BMC Ophthalmology

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Muller's muscle fibrosis is a possible predictive factor in the outcome of Muller's muscle-conjunctival resection

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Abstract

Background The purpose of our prospective study is to investigate the histopathology of Muller's muscle extracted after Muller's Muscle-Conjunctival Resection (MMCR) and to find the relationship between histopathological findings and the outcomes of ptosis surgery.

Methods Forty-seven patients with mild to moderate ptosis underwent MMCR surgery and pathological samples including conjunctiva and Muller's muscle were stained with Hematoxylin and Eosin (H/E) and Masson trichrome. The degree of muscle fibrosis and hypertrophy were evaluated.

Results The results indicated that an increase in the severity of fibrosis (for example, increase from mild to moderate), increases the 1 mm correction effect by 0.027 (CI = 0.002–0.052 and *p*-value = 0.033). There is no association between the 1 mm correction effect (*p*-value = 0.67), ptosis correction (*p*-value = 0.60), and post-operation difference between ptotic and normal eye (*p*-value = 0.90) with Muller's muscle hypertrophy. Also, there is no statistically significant association between Muller's muscle hypertrophy and 1 mm correction effect, ptosis correction, and post-operation difference according to the type of pathogenesis (aponeurotic; *p*-value = 0.123, congenital; *p*-value = 0.286, horner syndrome; *p*-value = 0.667).

Conclusions Following the increase in Muller's muscle fibrosis, the ptosis correction effect of MMCR surgery increases, but the presence or absence of hypertrophy of Muller's muscle is not correlated to the outcomes of surgery.

Keywords Muller's muscle-conjunctival resection, Ptosis, Pathology, Fibrosis

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Background

Muller's muscle-conjunctival resection (MMCR) was first described by Putterman and Urist in 1975 for the correction of mild to moderate ptosis [1]. It has been reported that this technique leads to predictable eyelid height with better contour (compared to levator muscle surgery) in cases with ptosis of less than 3 mm with good levator function, along with a positive phenylephrine test [2]. Shorter surgery time, less surgical tissue dissection, lack of skin incision and scar formation, and no tarsal instability are the main advantages of this method [3].

Studies have shown that MMCR is associated with a lower likelihood of requiring revision compared to levator muscle surgery [4–8]. Nonetheless, both techniques have reported failure rates, with anterior approaches showing rates of up to 30% [4–6], and MMCR up to 20%, in various studies [7, 8]. In MMCR, in the case of unexpected results, the result of the operation will be under-correction rather than over-correction; [9, 10] however, the reasons for under-correction have not been clearly defined so far. A better understanding of the causes of lower-than-expected results in this surgery can help in determining the plan for revision surgery [11]. Other applications of MMCR include the use of this surgical technique in the correction of remaining ptosis after external levator advancement surgery in cases of bilateral ptosis [12], as well as the treatment of contralateral eyelid drop because of Hering's effect following unilateral external levator advancement in the ptotic eye [13].

In the literature search, most of the studies on the subject of ptosis surgical treatment failure, have focused on revision surgeries and techniques [10, 14], and less on the pathophysiology and cause of surgical failure. Our hypothesis in this study was that maybe the histopathological findings of Muller's muscle (including the presence or absence of hypertrophy, the degree of fibrosis, etc.) can be associated with the outcome of MMCR surgery.

Methods

Design and setting

This was a prospective cohort study aimed at investigating the effect of Muller's muscle histopathology on the outcomes of MMCR surgery. The population of this study was all patients with mild unilateral ptosis (1–2 mm) and levator function ≥ 8 mm who were referred to the oculoplastic clinic of our hospital in 2022–2023.

The inclusion criteria were mild unilateral ptosis (1–2 mm), levator function ≥ 8 mm, no previous ptosis surgery, and age between 12 and 65 years. The exclusion criteria were the need for any emergency operation, bilateral ptosis, and the patient's lack of consent to continue cooperation (Fig. 1). The study was approved by our local ethics committee according to Helsinki's ethical principles (Approval ID: IR.TUMS.FARABIH.REC.1401.043). All methods and procedures were performed in accordance with the relevant guidelines and regulations.

To have a power of 80% to detect at least a correlation of 0.4 between the fibrosis and 1 mm correction effect with a type I error of 0.05 the sample size was calculated to be 46 patients, and we enrolled 47 subjects in our study.

Data collection

The data collection tool was a checklist that included different variables such as age, gender, levator function, pre- and post-operation margin reflex distance 1 (MRD1) of both eyes, ptosis amount, ptosis correction, 1 mm correction effect, post-operation difference, phenylephrine test (Phenylephrine 2.5% was administered twice to the superior fornix, five minutes apart, with MRD1 in the ptotic eye assessed five minutes after the second dose. "Full correction" was defined as MRD1 equivalence with the non-ptotic eye; an increase below that was labeled "under", and an increase above it as "over"), type of ptosis (congenital, aponeurotic, or Horner's), the amount of fibrosis, presence or absence of hypertrophy of Muller's muscle, and desirable result of surgery (surgery was considered successful if the MRD1 difference between the ptotic and non-ptotic eyes was within 0.5 mm at the final postoperative visit; differences exceeding 0.5 mm were classified as failures). Before and after surgery on every visit, the patient's head was placed behind the slit lamp, and slit photos were taken using a Nikon D5200 camera. The preoperative and the last photo (on the 90th day after surgery) were entered into the ImageJ software and the MRD 1 of each patient was measured with a difference of 0.1 mm. MRD1 measurements were standardized appropriately using a horizontal graduated caliper in photographs. All MRD1 measurements were performed by a single independent researcher (N.M.K) who was blinded to the histopathological results and surgical outcomes.

Surgical procedure

All patients who had the inclusion criteria underwent MMCR surgery under local anesthesia by an oculoplastic surgeon (S.M.R). In order to perform open MMCR surgery, after prepping and draping, the patient's upper eyelid was everted using a Desmarres retractor and anesthesia was injected into the sub-Muller level. A surgical incision was made at the upper edge of the tarsus and Muller's muscle along with the conjunctiva were dissected together. Using a Vicryl 5 – 0 double-armed S11 needle, Muller's muscle was connected to the upper edge of the tarsus in the center with a new insertion and exited from the skin in the upper eyelid crease. After adjustment (based on the amount of ptosis, levator muscle function,

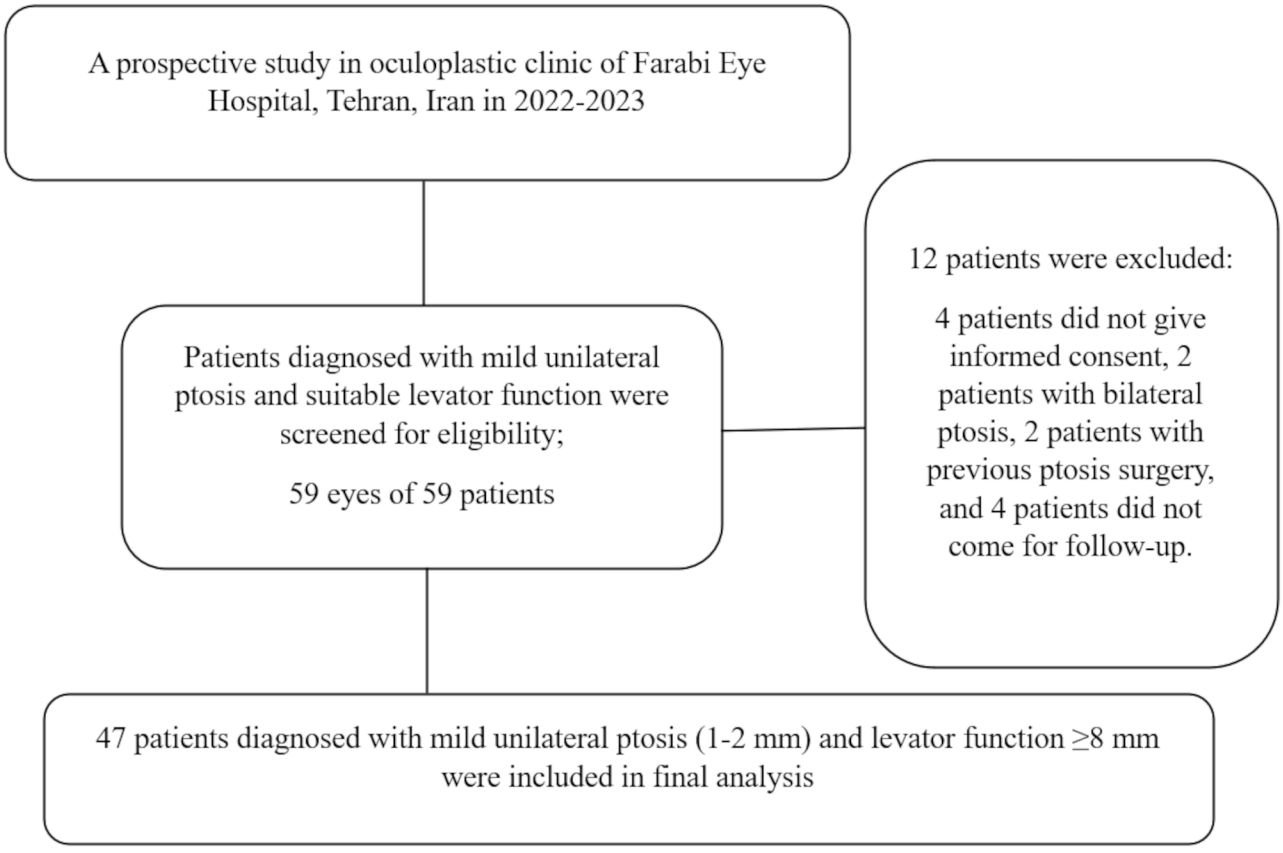

Fig. 1 Study flowchart

and ptosis pathogenesis), two similar sutures were added on the medial and lateral sides. After the final tightening of all three sutures, the distance from the Muller edge to the new insertion site was measured and then the excess muscle was resected. At the end of the procedure, Muller's muscle sample along with the conjunctiva was sent to the pathology laboratory.

The eye was patched and the day after the surgery, after removing the bandage, 0.5% chloramphenicol eye drops, 0.5% erythromycin eye ointment, and lubricant eye drops were prescribed for two weeks. Then, on the 7th, 28th, and 90th days after the surgery, the patient was visited and the results of the surgery on the last visit were included in the study.

Histopathological evaluation

All slides were evaluated by a well-experienced ocular pathologist (Z.N) who was blinded to the clinical and surgical outcomes. Muscular hypertrophy was identified by cell size increase based on standard histopathology definitions. Masson's trichrome staining highlighted fibrosis, differentiating collagen (blue) from muscle fibers (red). Fibrosis was classified into four categories: severe (>50% blue-stained), moderate (25–50% blue-stained), mild (<25% blue-stained), and no fibrosis (Figs. 2 and 3).

Statistical analyses

All statistical analyses were conducted using SPSS, version 24 (IBM Inc., Chicago, IL, USA). The normality of continuous data was evaluated using the Kolmogorov-Smirnov test and Q-Q plot subtly. The Chi-Square test and Fisher Exact Test were used for categorical data and the Mann-Whitney U Test, Spearman's correlation Test, and Univariate Analysis of Variance were used for quantitative variables. *P*-values < 0.05 were considered statistically significant.

Results

The findings revealed that of the 47 participants, the average age was 39 ± 11 years, with 42 individuals (89.4%) identifying as female. Congenital ptosis constituted the predominant etiology (51.1%), succeeded by aponeurotic ptosis (42.6%) and Horner syndrome (6.4%). The left eye was affected in 30 patients (63.8%). Regarding Muller's muscle fibrosis, 7 patients (14.9%) showed no fibrosis, 19 (40.4%) exhibited mild fibrosis, 11 (23.4%) indicated moderate fibrosis, and 10 (21.3%) presented with severe fibrosis. Hypertrophy of Muller's muscle was observed in 29 patients (63%), whereas 17 patients (37%) displayed no hypertrophy, and one patient (2.1%) was unable to be evaluated. The surgical success rate was

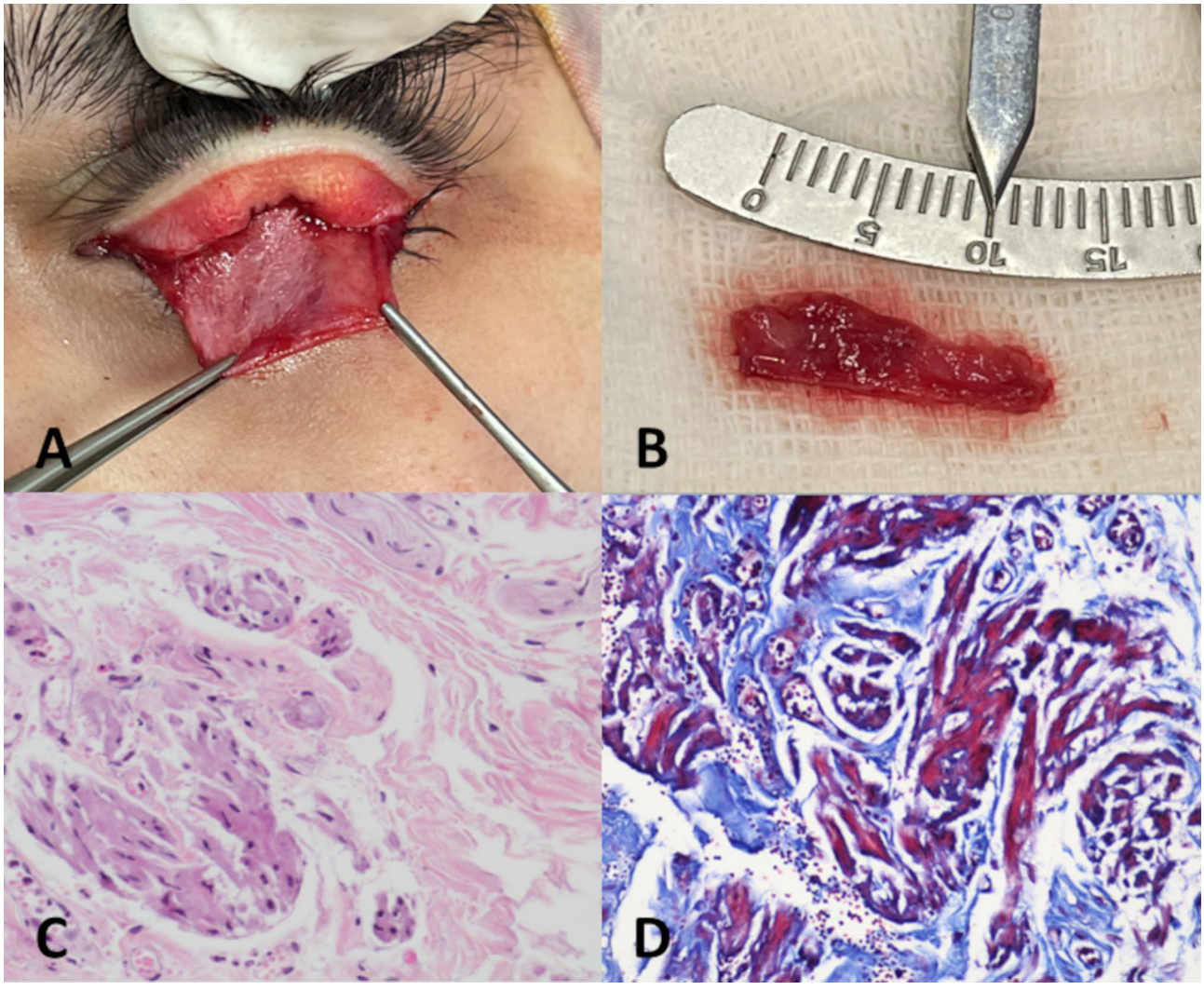

Fig. 2 **A**: Muller's Muscle-Conjunctival Resection (MMCR): preparation of a part of the conjunctiva and Muller's muscle for resection after tightening the sutures, **B**: Gross pathology view of the resected Muller's muscle, **C**: H/E staining of the pathological specimen, **D**: Masson's trichrome staining of the pathological specimen

78.7% (37 patients), with phenylephrine testing revealing full correction in 32 patients (68.1%), overcorrection in 11 (23.4%), and under-correction in 4 (8.5%). Additional clinical characteristics are detailed in Table 1 (Table 1).

The results of the study showed that there is no significant correlation between the age of patients and the success of surgery (p-value = 0.162), ptosis correction (p-value = 0.165), and 1 mm correction effect (p-value = 0.213). Also, these parameters (surgical success, ptosis correction, and 1 mm correction effect) are not correlated to the gender of the patients (p-value = 0.943, 0.201, and 0.102, respectively). There is no correlation between the age and gender of patients with the amount of fibrosis (p-value = 0.462 and 0.489, respectively) and hypertrophy (p-value = 0.542 and 0.885, respectively). There is no significant correlation between the amount of fibrosis and hypertrophy (p-value = 0.816). Also, no significant correlation was found between the amount of fibrosis and hypertrophy with the pathogenesis of ptosis (p-value = 0.761 and 0.176, respectively).

The result showed there is no association between the 1 mm correction effect (p-value = 0.67), ptosis correction (p-value = 0.60), and the post-operation difference between two eyes (p-value = 0.90) with Muller's muscle hypertrophy. Also, there is no statistically significant association between hypertrophy and 1 mm correction effect, ptosis correction, and post-operation difference according to the type of pathogenesis (aponeurotic; p-value = 0.123, congenital; p-value = 0.286, horner; p-value = 0.667). However, there is a positive and significant correlation between Muller's muscle fibrosis and ptosis correction (Correlation Coefficient = 0.314 and p-value = 0.033). The types of ptosis were not correlated with the outcome of surgery (aponeurotic;

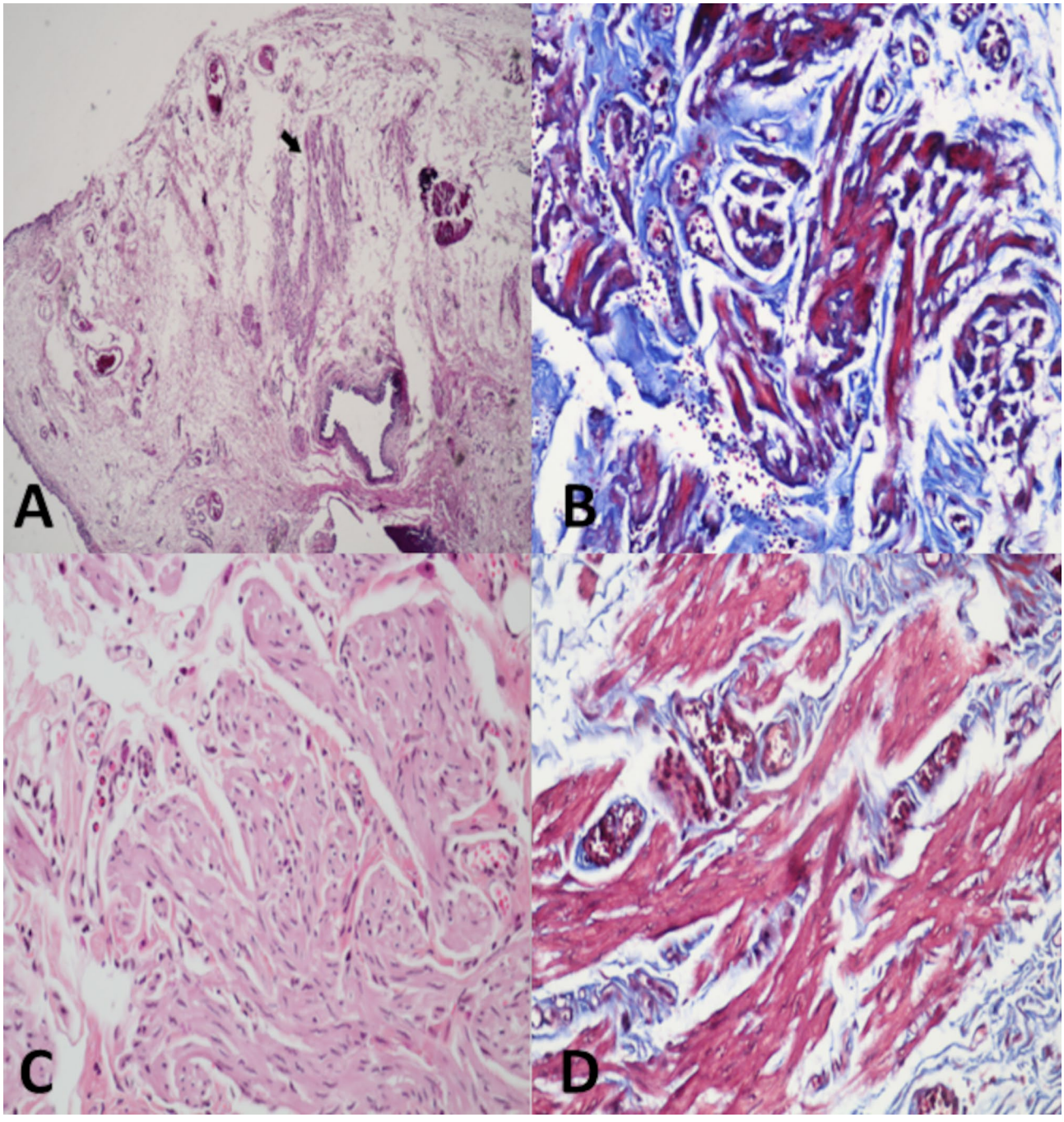

Fig. 3 Figures **A** and **B** show the fibrosis of the Muller's muscle. **A**: Histopathology examination shows atrophic smooth muscle (arrow) replaced in some area with fibrosis ((magnification × 40) (H/E staining)), **B**: Masson's trichrome stain shows red staining for muscle fiber and blue staining for fibrotic tissue (magnification × 400). Figures **C** and **D** show hypertrophy of the Muller's muscle. **C**: Hypertrophic Muller's muscle consists of undulating bundles of smooth muscle fibers (magnification × 400 (H/E staining)), **D**: Masson's trichrome staining shows muscle fibers in red. Collagen fibers are stained blue in the perimysium around the muscle bundles (magnification × 400)

p-value = 0.084, congenital; *p*-value = 0.230, horner; *p*-value = 0.333) (Table 2).

As you can see in Table 3, a grade increase in the degree of fibrosis (for example, an increase from mild to moderate) when the other factors are adjusted (adjusted for hypertrophy, age, gender, and the type of pathogenesis) increases the 1 mm correction effect by 0.027 (Confidence Interval (CI) = 0.002–0.052 and *p*-value = 0.033). No significant statistical correlation was observed between fibrosis rate and other factors. Also, the results showed that when other factors are adjusted, patients without hypertrophy (compared to patients with

Table 1 Clinical characteristics of participants

Variable	Mean	Range	Standard Deviation
Levator function (mm)	13	8–16	2
Muller resection (mm)	8.4	4–12	1.5
MRD1 normal preop (mm)	3.7	2.3–5.5	0.7
MRD1 ptotic preop (mm)	2.5	1–4	0.8
MRD1 normal postop (mm)	3.7	2.3–5.5	0.7
MRD1 ptotic postop (mm)	3.7	2–6	1.0
Ptosis amount (mm)	1.2	1–2	0.4
Ptosis correction (mm)	1.3	0.3–2.8	0.6
1 mm correction effect	0.15	0.05–0.34	0.08
Postop-difference (mm)	0.0	-1.8-0.8	0.6

Table 2 Correlation between fibrosis and outcomes of MMCR

Variables	Correlation Coefficient	*P*-value
1 mm correction effect	0.267	0.073
Ptosis Correction	0.314*	**0.033**
Postop-difference	-0.258	0.083
Success	-0.79	0.060

*Spearman's correlation Test

hypertrophy) have 1% less correction effect, which is not statistically significant (p-value = 0.673) (Table 3).

The results showed that there is no significant association between Muller's muscle fibrosis and hypertrophy with success in MMCR surgery according to pathogenesis (Table 4).

Discussion

Briefly, in our study, 47 patients with mild ptosis underwent MMCR surgery, and the pathology samples including conjunctiva and Muller's muscle were examined after hematoxylin-eosin (H/E) and Masson trichrome staining. The relationship between the presence or absence of hypertrophy and fibrosis of Muller's muscle fibers with the outcome and the success of the surgery was investigated and the results showed that with the increase in the fibrosis of Muller's muscle fibers, the effect of ptosis correction after MMCR surgery will increase. A moderate but statistically significant correlation was found between Muller's muscle fibrosis and ptosis correction (Correlation Coefficient = 0.314 and p-value = 0.033), suggesting that reduced muscle elasticity in fibrosis may help maintain surgical results.

New studies point to the importance of the Muller's muscle in eyelid function, but there is no comprehensive study that examines the pathology of the Muller's muscle, especially with aging [15]. Limited evidence suggests that, with increasing age, smooth muscle fibers in Muller's muscle may decrease and be replaced by fibrotic and adipose tissue. However, in our study, age was not

Table 3 Association between 1 mm correction effect with pathogenesis, phenylephrine test, hypertrophy, and fibrosis

Variables		B (Standardized Coefficient)	Standard Error (Confidence Interval)	*P*Value
Pathogenesis	*Aponeurotic*	-0.018	0.049 (-0.116-0.081)	0.719
	Congenital	-0.047	0.052 (-0.152-0.057)	0.367
	Horner	-	-	-
Phenylephrine test	*Full correction*	0.003	0.042 (-0.081-0.088)	0.935
	Over	0.015	0.049 (-0.084-0.114)	0.760
	Under	-	-	-
Hypertrophy	*No*	-0.010	0.024 (-0.059-0.039)	0.673
	Yes	-	-	-
Fibrosis		0.027	**0.012 (0.002–0.052)**	**0.034**

Table 4 Association between Muller's muscle fibrosis and hypertrophy with success in MMCR surgery according to pathogenesis of ptosis

Pathogenesis		***Fibrosis***					***Hypertrophy***		
		No	***Mild***	***Moderate***	***Severe***	***P value***	***No***	***Yes***	***P value***
Aponeurotic	***Failure***	1 (50%)	2 (20%)	1 (16.7%)	0 (0%)	0.318	1 (10%)	3 (30%)	0.712
	Success	1 (50%)	8 (80%)	5 (83.3%)	2 (100%)		9 (90%)	7 (70%)	
Congenital	***Failure***	0 (0%)	2 (25%)	1 (25%)	3 (37.5%)	0.281	0 (0%)	6 (35.3%)	0.907
	Success	3 (100%)	6 (75%)	3 (75%)	5 (62.5%)		6 (100%)	11 (64.7%)	
Horner	***Failure***	0 (0%)	0 (0%)	0 (0%)	0 (0%)	-	0 (0%)	0 (0%)	-
	Success	1 (100%)	1 (100%)	1 (100%)	0 (0%)		1 (100%)	2 (100%)	

found to be a significant factor in the degree of fibrosis, which may be due to the relatively young age range of our cohort. On the other hand, the anatomical position of the muscle also changes with age, and the migration of the muscle bulk will occur from the vicinity of the aponeurosis of the levator muscle towards the tarsal plate [15–18].

Muller's muscle not only causes upper eyelid elevation together with the levator muscle but also secondary to the function of sympathetic nerves, can play an important role in upper eyelid elevation alone, so involutional changes in Muller's muscle can cause the dysfunction of this muscle and the occurrence of ptosis. A hypothesis regarding the dysfunction of Muller's muscle is due to atherosclerosis and the narrowing of blood vessels supplying blood with sympathetic nerves during senescence [15].

The literature offers varying perspectives on how MMCR elevates the eyelid. Certain studies believe that the procedure achieves this by advancement the levator aponeurosis [19], whereas others maintain that lid elevation is due to the shortening of the posterior lamella and plication, rather than resection of the levator aponeurosis and muscle [20, 21]. This latter viewpoint is supported by observations that Müller's muscle can be easily separated from the aponeurosis, as well as histological evidence from cadaver eyelids showing an intact levator aponeurosis after undergoing MMCR [20]. Ultimately, MMCR likely works by shortening the posterior lamella, leading to both advancement of the levator palpebrae superioris muscle and plication of the levator aponeurosis [20, 21].

One possible explanation for the improved surgical outcomes observed in patients with fibrotic Muller's muscle is that fibrotic tissue may provide increased structural stiffness and reduced elasticity compared to non-fibrotic muscle. We hypothesize that in the absence of fibrosis, the greater elasticity of Muller's muscle might lead to postoperative elongation due to repetitive eyelid movements, thereby diminishing the surgical effect over time. In contrast, fibrotic muscle may resist such stretching, helping to preserve the eyelid position achieved during surgery. This concept, although not directly evaluated in our study, aligns with findings from prior research. Hussain et al. reported improved MMCR outcomes when epinephrine was added to the local anesthetic, attributing the effect to intraoperative tissue shrinkage, which may reflect a similar stiffening mechanism enhancing surgical efficacy [22].

Several studies have examined the pathology of the resected levator and Muller's muscle during ptosis surgery [15, 16, 23–28], but most of the studies have examined and compared the pathology results in various causes of ptosis, especially congenital and aponeurotic, and this study is novel in its way because examines the relationship between the pathologic findings and the surgical results.

In a study, Baytaroğlu et al. investigated light and electron microscope findings of levator muscle/aponeurosis and their relationship with clinical findings in congenital myogenic and aponeurotic blepharoptosis. In this study, quantitative and qualitative evaluations were made for muscle fiber morphology using light microscopy and electron microscopy on the tissue samples removed from the most proximal part of the aponeurosis during levator muscle/aponeurosis resection. The results of this study showed that no correlation was found between functional and microscopic parameters [29].

The study by Surve et al. examined microscopic and ultrastructural changes in the levator muscle in congenital ptosis, showing a predominance of fibrocollagenous tissue and a higher prevalence of muscle fibers in cases with moderate ptosis and greater levator function. Significant fibrosis was notably present in specific ptosis subtypes, especially among patients with the Marcus Gunn jaw-winking phenomenon. The study's findings support a dysgenesis theory, as opposed to active degeneration or inflammation, in the levator muscle [16]. Consistent with our findings, the study by Kasaee et al. on congenital ptosis surgery also demonstrated that a higher percentage of fibrosis in the levator muscle is significantly associated with surgical success, highlighting fibrosis as a potential predictor of positive surgical outcomes [30].

We initially intended to evaluate the macroscopic appearance of Muller's muscle samples to identify potential correlations with fibrosis severity. However, due to tissue manipulation, local anesthesia injection, and intraoperative bleeding, most specimens showed hematoma or distortion. These factors made macroscopic assessment unreliable and clinically uninformative, so we did not include such images in the study.

This study has several limitations. First, the sample size was relatively small and the study was conducted at a single center, which may limit the generalizability of the findings. Second, although our analysis identified a significant correlation between Muller's muscle fibrosis and ptosis correction, it is not feasible to predict surgical outcomes solely based on postoperative histopathological findings, as these are not available preoperatively. Third, the inclusion of patients with different etiologies of ptosis (aponeurotic, congenital, and Horner's syndrome) introduced heterogeneity into the sample. Nearly half of the patients had congenital ptosis, and the distribution among subgroups was uneven. This variability may have influenced both clinical and histopathological outcomes. However, our subgroup analysis did not reveal a statistically significant correlation between ptosis type and surgical success or fibrosis level. Although the majority of participants in our study were female (89.4%), we found

no statistically significant association between gender and the degree of Muller's muscle fibrosis or hypertrophy. Nevertheless, the gender imbalance may limit the generalizability of the findings, and future research with a more balanced population may help elucidate potential gender-related histopathological differences. Future studies may benefit from artificial intelligence-assisted histopathological analysis, especially given the ethical and practical limitations in obtaining normal Muller's muscle as control tissue. This approach could enable more objective and accurate quantification of muscle fibrosis and hypertrophy.

Conclusions

As a result, our study was an attempt to find the relationship between Muller's muscle histopathology and the results of MMCR surgery, which showed that following the increase in Muller's muscle fibrosis, the ptosis correction effect of the MMCR increases, but the amount of Muller's muscle hypertrophy was not correlated to the outcome of surgery.

Abbreviations

MMCR	Muller's muscle-conjunctival resection
MM	Millimeter
MRD1	Margin reflex distance 1
Preop	Pre-operation
Postop	Post-operation
H/E	Hematoxylin-eosin
CI	Confidence interval

Acknowledgements

The authors would like to appreciate the support and constructive comments of our methodology research office.

Author contributions

S.M.R prepared idea and performed surgeries and N.M.K prepared proposal, M.T.R, N.M.K and H.A collected data, Z.N reviewed histopathological samples, M.G and H.A analyzed data and H.A prepared and finalized draft. All authors read and proofed final article.

Funding

There were no specific funding sources for this study.

Data availability

On request from the corresponding author, data supporting the findings of this study will be made available.

Declarations

Ethics approval and consent to participate

The study had been approved by the local ethics committee of Tehran University of Medical Sciences according to Helsinki ethical principles. All methods and procedures were performed in accordance with the relevant guidelines and regulations of Tehran University of Medical Sciences. Written informed consent was obtained from all subjects, parents and/or their legal guardian(s).

Consent for publication

Not Applicable.

Competing interests

The authors declare no competing interests.

Received: 12 January 2025 / Accepted: 30 April 2025
Published online: 12 May 2025

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