Anatomic Research on the Pathological Observation of Orbicularis Oculi Muscles

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眼輪筋筋線維の病理学的所見による解剖学的研究

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ヒト眼輪筋の筋線維構成とその機能的特徴を明らかにするために，筋線維の横断面積，1 mm^2 中の筋線維数，筋線維総数，筋線維の太さ，密度等を sampling method，画像解析装置により計測し，算出した。さらに，筋の病理学的観察を実施し比較検討をおこなった。対象は，29歳から88歳までの解剖実習屍20体から得られた右側眼輪筋である。結果は次のごとくである。1) 筋の横断面積は，下瞼部が大きく性別では男性が大であった。2) 1 mm^2 中の数は，上瞼部が大で，性別では女性が大なる傾向があった。3) 総数では，下瞼部が多く，性別では男性が多かった。4) 太さは，下瞼部が大きく，性別では男性が大であった。5) 密度は，下瞼部が高く，男性が女性より高かった。6) 組織所見では，大小不同が多く，脂肪性の筋内膜増加，脂肪の周膜増加も多く認められたが，上・下瞼部，性別による差は認められなかった。以上の結果から、筋の一般的原則を改めて表したものであるが、男女間の表情の差が、眼輪筋の筋線維の性差となってあらわれていることも証明された。

Introduction

Orbicularis oculi muscles, which situates around the palebral fissure as muscles of facial expression, is very thin and encircles the palpebral fissure. This muscle is composed of three parts; the eyelid, orbit and pars lacrimalis, each part named after the region the muscle contains. Some descriptions give the m. depressor supercilii in addition to the above three[(1)], but generally the former description is frequent. The eyelid region begins in the inner palpebral ligament and ends in the outer part of the outer canthus. The orbit region begins in the nasal part of the frontal bone, the crista lacrimalis anterior of the upper jaw bone and the inner palpebral ligament

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and connects with the muscle fiber encircling the orbit. The pars lacrimalis begins in the crista lacrimalis posterior of the lacrimal bone, and the muscle fibers meet at the medial angle of the eye.

The action of the orbicularis oculi muscles is known to be mainly related to the movement of the eyelid. There are many research reports on other skeletal muscles such as the limb muscles, but there have been no reports on the orbicularis oculi muscles considering it as muscles of facial expression. Therefore, this research will examine the myofibrous organization of the orbicularis oculi muscles, such as the muscle fiber's cross-sectional area, the number of muscle fibers per square millimeter, the total number of muscle fibers, it's thickness, and density. By comparing the pathological observation of the muscle fibers and other muscles, I intended to clarify the functional characteristics of the orbicularis oculi muscles.

Materials and Methods

This research was conducted on 20 adult humans (12 male, 8 female, ages 29 through 88, average age 61.7) used for anatomy training at the School of Medicine. The right orbicularis oculi muscle was chosen for the specimen, fixed with 10% formalin. The middle part of the superior and the inferior was dissected along the eyelid and the orbit.

The extracted piece of muscle is fixed again with 10% formalin, then washed in water, dehydrated, and embedden in celloidin. Then it is sliced to 25 μm with a Jungian microtome, and stained with hematoxylin and eosin. Measurements on the muscle fiber were conducted by picking 100 points evenly spread over the cross-section of the 10～20 times enlarged photograph of the specimen. The number of the muscle fibers per 1mm^2 were determined by examining each of the 100 points with a microscope ($\times$400), counting the number of the muscle fibers within the 0.1mm^2 section of each point and adding them up. Furthermore, the total number of the muscle fibers was calculated by multiplying the total number of muscle fibers per 1mm^2 with the cross-sectional area of the muscle. To measure the thickness of the muscle fiber, 2 muscle fibers randomly chosen from each point was magnified 400 times and drawn using a microscopic drawing device. After using an X-Y Digitizer and a Programmable generator to measure the area of the 2 muscle fibers in the picture, the unit of measurement was converted into μm^2 and used as the thickness of the muscle fiber. In order to determine the density of the muscle fiber, the average thickness of the muscle fiber (μm^2) was multiplied by the number of muscle fibers per 1mm^2. Then the area occupancy ratio was calculated to get the density (%).

Results

I. Myofibrous organization

The cross-sectional area of muscle, the number of muscle fibers per $1mm^2$, the total number of muscle fibers, the thickness of the muscle fiber and its density for all the samples of orbicularis oculi muscles are as follows.

Table 1. Cross sectional area, number of muscle fiber per sq. mm, total number of muscle fibers.

muscle	sex	n	cross-sectional area (mm^2)			number of muscle fiber per sq. mm.			total number of muscle fibers		
			mean	max.	min.	mean	max.	min.	mean	max.	min.
superior	male	12	18.4	31.5	8.9	1781.5	2606	1250	33234.2	82245.3	16580.4
	female	8	17.0	23.0	12.3	1793.5	2484	1411	30086.2	40585.2	21103.8
	total	20	17.8	31.5	8.9	1786.3	2606	1250	31975.0	82245.3	16580.4
inferior	male	12	26.4	39.9	17.6	1646.6	2149	1270	42565.7	56819.5	29425.9
	female	8	18.1	30.0	11.4	1654.7	1979	1356	30830.5	59438.0	13085.1
	total	20	23.1	39.9	11.4	1649.9	2149	1270	37871.7	59438.0	13085.1

Table. 2 Mean size of muscle fiber, density of muscle fibers.

muscle	sex	n	size of muscle fibers (μ^2)			density of (%) muscle fibers
			mean	max.	min.	mean
superior	male	12	362.6	601.8	255.7	61.8
	female	8	309.6	417.8	184.1	53.2
	total	20	341.4	601.8	184.1	58.3
inferior	male	12	410.3	621.6	216.5	65.1
	female	8	389.0	539.5	214.2	61.9
	total	20	401.8	621.6	214.2	63.8

1) Cross-sectional area of muscle

Measurements obtained from the superior and the inferior showed an average of $18.4mm^2$ for the male superior, a maximum of $31.6mm^2$, and a minimum of $9mm^2$. The average area for the

female superior was 17.1mm^2, the maximum was 23mm^2, and the minimum was 12.4mm^2. The average for male and female combined was 17.9mm^2. With the inferior, the male average was 26.4mm^2, with a maximum of 40mm^2, and a minimum of 17.7mm^2. The female average was 18.2mm^2, the maximum 30.1mm^2, and the minimum 11.5mm^2. The average for both sexes was 23.1mm^2. On average, both the superior and the inferior were larger in male than in female. Between the superior and inferior, the inferior was larger for both male and female. Individually, the inferior was larger in 10 male specimens and 3 female specimens.

2) Number of muscle fibers per 1mm^2

The average number of muscle fibers in 1mm^2 of the male superior was 1781.6, with a maximum of 2606, and a minimum of 1250. The female average was 1793.5, with a maximum of 2484, and a minimum of 1411. In the inferior, the male average was 1646.7, the maximum was 2149, and the minimum was 1138. Female specimens had an average of 1654.8, with a maximum of 1979, and a minimum of 1356. On average, the number of muscle fibers were larger in the superior than the inferior for both male and female, the difference being approximately 140 fibers per 1mm^2. Individually, the number of muscle fibers of the inferior exceeded that of the superior in 3 male samples and 2 female samples. Between male and female, the average number of muscle fibers were slightly larger in female specimens for both the superior and the inferior. But the difference was merely 10 fibers per 1mm^2.

3) Total number of muscle fibers

The total number of muscle fibers in the orbicularis oculi muscle averages 33234 for the male superior, a maximum of 82245, and a minimum of 16580. The female average was 30086, the maximum was 40585, and the minimum was 21104. In the inferior, the male average was 42566, the maximum was 56820, and the minimum was 13085. For both male and female the total number of muscle fibers in the inferior was larger than that of the superior, the difference being over 9000 for male and over 700 for female specimens, showing a gap between sexes. Between sexes, male specimens had more muscle fibers in both the superior and the inferior, the difference being 3000 in the superior and a little under 11000 in the inferior showing a large gap in the inferior.

Individually, there was a male sample of the superior which had over 80000 muscle fibers, more than 2.7 times the male average. On the other hand, one female inferior had 13000 fibers, less than 1/2 the female average.

4) Thickness of the muscle fiber

Of all the samples of the orbicularis oculi muscles, the average thickness of a muscle fiber in the male superior was 362.7 μm^2, the maximum was 602 μm^2, and the minimum was 256 μm^2. The female average was 309.6 μm^2, the maximum was 418 μm^2, and the minimum was 184 μm^2. The average for male and female combined was 341 μm^2. In the inferior, the male average was 410.3 μm^2, the maximum was 622 μm^2, and the minimum was 217 μm^2. The female average was 389 μm^2, the maximum was 540 μm^2, and the minimum was 214 μm^2. The average for both sexes was 402 μm^2. In both the superior and the inferior, male samples tended to be thicker than female samples. Furthermore, when comparing the superior and the inferior, the inferior was larger than the superior for both sexes. Individually, there ware 2 examples with thicknesses in the 600 μm^2 level and 3 examples in the 500 μm^2 level for male samples of the inferior and superior combined, indicating a wider range of diversity between individual samples compared to females which had 1 example with a thickness in the 500 μm^2 level.

5) Correlation between the cross-sectional area of the muscle and the number of muscle fibers per 1mm^2, the total number of muscle fibers and the thickness of a muscle fiber.

When observing the correlation of the cross-sectional area of a muscle with the number of muscle fibers per 1mm^2 and the total number of muscle fibers, there were 3 examples (2 male, 1 female) of the superior which had both above average cross-sectional area and above average number of muscle fibers per 1mm^2. 5 samples (3 male, 2 female) had above average cross-sectional area and below average number of muscle fibers per 1mm^2. 7 samples (5 male, 2 female) had below average cross-sectional area and above average number of muscle fibers per 1mm^2. 5 samples (2 male, 3 female) had below average cross-sectional area of muscle and below average number of muscle fibers per 1mm^2. In the inferior, there were 7 examples (5 male, 2 female) with above average cross-sectional area of muscle and above average muscle fibers per 1mm^2. 3 samples (3 male) had above average cross-sectional area of muscle and below average number of muscle fibers per 1mm^2. 4 examples (3 male, 1 female) had below average cross-sectional area of muscle and above average number of muscle fibers per 1mm^2. 6 samples (1 male, 5 female) had below average cross-sectional area of muscle and below average number of muscle fibers per 1mm^2.

Next, there is the relationship between the cross-sectional area of muscle and the total number of muscle fibers. In the superior, there were 6 samples (4 male, 2 female) which had above average cross-sectional area of muscle and above average total number of muscle fibers. 2 examples

(1 male, 1 female) had above average cross-sectional area of muscle and below average total number of muscle fibers. 3 samples (2 male, 1 female) had below average cross-sectional area of muscle and above average total number of muscle fibers. 9 examples (5 male, 4 female) had below average cross-sectional area of muscle and below average total number of muscle fibers. In the inferior, 9 examples (7 male, 2 female) had above average cross-sectional area of muscle and above average total number of muscle fibers. 1 male sample had above average cross-sectional area of muscle and below average total number of muscle fibers. 10 samples (4 male, 6 female) had below average cross-sectional area of muscle and below average total number of muscle fibers. None of the samples of the inferior had below average cross-sectional area of muscle and above average total number of muscle fibers.

In the correlation between the cross-sectional area of muscle and the thickness of the muscle fiber, there were 4 examples (3 male, 1 female) of the superior which had above average cross-sectional area of muscle and above average thickness of the muscle fiber. 4 samples (2 male, 2 female) had above average cross-sectional area of muscle and below average thickness of the muscle fiber. 4 samples (3 male, 1 female) had below average cross-sectional area of muscle and above average thickness of the muscle fiber. 8 samples (4 male, 4 female) had below average cross-sectional area of muscle and above average thickness of the muscle fiber. In the inferior, there were 5 male samples with above average cross-sectional area of muscle and above average thickness of the muscle fiber. 5 samples (3 male, 2 female) had above average cross-sectional area of the muscle and below average thickness of the muscle fiber. 4 samples (1 male, 3 female) had below average cross-sectional area of the muscle and above average thickness of the muscle fiber. 6 samples (3 male, 3 female) had below average cross-sectional area of the muscle and below average thickness of the muscle fiber.

6) Density of muscle fibers

In the comparison of the muscle fiber density of the orbicularis oculi muscles, the average for the male superior was 61.8%, the maximum was 75.3%, and the minimum was 47.5%. The female average was 53.2% with a maximum of 67.1% and a minimum of 42.6%. In the inferior, the male average was 65.2% with a maximum of 83.7% and a minimum of 36.3%. Both male and female samples had a higher density in the inferior than in the superior. Density in male samples exceeded female samples in both the superior and the inferior.

Individually, differences of density between specimens were small in the superior of both male and female. Samples of the inferior, especially in male specimens had a large diversity in

density, the maximum being 83.7% and the minimum 36.3% showing a gap of approximately 2.3 times.

II. Pathological observation

When pathologically observing the superior and the inferior of each specimen according to references[(2),(3)], samples of the superior had 12 examples of irregular size, 4 examples of vacuolation, 4 examples of hyaline degeneration, and 3 examples of deformation. There were 12 examples showing an increase in endomysium, of which 8 examples were aliphatic, and 4 examples

Table. 3 Orbicularis Oculi (superior)

		♂												♀							
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Cell	1. irregular size		○	○		○	○	○	○	○	○	○			○		○	○			
	2. metamorphosis														○			○	○		
	3. atrophy												○								
	4. vacuolar degeneration			○		○		○								○					
	5. hyaline degeneration			○			○					○				○					
	6. fatt degeneration																				
Endomay -sium	7. fat replacement	○			○				○			○		○				○	○	○	
	8. fibrous tissue replacement			○							○					○	○				
Perimysium internum	9. fat replacement				○	○		○			○			○			○			○	○
	10. fibrous tissue replacement		○										○			○					

Table. 4 Orbicularis Oculi (inferior)

		♂												♀							
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Cell	1. irregular size	○		○			○		○	○		○	○			○	○		○		○
	2. metamorphosis					○					○				○	○		○		○	
	3. atrophy												○								
	4. vacuolar degeneration																○				
	5. hyaline degeneration					○				○		○		○			○				
	6. fatt degeneration																				
Endomay -sium	7. fat replacement				○				○			○	○	○			○	○			○
	8. fibrous tissue replacement			○		○		○		○						○	○		○		
Perimysiumi nternum	9. fat replacement		○	○							○	○	○	○	○			○		○	
	10. fibrous tissue replacement					○	○														

were of connective tissue nature. 11 examples showed an increase in perimysium interum, of which 8 examples were aliphatic, and 3 examples were of connective tissue nature. In the inferior, 12 samples had irregular size, 6 samples were deformed, and 5 samples had hyaline degeneration. 15 samples had an increase in endomysium, of which 8 were aliphatic, and 7 were of connective nature. 11 samples had an increase in perimysium interum, of which 9 were aliphatic, and 2 were of connective nature. No great differences were found within the samples of the superior nor in the samples of the inferior, except for 3 samples which differed in deformation and connective tissue natured increase in the endomysium.

Discussion

The function of the musculus orbicularis oculi is said to be closing the eyelids and opening the orbit region and the pars lacrimalis. The nerves controlling the musculus orbicularis oculi are the temporal branch and the cheeckbone branch of the facial nerves. When observing the upper and lower eyelid separately, the upper palpebral muscles usually opens the palpebral fold, but when opening it wider, the frontal muscles pulls the eyebrows up and the palpebral region is said to be involuntary. In addition, the lower eyelid is said to flexibly return to its original state, but the movement is small[4].

For both male and female, the cross-sectional area of the muscle in the inferior was smaller than that of the superior. Individually, 13 examples had larger inferiors, and 7 examples had larger superiors. This shows that there is a meaningful difference between the superior and the inferior ($P<0.05$), and judging from the movement of the superior and the inferior, it is thought to be indicating a functional difference. Furthermore, male samples were larger than female samples for both the superior and the inferior. However, though a meaningful difference was noticed between the superior and the inferior, there was little difference between sexes and age, with the conclusion that there is no difference in the cross-sectional area of muscle resulting from sex or age difference.

The number of muscle fibers per $1mm^2$ was larger in superior than the inferior for both sexes, and although the difference between sexes was slight, females inclined to have more fibers than males. This shows that like other muscles, the number of muscle fibers per $1mm^2$ in the orbicularis oculi muscles is larger in female than in male[5]. According to research on the changes of the cross-sectional area of the skeletal muscles[6],[7], a sample with a large cross-sectional area of muscle has a small number of muscle fibers per $1mm^2$ and thick muscle fibers which indicates

developed muscles. The same tendency was noticed in the orbicularis oculi muscles, but the difference was not distinct enough to indicate a difference in the movement of the superior and inferior.

The total number of muscle fibers was larger in the inferior for both sexes. The correlation was especially close in males ($P < 0.05$) indicating a meaningful difference but there was no such distinction in females.

Muscle fibers were thicker in the inferior for both sexes, showing a distinct difference between the inferior and superior ($P < 0.1$). Muscle fibers are generally thick in muscle groups that can exhibit instantanious maximum muscle power and thin in those that work continuously. Muscle groups related to minute movements such as those controlling facial expression have thin muscle fibers[8),(9)]. The muscle fibers in the orbicularis oculi, even in comparison with other muscle groups shown above, is extremely thin, indicating that the orbicularis oculi muscles are related to continuous motion and minute movements.

When observing the relationship of the cross-sectional area of a muscle fiber in regard to the number of muscle fibers per $1mm^2$, the total number of muscle fibers and the thickness of muscle fibers, the cross-sectional area of muscle and the number of muscle fibers per $1mm^2$ had slight negative correlation in the superior and no significant inclination in the inferior, compared to the reports of negative correlation in past research[10),(11)]. Reports from past research also show that the total number of muscle fibers tend to have a slight positive correlation, and the thickness of the muscle fiber tend to have a positive correlation. In this research however, the total number of muscle fibers showed a very distinct positive correlation to the cross-sectional area in both the superior and the inferior and there was a significant difference for both ($P < 0.01$). Furthermore, the thickness of a muscle fiber had a slight positive correlation in the superior but did not have a clear relationship in the inferior, showing no correlation as a whole. Judging from these results, the orbicularis oculi muscle showed a more or less close result to the other skeletal muscles.

In comparison with other muscles of humans using hematoxylin and eosin staining, the number of muscle fibers per $1mm^2$ was largest and the muscle fiber tended to be the thinnest. This can be said to be indicating a significant characteristic of the myiofibrous organization and was anticipated that this might have something to do with the fact that orbicularis oculi muscles are functionally related to the movement of the eyelids.

In the pathological observation, both superior and inferior showed much irregularity of size followed by many samples with increased endomysium and perimysium. There is a report that

endomysium increases of connective tissue nature was found in the skeletal muscles of athletes and that it is due to excess amounts of exercize[12], but in the orbicularis oculi muscles, such a relationship with the function of the muscles could not be verified. Furthermore, nothing significant could be found in the relations between pathological observation and age such as the relationship with aging and muscular atrophy, nor was there any significant substance in the relationship between pathological observation and sex.

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