to study the properties of the connective tissue structure of upper eyelid skin in POAG patients.

Materials and methods

Forty-seven patients aged 47–91 underwent blepharoplasty during which eyelid skin samples were taken. This study was performed according to the tenets of the Declaration of Helsinki and was approved by the local ethical committee. Informed consent was obtained from all individual participants included in the study.

Hypotensive treatment of POAG patients included eye drops that contained beta blockers and/or carbonic anhydrase inhibitors. Patients who received prostaglandin analogs (PGA) were excluded from the study, as PGA may affect the ECM state of upper eyelids of POAG patients: Long-term use of PGA is associated with the involution of dermatochalasis, orbital fat atrophy, mild enophthalmos, flattening of lower eyelid bags, inferior scleral show and tight orbits [40–43].

The patients were divided into three groups depending on the age and POAG presence: group 1 (16 patients aged 47–60 without POAG), group 2 (12 patients older than 60 without POAG) and group 3 (19 patients older than 60 with POAG of stages II and III).

The median age and interquartile range (25–75%) of the three groups were, respectively, 55 (54–55.5) years, 73 (72–76.5) years and 74 (70–80.5) years. Age differences between groups 1 and 2 and groups 1 and 3 were statistically significant ($p < 0.05$).

All patients received a standard ophthalmological examination supplemented with 24-h tonometry according to Maklakov, computer perimetry using a Humphrey Visual Field Analyzer 750i (Carl Zeiss, Germany) and optical coherent tomography of the retina and the optic nerve on an RTVue-100 tomograph (Optovue, USA) aimed at diagnosing POAG.

Biomechanically related structural properties of eyelid skin samples were tested by DSC technique on a Phoenix DSC 204 device (Netzsch, Germany).

The temperature and energy scales were calibrated according to the manufacturer's guidelines with zinc, gallium, indium and naphthalene as standards. The soaked samples were blotted, hermetically sealed into 20-µl aluminum DSC pans and heated in the calorimetric cell from 25 to 95 °C at a constant rate of 10 °C/min with an empty aluminum pan as reference. The endothermic peaks of the DSC thermogram were monitored. The peak temperature and area correspond to denaturation temperature and enthalpy [38]. All measures were taken at least twice to ensure reproducibility. After heating, the specimens were dried and the dry weights were recorded with 1-µg accuracy using Mettler balances. The denaturation enthalpy (ΔH_d) was normalized to dry weight.

The obtained data were statistically processed. The significance of differences between numerical features was estimated by nonparametric methods due to the small size of the groups studied. The determined parameters included the median, the minimum and maximum values, quartiles (25.0 и 75.0), the significance level of differences between the groups using the Mann–Whitney U test ($p < 0.05$ corresponds to significant difference) and Spearman's correlation in R (values from -1, reverse correlation, to $+1$, direct correlation; the closer to zero, the weaker the link, $p < 0.05$ corresponds to significant correlation).

Results

The medians and the interquartile range of thermodynamic parameters T_d and ΔH_d of collagen denaturation of eyelid skin samples of patients of various ages without POAG are presented in Table 1.

Table 1 and Fig. 1 show that T_d of eyelid skin grows with age significantly, which testifies to an increase in cross-linkage of tissue collagen and agrees with a regularity noted in the literature: age-related increase of cross-linkage level in CT [44, 45]. Spearman's correlation between the age and the T_d value is direct, of medium force ($R = 0.638$) and significant ($p < 0.05$).

In contrast to T_d, the enthalpy demonstrates a weakly decreasing trend (Fig. 2); Spearman's correlation coefficient ($R = -0.321$) shows a weak reverse correlation ($p > 0.05$) between the age and ΔH_d, which probably indicates the known process of age-related reduction of collagen content in CT [46].

The results obtained from the study of eyelid skin samples of senior patients with and without POAG are given in Table 2.

The value of ΔH_d of eyelid skin of POAG patients is significantly higher than the respective parameter of patients of the same age without POAG. In POAG, a slight increase was also found for T_d, but in this case, no statistically significant difference from the value obtained in similarly aged patients without POAG was detected. However, the graphs showing the dependence of these parameters on the age illustrate a sufficiently clear growing trend in POAG (Figs. 3, 4).

The revealed trend can be traced clearly in a comparative analysis of all data obtained: The median and the interquartile range of ΔH_d and T_d of patients without and with POAG aged 47 to 86 are, respectively, equal to 7.79 (6.9–10.17) and 66.6 (61.2–67.3), 11.40 (9.0–14.9) and 67.7 (67.05–68.45); the

Table 1 Medians and the interquartile range of ΔH_d (J/g of dry weight) and T_d (°C) of eyelid skin samples of patients of various ages without POAG

Patients	ΔH_d	T_d
Group 1, $n = 16$ 55 (54–55.5) years	8.41 (7.42–10.25)	66.55 (59.9–66.7)
Group 2, $n = 12$ 73 (72–76.5) years*	7.10 (5.76–10.17)	67.35 (67.0–68.03)*

*The differences between the respective parameters of groups 1 and 2 are significant, $p < 0.05$

ΔH_d denaturation enthalpy, T_d endothermic peak

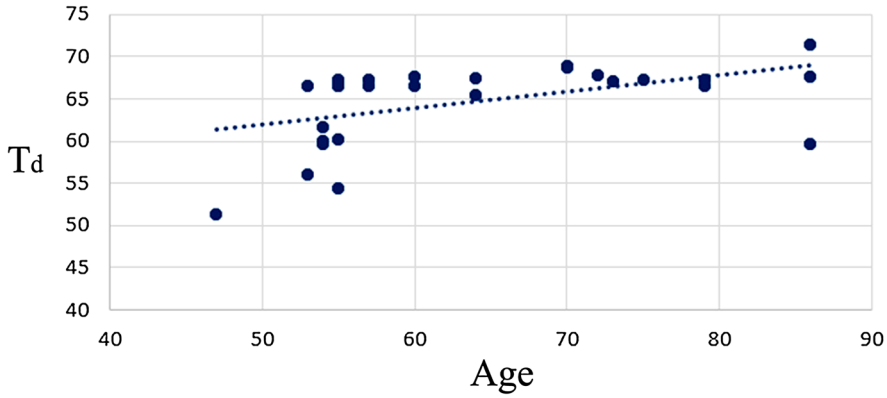

Fig. 1 Dependence of T_d (°C) of eyelid skin samples on the age of patients without POAG, Spearman's correlation in $R = 0.638$

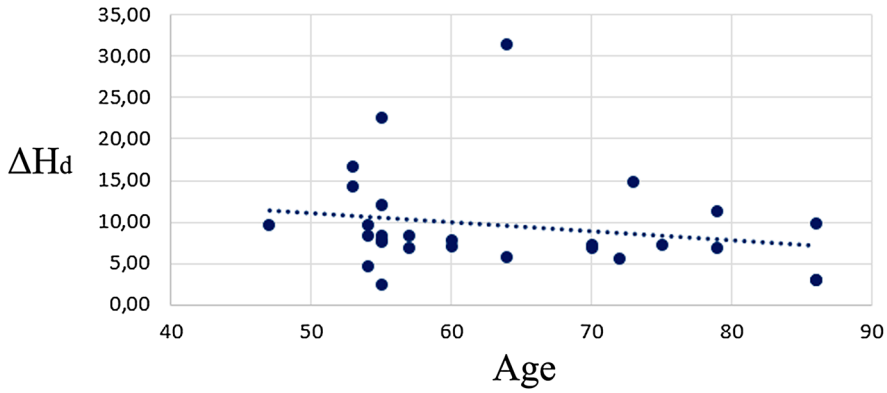

Fig. 2 Dependence of ΔH_d (J/g of dry weight) of eyelid skin samples on the age of patients without POAG, Spearman's correlation in $R = -0.321$

Table 2 Medians and the interquartile range of ΔH_d (J/g of dry weight) and T_d (°C) of eyelid skin samples of patients over 60 with and without POAG

Patients	ΔH_d	T_d
Group 2 (without POAG), $n = 12$	7.10 (5.76–10.17)	67.35 (67.0–68.03)
73 (72–76.5) years		
Group 3 (with POAG), $n = 19$	11.40 (9.0–14.9)*	67.70 (67.05–68.45)
74 (70–80.5) years		

*The difference of ΔH_d between groups 2 and 3 is significant, $p < 0.05$

ΔH_d denaturation enthalpy, T_d endothermic peak

difference between the respective parameters is significant, $p < 0.05$.

Figures 5 and 6 represent the dependencies of ΔH_d and T_d of eyelid skin samples on the age of all patients included in the study. These dependencies clearly reflect the difference of the measured parameters of eyelid skin ECM in patients with and without POAG.

The comparison of the medians and the interquartile ranges of thermodynamic parameters of collagen denaturation—ΔH_d and T_d of eyelid skin samples of patients of all three groups, presented in the diagrams, demonstrates a ΔH_d reduction with age in normal patients and an increase in POAG patients. As for T_d, it shows an age-related growth, which is especially pronounced in POAG patients (Figs. 7, 8).

Discussion

Our study has demonstrated that, normally, T_d of eyelid skin is growing, which agrees with the known regularity that connective tissue is subject to age-

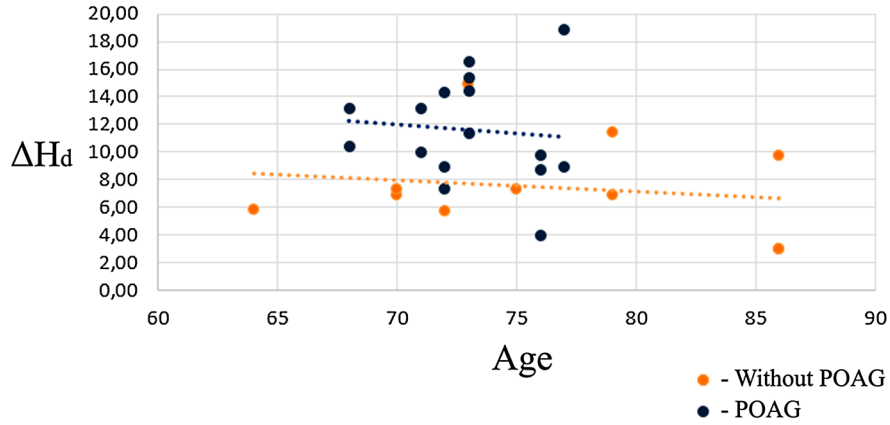

Fig. 3 Dependence of ΔH_d (J/g of dry weight) of eyelid skin samples on the age of patients over 60 with and without POAG

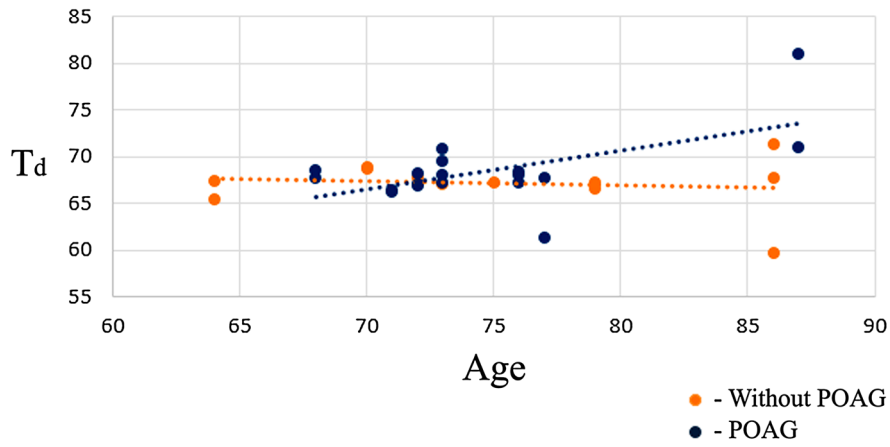

Fig. 4 Dependence of T_d (°C) of eyelid skin samples on the age of patients over 60 with and without POAG

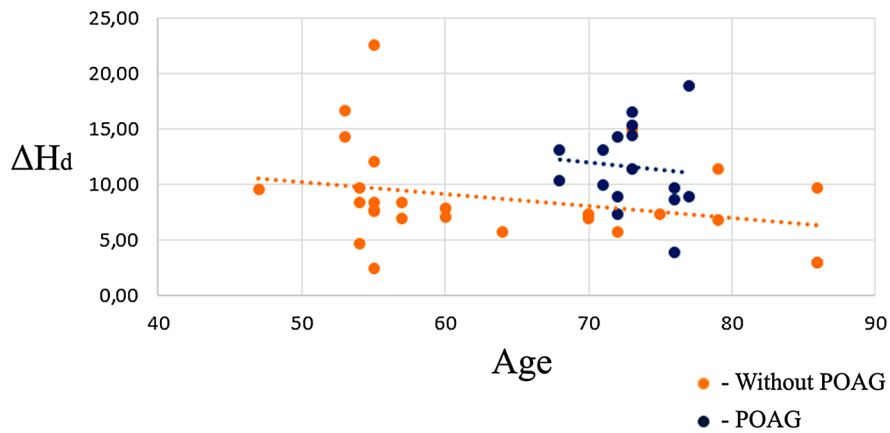

Fig. 5 Dependence of ΔH_d (J/g of dry weight) of eyelid skin samples on the age of patients with and without POAG

related increase in the quantity of collagen cross-links which are mainly formed by non-enzymatic glycation [46, 47]. Similar results were obtained from studies of human skin in other areas of body surface, rat skin, and human sclera and Tenon's capsule [12, 23, 35, 44, 48]. Unlike this, ΔH_d is hardly changing with age. At the same time, in POAG patients both parameters are growing, which agrees with the data obtained from a study of the sclera of glaucomatous eyes [12, 23, 24]. Increasing T_d and ΔH_d in the sclera may be associated with a higher content of insoluble collagen and a higher quantity of intra- and intermolecular stabilizing

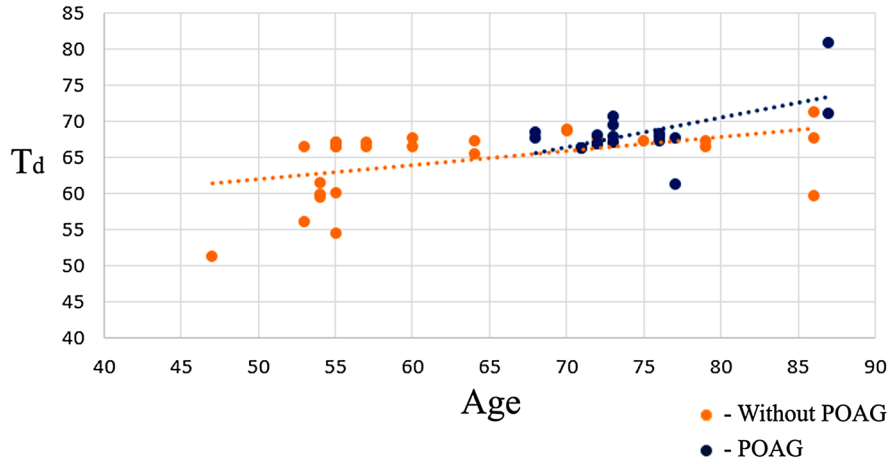

Fig. 6 Dependence of T_d (°C) of eyelid skin samples on the age of patients with and without POAG

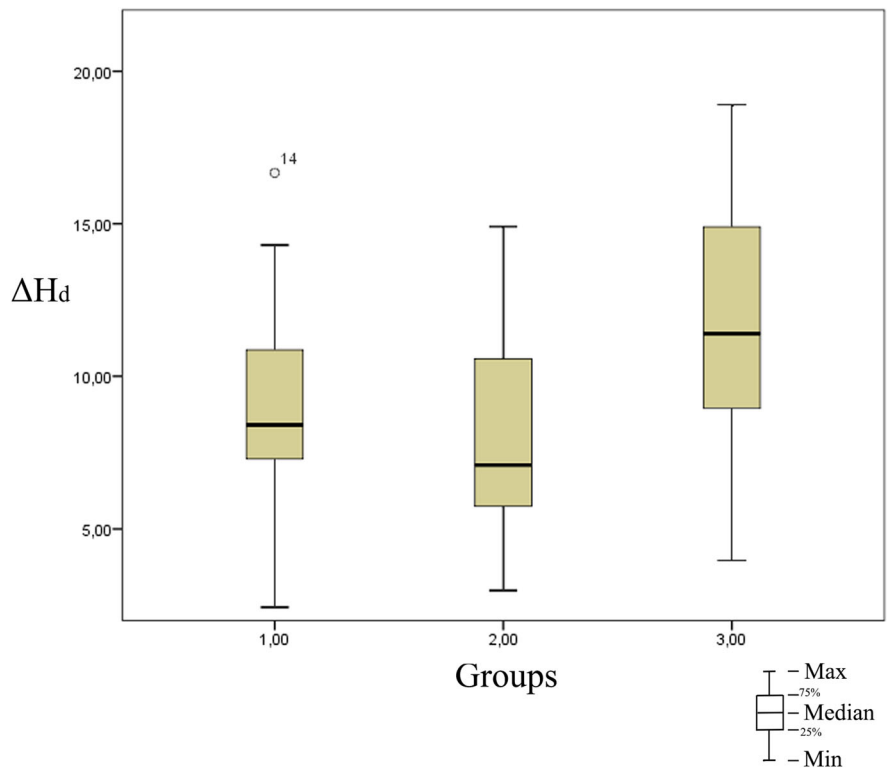

Fig. 7 Medians and interquartile ranges of ΔH_d (J/g of dry weight) of eyelid skin samples on the age of patients of the three groups

links in collagen structures [12, 44–47]. Such changes in POAG sclera are not only caused by the accumulation of cross-links characteristic of natural aging processes and formed through glycation, but also by links formed by other processes, possibly by processes of enzymatic cross-linking due to transglutaminase activation [24]. There is no doubt that aging affects the structure of the matrix of scleral connective tissue, but the advance of glaucomatous processes coincides with more pronounced disorders of corneoscleral shell metabolism, which may lead to permeability and damping capacity loss and to increased rigidity of the sclera. The results obtained by DSC of the sclera explicitly demonstrate that, specifically, glaucoma-induced ECM remodeling takes place in POAG. In particular, this concerns the differences of sclera T_d and ΔH_d between different age-groups (within the limits of 0.8–1.0 °C and 0.3–2.1 J/g of dry weight,

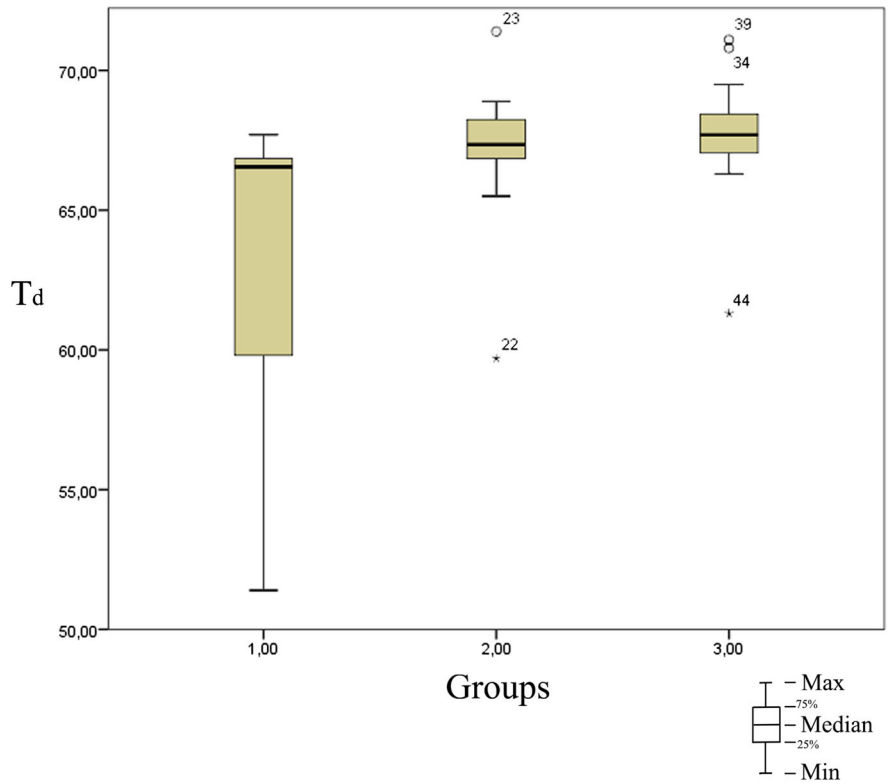

Fig. 8 Medians and interquartile ranges of T_d (°C) of eyelid skin samples on the age of patients of the three groups

respectively) which proved to be smaller than the differences associated with glaucoma progression (within the limits of 1.9–2.7 °C and 4.0–7.5 J/g of dry weight, respectively) [23].

The results we obtained demonstrate the existence of pathological changes in the ECM of upper eyelid skin of glaucoma patients. These changes are analogous with those of glaucomatous sclera, which corroborates the viewpoint that the glaucomatous process is a pathology associated with a systemic disturbance of the connective tissue. The absence of a direct influence of increased IOP on upper eyelid structures permits us to view the manifestation of CT pathology, both systemic and organ type (at eye level) as a possible initial risk factor for glaucoma development.

Conclusion

In the study of skin samples of the upper eyelid of patients without POAG, we established a significant age-related increase in T_d, while ΔH_d of the denaturation process decreased slightly. In POAG, ΔH_d shows a statistically significant growth, whereas T_d tends to grow as compared to the respective parameters of eyelid skin of patients of same age with no POAG. This is an evidence of ECM remodeling, in particular of the accumulation of collagen in this tissue and the increase in the quantity of its intra- and intermolecular cross-links. The revealed structural features of eyelid skin of eyes with glaucoma coincide qualitatively with the features of glaucomatous sclera discovered earlier. This coincidence is an evidence of the fact that the pathology of the connective tissue in POAG probably exists on the organ level and the systemic level. It may be unrelated to the effect of increased IOP and can be viewed as an independent risk factor for glaucoma development. In our opinion, this direction of research into POAG pathogenesis is promising and needs to be continued.

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Compliance with ethical standards

Conflict of interest The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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