Ophthalmol Ina 2023;Supplement Ed.: 165-170

ORIGINAL ARTICLE

THE EXPRESSION OF ALPHA-SMOOTH MUSCLE ACTIN IN CONTRACTED SOCKET

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ABSTRACT

Purpose: *A contracted socket is considered a common complication in anophthalmic patients, with the resulting poor fitting of an ocular prosthesis. In severe cases where patients have had multiple previous reconstructive attempts, further socket contraction may result from fibrosis, leading to eventual surgical failure. Alpha-smooth muscle actin (α-SMA) is often used to identify pathologic fibroblasts. It may serve as a reliable marker for myofibroblast cells. The aim of the study was to find α-SMA protein expression in myofibroblasts derived from orbital socket contracture.*

Methods: *In this study, we observed five patients with contracted sockets who underwent fornicoplasty and buccal graft surgery from October 2022 to February 2023. Tissue samples were taken from the conjunctival fornix area during the surgery and then examined for α-SMA expression.*

Result: *In this study, there were 2 out of 5 (40%) positive α-SMA expressions with history of recurrent contraction, 2 (40%) negative expressions with no history of recurrent contraction, and 1 result that could not be assessed due to the limited number of samples containing fibroblasts.*

Conclusion: *α-SMA levels can be detected in patients with contracted sockets. Patients with a history of recurrently contracted sockets have a tendency to express positive α-SMA. This may allow for the need for the administration of antimetabolites in patients with recurrent contracted sockets to avoid recurrence of this contracted socket.*

Key words: *alpha-SMA, contracted socket, myofibroblast*

INTRODUCTION

The goal of ocular evisceration surgery is to create a well-fitted ocular prosthesis with a deep fornix and appropriate conjunctival covering that resembles the eye as naturally as possible [1]. Poor cosmesis, psychological harm to patients, extra injury, and infection can all be brought on by an ill-fitting prosthesis, in addition to these other negative effects [2].

After socket surgery, socket contracture is a common condition. The shortening of the orbital tissue due to fibrosis is what causes the contracted socket [3]. Shortness of the conjunctival lining with shallow or absent fornices can happen under a variety of circumstances, such as previous external beam radiation, multiple orbital implant extrusions, immunologic conditions like mucous membrane pemphigoid or Stevens Johnson syndrome, or after thermal or chemical trauma [1].

Management of acquired anophthalmic socket contracture mainly involves replacement of the cicatricial conjunctival tissue with a healthy graft to re-establish a deep fornix that allows a proper subsequent fitting of an ocular prosthesis [4]. For socket reconstructive surgery to be successful, postoperative conjunctival fibrosis prevention is essential. Repeated socket contraction, however, can happen even after diligent and cautious surgical interventions [5].

Myofibroblasts have since been identified in nearly all fibrotic conditions marked by tissue remodelling and retraction. Therefore, healing and normal fibroblasts may exhibit differences in phenotypic expression, such as cell proliferation, collagen synthesis, and α-smooth muscle actin (α-SMA), which is a specific marker of myofibroblasts. While some degree of fibroblast contraction is necessary for wound closure, excessive fibroblast contraction can lead to the formation of scar tissue. It has been suggested that during this period of excessive contraction, fibroblasts increase the expression of α-SMA [6].

Therefore, the purpose of this study was to find α-SMA protein expression in myofibroblasts derived from orbital socket contracture. In contrast to the possibility of stronger contraction forces produced by a higher expressed level of α-SMA, the likelihood of repeatedly contracted sockets increases in this situation.

METHODS

Patients

This consecutive study observed five patients with severely contracted sockets who underwent fornicoplasty and buccal graft surgery. Patients came to our tertiary referral center at Sardjito General Hospital in Yogyakarta between October 2022 and February 2023. The patient had to meet the following criteria to be included: ocular socket contracture (grades 1–5) and a clinical need for surgical socket reconstruction.

The socket contracture severity was classified according to Tawfik et al [7] into 5 grades. Grade 1: minimal contraction with shallow or shelved lower fornix, grade 2: mild contracture with loss of the inferior and/or the superior fornix, grade 3: more advanced cicatrization involving the entire fornices, grade 4: severe phimosis of the palpebral fissure both vertically and horizontally, grade 5: recurrent contracted socket [8].

Surgery

All patients were operated under general anaesthesia. The procedure was carried out by three ophthalmologists from reconstructive and oculoplastic subdivision. The fibrotic tissue that

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was taken from the lower conjunctival fornix area of the constricted socket served as the study's sample.

Immunohistochemistry

Four micro meter sections of formalin-fixed and paraffin-embedded conjunctival samples were prepared. Immunohistochemical staining was performed using a primary clone antihuman monoclonal α-SMA antibody, prediluted to 1:50 dilution in 1% bovine serum albumin. Avidin–Biotin immunoperoxidase complex technique was used, by applying a super sensitive detection kit according to the manufacturer instructions to detect the bound antibody. The prepared tissue sections were fixed on poly-L-lysine coated slides overnight at 37°C. They were deparaffinized and rehydrated through graded alcohol washes, then the sections were heated in a microwave oven in 10 mM citrate buffer (pH 6.0) for 10 minutes. After the blocking of endogenous peroxidase and incubation in Protein Block Serum-Free Solution for 20 minutes, the sections were incubated with α-SMA antibody at room temperature. Biotinylated immunoglobulin and streptavidin conjugated to horseradish peroxidase were then added, and then chromogen was used to form an insoluble brown product. Finally, the sections were counterstained with haematoxylin and mounted. Vascular smooth muscle was used as an internal control [9].

Statistical data analysis

We provided the data on an Excel spreadsheet (Excel 2010, Microsoft Corporation, Redmond, WA, U.S.A.), and statistical analysis was conducted with the SPSS software version 23 for Windows (IBM Corporation, New York, NY, U.S.A.).

RESULTS

This study observed five patients who underwent fornicoplasty and buccal graft surgery. Table 1 shows the demographic data and preoperative characteristics of patients. The mean age was 48.8 ± 13.84. Four patients (80%) are men, and one (20%) is a woman. In this small sample study, we found that ocular trauma (100%) was the main cause of anophthalmia. For the number of prior surgeries, patients range from 0 to 1, with signs of recurrence. One patient with grade 3 socket contracture, two patients with grade 4, and two patients with grade 5, based on Tawfik classification. The duration of anophthalmia ranged from 33 to 66 years, with a mean of 39.6 ± 15.61. 3 of 5 patients use the prosthesis every day during the day, but 2 patients never use the prosthesis.

Two out of five (40%) positive α-SMA expressions with a history of recurrent contraction, two (40%) negative expressions without a history of recurrent contraction, and one result that could not be evaluated because of the small number of samples including fibroblasts.

Table 1. Preoperative data of Patients Included in the Study

Age (years)	48.8 ± 13.84 (Range: 30-66)
Sex	
Males	4 (80%)
Females	1 (20%)
Causes of anophthalmia	
Ocular trauma	5 (100%)
Number of prior surgeries	0.4 ± 0.55 (Range: 0-1)
Grade of contracted socket	4.2 ± 0.84 (Range: 3-5)
Duration of anophthalmia	39.6 ± 15.61 (Range: 33-66)
Prothesis use	
Yes	3 (60%)
No	2 (40%)

Graph 1. Expression of α-SMA

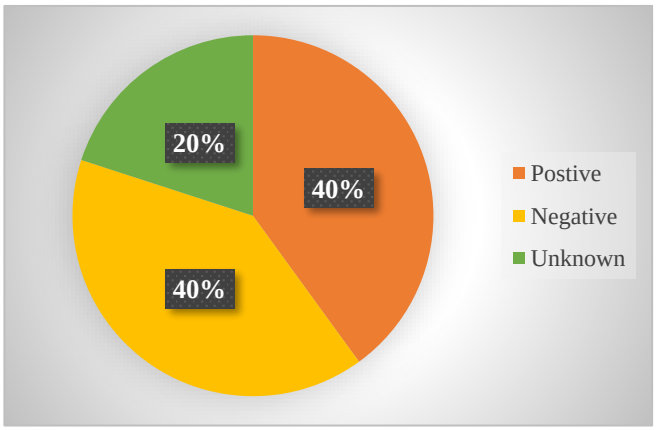

Table 2. Study of correlation

STUDY OF CORRELATION	Correlation Coefficient	P-Value
Age and α-SMA expression	0.18	0.893
Recurrency and α-SMA expression	0.190	0.663
Grade of contracted socket and α-SMA expression	0.020	0.886
Prothesis use and α-SMA expression	0.429	0.513
Duration of anophthalmia and α-SMA expression	0.225	0.635

Linear regression was done to analyze the correlation between some variables and the expression of α-SMA. The correlation between age and the expression of α-SMA was not

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significant (p=0.893). Followed by recurrency of contracted socket (p=0.663), grade of contracted socket (p=0.886), history of prothesis use (p=0.513), and also duration of anophthalmia (p=0.635), we found that these variables were not significant with the correlation of the expression of α-SMA.

DISCUSSION

Socket contracture may result from a variety of factors, including fibrosis occurring due to the initial trauma, a poor surgical technique with excessive dissection of the conjunctiva or Tenon's capsule, multiple socket procedures, lower lid laxity causing a shallow inferior fornix, alkali burns, cicatrizing diseases of the conjunctiva, or irradiation. Enucleation itself is an obvious cause for contraction, as the closure of the corneal defect results in inevitable foreshortening of the conjunctiva. The lack of an expanding force such as the absence of a prosthesis or a cosmetic shell may also play a role [7].

The presence of myofibroblasts, which are the cause of socket contraction, is supported by the observation of α-SMA expression in patients with a history of recurrently contracted sockets. An insufficient number of samples, however, might also be a result of the tissue's limited myofibroblasts, which undoubtedly influence the expression of this α-SMA. The significance of the obtained correlation values can also be impacted by the limited sample size. The proper correlation between each variable and α-SMA expression is anticipated to be able to be described by a comparable investigation with a bigger sample size.

With this expression of α-SMA, it is envisaged that in the future, antimetabolite injections can be given to the patient's socket area as a prophylactic therapy for repeatedly contracted sockets. Of course, further research is required, ideally with multiple institutions and a considerably bigger sample size. Numerous investigations towards fibrosis prevention have been conducted. According to Tawfik HA et al, a single injection of MMC, 5-fluorouracil, and triamcinolone to mice during socket surgery produced encouraging results in terms of lowering myofibroblast activity [9]. Dewi et al [10] study, passage number increased whereas TGF-β1 could decrease α-SMA expression levels in dose-dependent fashion, however, both did not affect morphology of myofibroblast culture derived from orbital socket contracture.

Another issue lies with the pathogenesis of socket contraction. A lot of contracted sockets are ischemic sockets with insufficient vascularization and it would seem counterintuitive to inject these sockets with wound modifiers that in essence would also lead to inhibition of new vessel formation where they are eagerly needed [11].

CONCLUSION

Patients with constricted sockets can have elevated levels of α-SMA. Patients who have experienced recurrently constricted sockets are more likely to display positive α-SMA. As a result, it could be necessary to administer antimetabolites to patients who frequently experience contracted sockets in order to prevent the recurrence of such a contracted socket. Though a larger sample size could be required for this study.

CONFLICT OF INTEREST

There is no conflict of interest regarding the manuscript.

FUNDING

The authors are responsible for the study's funding without the involvement of a grant, scholarship, or any other funding resource**.**

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