Histopathologic Characteristics in Blepharochalasis with Blepharoptosis

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SYNOPSIS Some new information about in blepharochalasis with blepharoptosis has been obtained by histological, histochemical, and immunogold electron microscopical examination. The observations suggest the abnormal changes on the eyelid are related to the involvement of immuno-pathogenetic mechanisms.

【Abstract】

Purose: To analyze the pathological and immunological findings in skin with orbicularis oculi muscle as well as levator aponeurosis speicmens in blepharochalasis with blepharoptosis.

Methods: A prospective case-control study performed of 11 consecutive patients in blepharochalasis with blepharoptosis was analyzed. All the samples obtained during surgery from the department of Ophthalmology, Beijing Tongren Hospital between Jan. 2019 to Dec. 2022. There were skin and orbicularis oculi muscle 10 cases, and levator aponeurosis 8 cases from blepharochalasis with blepharoptosis. Hematoxylin-eosin, Van Gieson stains, immunohistochemistry and colloidal gold-labeled pre-embedded indirect immunogold electron microscopy (Gold-IIEM) were performed to analyze the characteristics of the samples. Normal samples were obtained from the donors in the eye bank of Beijing Tongren Hospital as control group.

Results: Hematoxylin-eosin and Van Gieson stains showed a marked loose and decrease of collagen and elastic fibers of the upper eyelid. Muscles atrophy, derangement and rupture of levator aponeurosis. The two experimental groups got a same result on immunohistochemical staining that demonstrated a difference in level of immunoglobulin, including IgA, CD3+T cell MMP-9 and type III collagen ($P < 0.05$).Gold-IIEM showed that a remarkable decrease of the

density between collagenous fibers in the skin and orbicularis oculi muscle experimental group. The mean gap between collagen fibers was 0.15±0.03μm in blepharochalasis group and 0.10±0.02μm in the control group. MMP-3 and MMP-9 colloidal gold existed in the basal membrane cells and fibroblasts around the collagen.

Conclusions: The blepharoptosis in blepharochalasis appears abnormal histopathological changes on skin and orbicularis oculi muscle as well as levator aponeurosis include perivascular inflammatory cell infiltration, suggest that the immuno-pathogenetic mechanisms with the involvement of cell-mediated immunoresponses might play a substantial role in the pathogenesis of the disease.

[Key words] Blepharochalasis; Ptosis; Histopathology; Immunogold electron microscopic

Introduction

Blepharochalasis (BC) is a rare eyelid disorder, which characterized by exacerbations and remissions of painless edema of the eyelids, tends to be bilateral and is more prevalent in the upper eyelid[1-3]. BC is generally beginning during adolescence, and a large series of cases−based epidemiological study estimated the mean age of onset in North China was 10.09 years[4].

Blepharochalasis can cause various symptoms such as thinned and wrinkled eyelids, blepharoptosis, acquired forms of blepharophimosis, lower lid retraction, pseudoepicanthal folds, proptosis, prolapse of orbital fat or lacrimal tissue, of which blepharoptosis is the most common manifestations. Blepharoptosis was found to be present 70%～80% of the British with BC from 1977 to 2006[3], and 47.31% of those clinically confirmed BC patients in Tongren hospital from 2005 to 2019[4]. It's reported that blepharoptosis in BC is secondary to aponeurotic disinsertion by recurrent bouts of edema and stretching, whereas the levator function itself is preserved. Although certain histopathologic changes including reduced number of elastic fibers and chronic inflammation have been described in some reports, the etiology of blepharochalasis has yet to be fully elucidated, but histopathologic examinations indicate that an immune-related mechanism might play a substantial role in the pathogenesis of the disease [6,7].

Therefore, this study is designed to detect the characteristic immunopathology of skin and orbicularis oculi muscle as well as levator aponeurosis tissues in patients with blepharoptpsis in BC, and discuss the possible associations of immune response

in the pathogenesis of BC.

Materials and methods

This study was undertaken in agreement with the basic principles of Helsinki Declaration，and was gained according to medical ethics and morality by the permission of the Beijing Tongren Hospital Institutional Review Board and the Ethical Committee. Ensure that informed consent provided for all participants is the basis for this study.

11 consecutive patients (15 eyes) with blepharoptosis in BC at quiescent stage and treated by surgery from 2009 to 2019 were enrolled in this study. Of those, 7 were in men and 20 in women aged from 18 to 29 (average age of 10.59). All patients were hospitalized and evaluated with routine ophthalmologic examination to rule out other eye disease. General check–up and blood biochemical examination as well as thyroid function were used to identify autoimmune diseases, haematological diseases, metabolic disease and infections which excluded from experimental group. CT scans of orbital measured before treatment showed no orbital neoplasms.

18 samples with blepharoptosis in BC were divided into two groups: skin and orbicularis oculi muscle group (10 cases), levator aponeurosis group (8 cases). For treatment, surgery was the primarily choice, and surgical approaches were taken according to severity of blepharoptosis, such as skin excision, blepharoplasty, and levator shortening or plication. All surgical should be performed when BC is in a quiescent stage[6]. There were 5 cases normal samples obtained from the donors in the eye bank of Beijing Tongren Hospital, including 3 in male and 5 in female aged from 18 to 25 years, from which skin and orbicularis oculi muscle as well as levator aponeurosis tissues are removed in order to prepare the individual anatomical structures.

Histopathological examination was performed on 18 specimens with BC using haematoxylin-eosin and Verhoeff-Van-Gieson. Immunohistochemistry staining was performed on 18 specimens using primary antibodies for IgA, IgM, IgG, CD3, C1-inhibiter, CD20, MMP-9, MMP-3, collagen type IV and collagen type III [8,9].

Normal controls were used for all antibodies. Additional，another part of specimens (skin and orbicularis oculi muscle 1 case and levator aponeurosis 4 cases) were cut into 2*2*2mm^3 sections in a cryostat for colloidal gold-labeled pre-embedded indirect immunogold electron microscopy (Gold-IIEM). Immunoelectron microscopy using MMP-3, MMP-9 as primary antibodies, and the ultra-small colloidal gold-labeled (10nm) as secondary antibody. Ultrathin sections of 80nm were examined under an electron microscope (JEOL JEM-1400 PLUS)[10]. Normal controls were used for all antibodies.

Results of immunohistochemical staining were studied by semi-quantitative analysis according to the method described by Allred et al. in brief: the proportion of positive stained cells was rated as 0=between 0% and 5% positive, 1=between 6% and 25%, 2=between 26% and 50%, 3=between 51% and 100%. In addition, the intensity score was made on the basis of the average intensity of staining: 0=negative, 1=week, 2=intermediate，3=strong. The intensity score and the proportion score were multiplied to obtain the total score as 0,1+,2+,3+：0=0 and 1, 1+=2 and 3, 2+= 4 and 6， 3+=＞6[8]. All histopathologic examinations were performed and read by three experienced pathologists (Xu X, Li Y, Ding J).

Statistics

To investigate the difference in the expression levels of relative pathological factors between BC tissues and normal control group the nonparametric Wilcoxon signed-ranks test was carried out for statistical analysis by using SPSS 22.0 Software. When a value of $P<0.05$ was required for statistical significance.

Result

Histologic Analysis

Compared with the control group, the upper eyelid skin in BC group appeared to edema, atrophy, a marked loose and decrease of collagen and elastic fibers（Fig.1 A and B）, and a peri-vascular inflammatory infiltrate with macrophage, lymphocytes plasma cells and eosinophils (Fig.2E). Orbicularis oculi muscle atrophy derangement,

and focal lymphocytic infiltration within the light microscope (Fig.2 A and C).

As for the levator aponeurosis, it is found that muscles atrophy, muscle fiber derangement, striation of myofibril dissolved, and dehiscence of sarcolemma, as well as aponeurotic disinsertion compared to the control group (Fig.1 C and D, Fig.2 B, D and F).

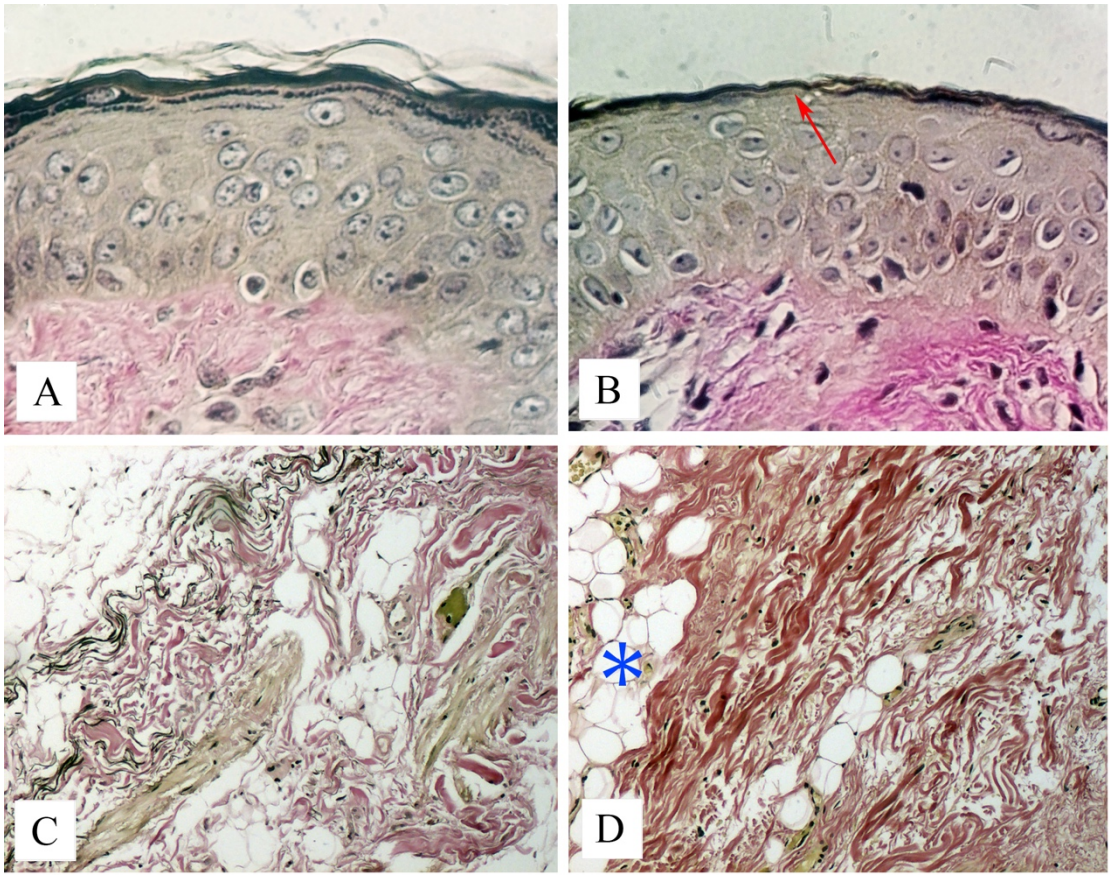

FIG. 1. Verhoeff-Van-Gieson staining of skin and orbicularis oculi muscle and levator aponeurosis A, Normal control of skin and orbicularis oculi muscle（Van Gieson x100）. **B,** Skin and orbicularis oculi muscle with blepharoptosis in BC, finding thinned dermis and irregular dermal-epidermal junction（Van Gieson x100）. **C,** Normal control of levator aponeurosis（Van Gieson x40）. **D,** Levator aponeurosis with blepharoptosis in BC, showing the decrease of elastic fibers, collagen hyperchromatic, and fatty infiltration（*）（Van Gieson x40）.

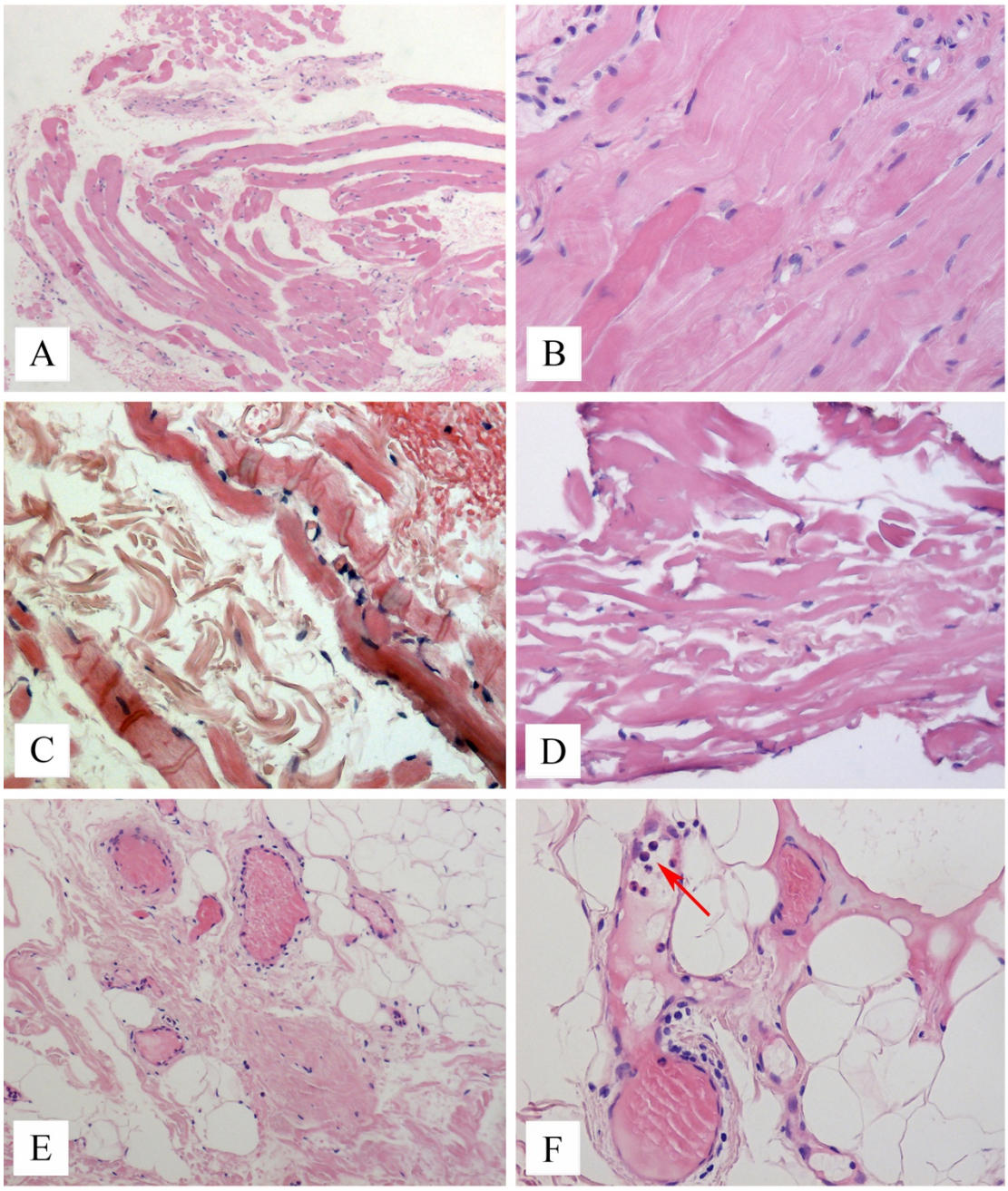

FIG. 2. Hematoxylin-eosin staining of orbicularis oculi muscle and levator aponeurosis **A,** Normal control of orbicularis oculi muscle（HEx100）. **B,** Normal control of levator aponeurosis （HE x 40）. **C,** Orbicularis oculi muscle with blepharoptosis in BC, showing the uneven coloration of collagen fibers, muscle fiber derangement, striation of myofibril dissolved, and dehiscence of sarcolemma（HEx100）. **D,** Levator aponeurosis with blepharoptosis in BC, showing the uneven coloration and diameter variation of muscle fibers, and dehiscence of sarcolemma（HEx40）, **E,** Skin and orbicularis oculi muscle with blepharoptosis in BC, finding a peri-vascular inflammatory infiltrate with macrophage, lymphocytes plasma cells and eosinophils（HEx40）. **F,** Levator aponeurosis with blepharoptosis in BC, showing the damages to the structure of vascular, eosinophils and lymphocyte increased around it（↑）（HEx40）.

Immunohistochemical Analysis

Among the 10 skin and orbicularis oculi muscle tissues, no significant differences of IgG, IgM, CD20, C1-inhibitor and MMP-3 were found compared to the normal control. Comparatively, IgA (P=.001), CD3 (P=.001), MMP-9 (P=.001), as well as collagen type III (P=.002) were statistically significant when compared with normal control group (Table 1, Fig.3).

Among the levator aponeurosis group, there was an increase in the expression of IgA (P=.003), CD3 (P=.002), MMP-3 (P=.036), and MMP-9 (P=.002), as well as a decrease of collagen type III (P=.001). Whereas no significant changes in other proteins (Table 2, Fig.4).

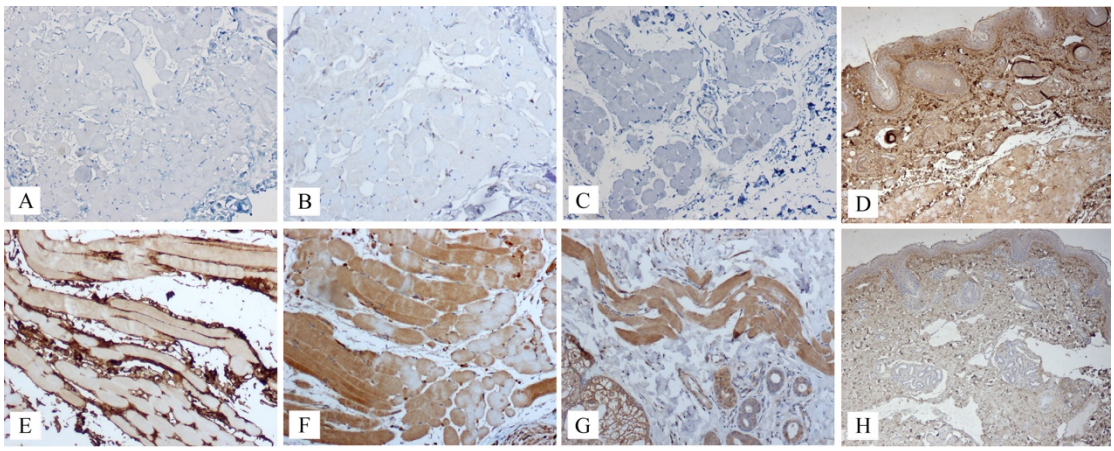

FIG. 3. Immunohistochemical staining of relative proteins expression of BC in orbicularis oculi muscle (DAB×100). A, Normal control stained by IgA. B, Normal control stained by CD3. C, Normal control stained by MMP-9. D, Normal control stained by type III collagen. E, Orbicularis with blepharoptosis in BC stained by IgA. F, Orbicularis with blepharoptosis in BC stained by CD3. G, Orbicularis with blepharoptosis in BC stained by MMP-9. H, Orbicularis with blepharoptosis in BC stained by type III collagen.

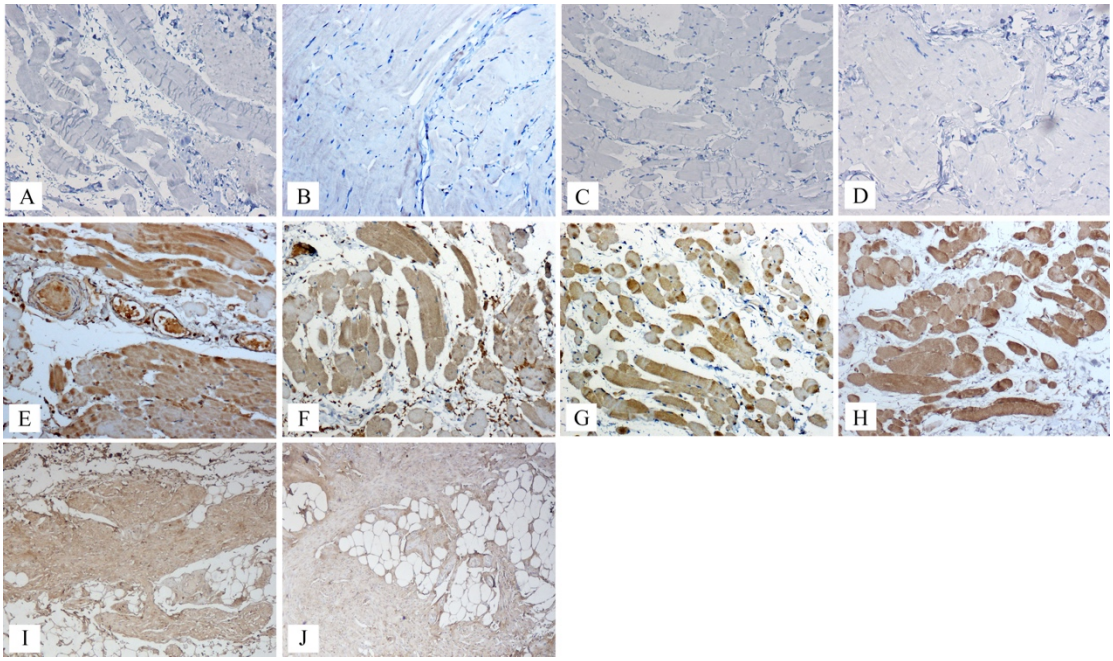

FIG. 4. Immunohistochemical staining of relative proteins expression of BC in levator aponeurosis (DAB× 100). A, Normal control stained by IgA. **B,** Normal control stained by CD3. **C,** Normal control stained by MMP-3. **D,** Normal control stained by MMP-9. **E,** Levator aponeurosis with blepharoptosis in BC stained by IgA. **F,** Levator aponeurosis with blepharoptosis in BC stained by CD3. **G,** Levator aponeurosis with blepharoptosis in BC stained by MMP-3. **H,** Levator aponeurosis with blepharoptosis in BC stained by MMP-9. **I,** Levator aponeurosis with blepharoptosis in BC stained by type III collagen. **J,** Levator aponeurosis with blepharoptosis in BC stained by type III collagen.

TABLE 1. Immunohistochemical staining of relative proteins expression in skin and orbicularis oculi muscle (Wilcoxon signed-ranks)

	Patients					Control					Z	P
Protein	number	+++	++	+	-	number	+++	++	+	-		
IgA	10	6	4	0	0	5	0	0	0	5	-3.224	.001
IgG	10	2	3	5	0	5	1	3	0	1	-.453	.650
IgM	10	2	5	3	0	5	1	0	2	2	-1.660	.097
CD3	10	2	5	3	0	5	0	0	0	5	-3.193	.001
CD20	10	0	2	0	8	5	0	0	0	5	-1.038	.299
C1-inh	10	0	6	3	1	5	1	1	1	2	-.589	.556
MMP-3	10	0	2	2	6	5	0	0	0	5	-1.576	.115
MMP-9	10	6	3	1	0	5	0	0	0	5	-3.237	.001
Collagen III	10	0	1	8	1	5	2	3	0	0	-3.159	.002

TABLE 2. Immunohistochemical staining of relative proteins expression in levator aponeurosis (Wilcoxon signed-ranks)

	Patients					Control					Z	P
Protein	number	+++	++	+	-	number	+++	++	+	-		
IgA	8	5	2	1	0	5	0	0	1	4	-2.989	.003
IgG	8	4	2	1	1	5	2	0	3	0	-.728	.467
IgM	8	3	3	1	1	5	3	0	1	1	-.687	.492
CD3	8	0	5	3	0	5	0	0	0	5	-3.122	.002
CD20	8	0	0	0	8	5	0	0	0	5	.000	1.000
C1-inh	8	0	1	3	4	5	0	1	1	3	-.162	.871
MMP-3	8	1	2	2	3	5	0	0	0	5	-2.094	.036
MMP-9	8	4	4	0	0	5	0	0	0	5	-3.103	.002
Collagen III	8	0	0	6	2	5	5	0	0	0	-3.183	.001

Immunoelectron microscopy (Gold-IIEM) Analysis

Immunoelectron microscopy (Gold-IIEM) was used to probe the subcellularly located of MMP-3 and MMP-9 in the lesion tissues. Gold-IIEM showed collagen fiber well organized with cross-striations and normal myocytes in normal aponeurosis, and no gold particles of MMP-3 or MMP-9 were observed on the control group.

By contrast, observations by electron microscope showed that collagen and muscle fibers were disorganized, the structure were vague and arranged disorderly. A remarkable decrease of the density in collagenous fibers, that the mean gap between collagen fibers was 0.15±0.03μm in BC group and 0.10±0.02μm in the control group (Fig.5). As for the 4 blepharoptosis samples, it appeared that the colloidal gold existed in the basement membrane cell and fibroblasts, which localized very closely to the collagen and elastic fiber. The density of MMP-3 gold particles was relatively lower than MMP-9 (Fig.6 and 7).

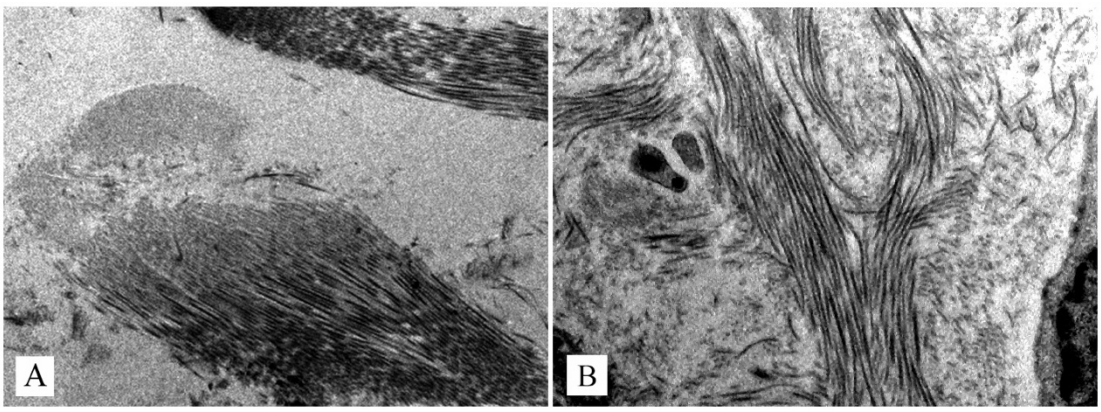

FIG. 5. The density of collagenous fibers observed by Electron microscopy A, Gold-IIEM revealed that normal collagen fibers were more densely and neatly arranged. **B,** Gold-IIEM revealed that collagen fibers were disorganized with dilated and low electron-densed interfibral spaces in skin and orbicularis oculi muscle with blepharoptosis in BC（1bar=5μm）.

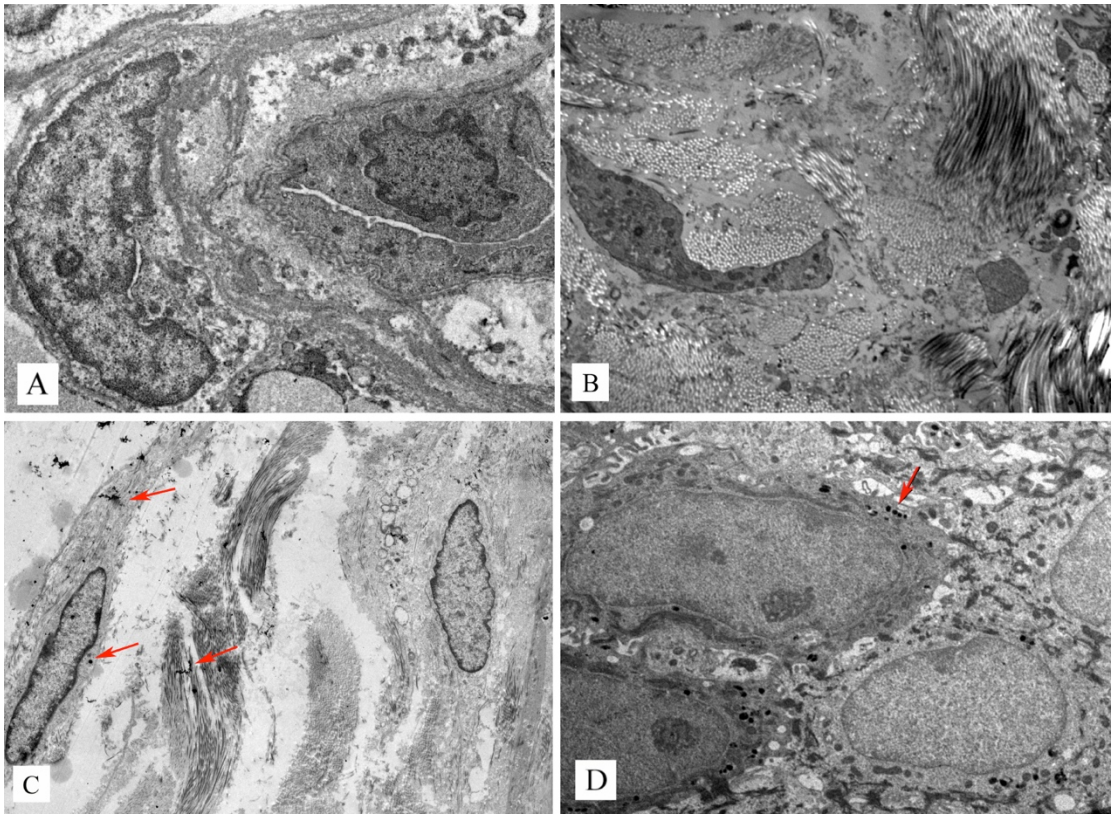

FIG. 6. Skin and orbicularis oculi muscle observed by Gold-IIEM A, Normal control group labeled by MMP-3. **B,** Normal control group labeled by MMP-9. **C,** Skin and orbicularis oculi muscle with blepharoptosis in BC labeled by MMP-3, showing that the immunogold was distributed in the fibroblast around the collagen and elastic fibers. **D,** Skin and orbicularis oculi muscle with blepharoptosis in BC labeled by MMP-9, showing gold particles were localized perinuclear in basement membrane cell, which distinguished from melanin granules（1bar=2μm）.

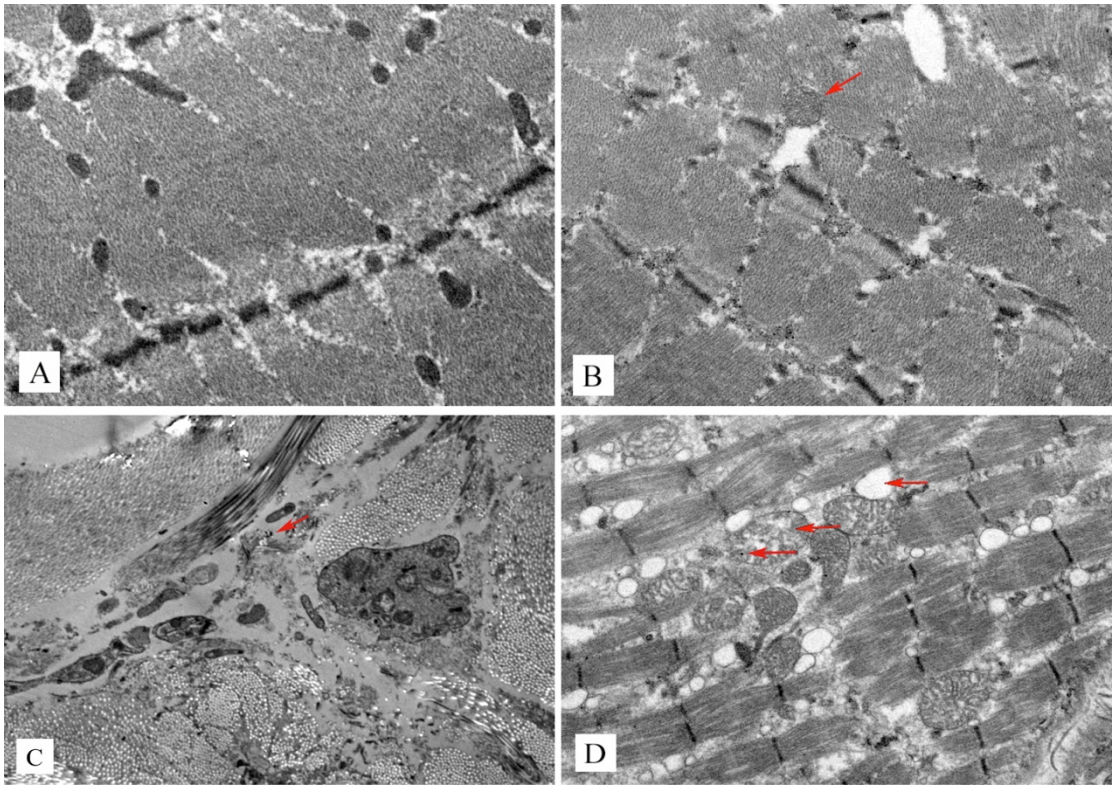

FIG. 7. Levator aponeurosis observed by Gold-IIEM A, Normal control group labeled by MMP-3. **B,** Normal control group labeled by MMP-9(↑Normal mitochondria). **C,** Levator aponeurosis with blepharoptosis in BC labeled by MMP-3, showing that the immunogold was distributed around the collagen fibers. **D,** Levator aponeurosis with blepharoptosis in BC labeled by MMP-9, showing mitochondria swelling, cristae breaking, and gold particles distribution（1bar=2μm）.

Discussion

In most recent studies about the blepharochalasis showed evidence of inflammation process [11,12]. We find a peri-vascular inflammatory infiltrate with macrophage and eosinophils，which play an important role in the nonspecific immunology. Grassegger et al found that IgA antibodies were directed against elastic fibers[13,14]. In our study, immunostaining for IgA in skin and orbicularis muscle and levator aponeurosis with blepharoptosis in BC was statistically significant when compared with normal controls. As we know, in the eyelid, the majority of IgA is secreted by the plasma cells in lacrimal gland. Therefore, we suppose that with uncertain trigger condition, the non-specific immune system attacks the elastic fibers, and then induces the lacrimal gland producing plasma cells to produce antibodies to reach the purpose of protecting. On the other hand, our results showed that CD3

antibody was significantly higher than the controls, but the expression of CD20 in the experimental groups was as normal control. CD3 could be used in the detection of T-lymphocytes, while CD20 to achieve selective deposition of B-lymphocytes. According to this, we suggest that T cell may play an important role in the immune responses. However, all the samples collected in a quiescent stage that could not be ruled out to explain why CD3 higher than CD20. Besides, our results showed that the type III collagen staining was comparable with normal controls in skin and orbicularis oculi muscle group as well as levator aponeurosis. Whereas, type IV collagen in the experimental groups were all absent，neither nor the control group. We attributed this to incorrect disposal of specimen, since different methods of processing tissues have great effect on the quality of sections and a few papers have suggested that frozen section is more suitable for type IV collagen staining.

In the study by Motegi et al[15], it was suggested that the degradation of elastic fibers might associated with the overexpression of MMP-3 (stromelysin-1) and MMP-9 in the development of blepharochalasis. The matrix metalloproteinases (MMPs) are a kind of elastase that consists of more than 25 members. MMPs play a role in the degradation of extracellular matrix components, as well as involve in cell-signaling and gene regulation activities. Additionally, it was suggested that during inflammation T cell may produce MMP-3 and MMP-9. MMP-3 preferentially degrades proteoglycans and structural glycoproteins. MMP-9 also known as gelatinase B plays an important role in the balance of ECM degradation and remodeling. Its specific substrates are mainly type Ⅳ, Ⅴ, Ⅶ, Ⅹ, Ⅺ collagen, proteoglycans, fibronectin, laminin and elastin[15]. In the present study, the expression of MMP-3 and MMP-9 in the lesion tissue was significantly higher than in the control group. In addition, the significance of MMP-9 expression was higher than MMP-3 in skin and orbicularis oculi muscle (P=0.001 and P=0.115, respectively), levator aponeurosis (P=0.002 and P=0.036, respectively). It has been reported that MMP-3 is a stromelysin with high proteolytic efficiency and activates a few proMMPs, so that it may act as an effective activator of MMP-9. We suggested that both MMP-3 and MMP-9 play important roles in the promotion of inflammatory infiltrates. The

substance in a way that provokes the immune system, leading to irritated skin inflammation and activated MMP-3, then additionally activates MMP-9. Besides that, all our samples collected from a quiescent stage, it might also be possible to explain MMP-9 higher than MMP-3. However, expression of MMP-3 responds to various stimuli, including growth factors, IL-1, tumor promoters and oncogene products[15]. The exact stimuli and mechanisms of MMPs require further investigation.

Grassegger et al. [14] have found that IgA antibodies seemed to be concentrated at the margin of the elastic fiber and change the structure of it. A few years later, Kaneoya et al. [16] reported that elastin mRNA expression in the patient's fibroblasts was not decreased compared with that in control group, suggesting that elastin continued to produce but increased destruction in lesion eyelid. Moreover, using a pre-embedding immunoelectron microscopy with colloidal gold-labeled as probes (gold-IIEM) as well as primary antibodies against MMP-3 and MMP-9, we provide the clear pictures on the precise localization of antigens. Our study showed that a remarkable decrease of the density in collagenous fibers and plenty of colloidal gold existed in the basement membrane and fibroblasts around the collagen. All of these indicating that during the swelling of elastic tissue, the nonspecific immune response disintegrating the elastin, and a change of the elastic fiber structure may exposure antigenic sites, then followed by an immune response that associated with the IgA and T cell, as well as overexpression of MMP-3 and MMP-9 may play an important role in the development of BC. Finally, leading to the unbalance between elastic and collagen production and destruction exists in the lesion eyelid. What's more, Karaconji et al. have found doxycycline can use for the treatment of blepharochalasis via inhibition of matrix metalloproteinases[17]. All of this suggests the involvement of immunopathogenetic mechanisms.

In summary, the current results suggest that the cell-mediated immunity inflammation processes play the major role in triggering the exacerbations and remissions edema of the eyelids and the degradation of elastic fibers. Experimental samples with blepharoptosis in BC were selected as the most common clinical manifestation: skin and orbicularis oculi muscle group and levator aponeurosis group.

Despite the different histological structure，the two group almost got a same result on immunohistochemical staining. Such an increased level of immunocytes, including IgA, $CD3^{+}$T cell, MMP-3 and MMP-9 may originate due to the mechanical distortion of elastic fibers and the exposure of antigenic sites. However, the unclear triggers and the cascade reaction of immune processes，which deserve further investigation.

Conflicts of interest

There is no conflict of interest regarding the publication of this paper.

Reference:

[1] Koursh D M, Modjtahedi S P, Selva D, et al. The blepharochalasis syndrome[J]. Survey of Ophthalmology, 2009, 54(2):235-244 .

[2] Ortiz-Perez S, Patel BC. Blepharochalasis Syndrome. 2022 Aug 1. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan–.

[3] Machado Á, Machado S, Selores M. Blepharochalasis. Acta Med Port. 2019 Jun 28;32(6):471.

[4] Zhou J, Ding J, Li D. Blepharochalasis:Clinical and Epidemiological Characteristics, Surgical Strategy and Prognosis-- A Retrospective Cohort Study with 93 Cases[J]. BMC Ophthalmology, 2021, 21(1).

[5] K Sankha, M Rajesh Kumar, D Goutam, et al. Unilateral blepharochalasis of right eye. Indian Journal of Dermatology, 2014, 59(5):525-526.

[6] Li D M, Chen T, Hou Z J, et al. Clinical features of blepharochalasis and surgical treatment of associated deformities[J]. Chinese journal of ophthalmology, 2012, 48(8):696-700.

[7] Dantas SG, Trope BM, de Magalhães TC, Azulay DR, Quintella DC, Ramos-E-Silva M. Blepharochalasis: A rare presentation of cutis laxa. Actas Dermosifiliogr (Engl Ed). 2019 May;110(4):327-329.

[8] Ingrid H O. Immunohistochemistry (IHC)[M]// The Dictionary of Genomics, Transcriptomics and Proteomics. Wiley−VCH Verlag GmbH & Co. KGaA, 2015.

[9] Qureshi A , Pervez S . Allred scoring for ER reporting and it's impact in clearly distinguishing ER negative from ER positive breast cancers[J]. Journal of the Pakistan Medical Association, 2010, 60(5):350-353.

[10] Yi H, Leunissen J, Shi G, et al. A novel procedure for pre-embedding double immunogold-silver labeling at the ultrastructural level[J]. Journal of Histochemistry & Cytochemistry, 2001, 49(3):279-284.

[11] Xin Y, Xu XL, Li Y, Ding JW, Li DM. [Clinical histopathologic characteristics of lacrimal glands in lacrimal gland prolapse with blepharochalasis]. Zhonghua Yan Ke Za Zhi. 2020 Mar 11;56(3):205-210. Chinese.

[12] T Yasuhiro, Z Xiaodong, M Hidenori, et al. Recurrent ptosisi in a patient with blepharochalasis:clinical and histopathologic findings. J Craniofacial Surgery, 2015, 26(1): 52-53.

[13] O Mikio，Y Toshiyuki，Y Keiko. Blepharochalasis showing prominent infiltration of neutrophils in dermal vessels. J Dermatology, 2014, 41(11): 1036-7.

[14] Grassegger A, Romani N, Fritsch P, Smolle J, Hintner H. Immunoglobulin A (IgA) deposits in lesional skin of a patient with blepharochalasis. Br J Dermatol. 1996 Nov;135(5):791-5.

[15] Motegi S, Uchiyama A, Yamada K, Ogino S, Takeuchi Y, Ishikawa O. Blepharochalasis: possibly associated with matrix metalloproteinases. J Dermatol. 2014 Jun;41(6):536-8.

[16] Kaneoya K, Momota Y, Hatamochi A, Matsumoto F, Arima Y, Miyachi Y, Shinkai H, Utani A. Elastin gene expression in blepharochalasis. J Dermatol. 2005 Jan;32(1):26-9.

[17] Karaconji T, Skippen B, Di Girolamo N, Taylor SF, Francis IC, Coroneo MT. Doxycycline for treatment of blepharochalasis via inhibition of matrix metalloproteinases. Ophthalmic Plast Reconstr Surg. 2012 May-Jun;28(3): e76-8.