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Form Deprivation Myopia Effect in Axial Length and Refraction Status in *Oryctolagus cuniculus* as Animal Model: In Vivo Experimental Study

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ABSTRACT

Background: Myopia is an ocular disorder that occurs around the world with unknown underlying causes. FDM is a method that is conducted as experimental myopia research in animal models. The deprivation in certain periods is resulting myopic eyes and changes that occur anatomically or molecularly. ***Objective:*** Observing the effect of Form Deprivation Myopia (FDM) on axial length and refraction status in *Oryctolagus cuniculus* (rabbits). ***Method:*** A total of 16 rabbits were divided into two groups selected randomly. Deprivation for four weeks is given at the right eye of the treatment group with an adhesive bandage. After four weeks of deprivation, the axial length and refraction status are examined to see if the differences occurred compared to the initial condition. ***Results:*** The axial length parameter after four weeks of deprivation shows the treatment group is significant from week 0 to week 4 (p= 0.002). The control group shows significant results (p=0.034). However, the mean value of the treatment group is larger than the control group (0.6500>0.3812). The refraction status results after four weeks of deprivation show the difference is significant in the treatment group (p= 0.000). No significant result from the control group (p>0.05). ***Conclusion:*** The effects from the result of 4 weeks of deprivation in the rabbit eye, such as axial length elongation and status refraction changes, indicate myopia condition. Although the control group also experienced changes in axial length and status refraction, the result was still not as significant as the treatment group.

Keywords: animal model; axial length; Form Deprivation Myopia (FDM), refraction status.

INTRODUCTION

Myopia is an ocular disorder that is also epidemic and very common to occur around the world. Unfortunately, the mechanism of how myopia occurs is still confusing. The researchers believe it appears that both genetics and environment play a role in the occurrence of myopia [1]. As myopia causes global issues, it can be seen at an early age that showed in the year 2000, the occurrence of myopia is 22,9% and will increase to 49,7% in the year 2050 [2].

In Indonesia, the prevalence of refractive error takes place in the first place and reaches 25 percent of the total population [3]. Refractive errors, known as vision problems, cause visual disturbances and inhibition to have clear vision. Refractive errors consist of four types that are myopia, hyperopia, astigmatism, and presbyopia [6].

Myopia is a serious condition because people mostly neglect it and underestimate it just because it can simply be corrected or handled by using contact lenses, spectacles, and refractive surgery [2].

Much experimental research has been conducted previously in animal models to help the medical world always improve knowledge about myopia. Even though experimental myopia in the animal model is only done in the laboratory, the impact on human knowledge is abundant due to the anatomical and structural similarities between the animal eye and human eye, similarities of the mechanism of myopia, similar humanlike pathogenesis, and presence of accommodative response. Previously, many experimental myopia research in animal models such as chicks, guinea pigs, rabbits, rhesus monkeys, mice, marmosets, etc [4,5].

The most common methods of experimental in animal models to develop myopia are form deprivation myopia (FDM) using occlusion in the eye and lens-induced myopia (LIM) using a lens applied in the eye [5]. The result of an experiment in both FDM and LIM shows the elongation of the axial length, error refraction status, and abnormally scleral growth. Those parameters are shown as an indication of myopia progression [4].

Many experimental research using animal models use rabbit (Oryctolagus cuniculus), such as previous research conducted by Nie et al., 2012 and Kusumawardhany et al., 2019. Rabbit's eye is similar in anatomical structure to human eye structure [7]. Thus, this research will be the alternative for further research about myopia experiments using the animal model, which is rarely done in Indonesia.

METHOD

This study is a true experiment with an in vivo randomized control group design. The experiment examines the axial length and refraction status of New Zealand white rabbit (Oryctolagus cuniculus) eyeballs and is divided into two groups: the treatment group and the control group. Both groups were observed for four weeks. The treatment group was deprived of the right side of the eye, while the control group was left untreated. Deprivation was performed with the bandages attached to the rabbit's eye with adhesive tape.

Before the deprivation was performed, initially, both groups' axial length and refraction status were measured using the biometer immersion for the axial length and streak retinoscopy for the refraction status. The final measurement was conducted by the end of 4th week.

The population of this study was the New Zealand white rabbits that were obtained from the Institute of Tropical Disease (ITD), Universitas Airlangga. It ages 3-4 months old and weighs 2-4 kilograms in healthy condition with normal intraocular pressure (18-21 mmHg). The data of the research were collected, grouped, and analyzed the normality by the Saphiro-Wilk Test. If the data gives the result distributed normally, it will be compared by T-Test. If the data is not distributed normally, it will be tested with Mann-Whitney or Wilcoxon test.

RESULT

A total of sixteen rabbits with the inclusion criteria were divided into two groups. The rabbits' eyes were assessed in terms of their axial length and status refraction in week zero before the deprivation was given and four weeks later to see the differences caused by the deprivation.

Axial length

The differences in the axial length of both groups from the initial and the fourth-week measurements are shown in Table 1.

TABLE 1: Median, mean, and standard deviation of axial length in week 0 and week 4.

Groups	N	Axial Length (mm) week 0	Axial Length (mm) week 4
		Median; Mean ± SD	Median; Mean ± SD
Control	8	15,30; 15,30 ± 0,10	15,54; 15,68 ± 0,47
Treatment	8	15,39; 15,58 ± 0,48	16,00; 16,23 ± 0,49

Initially, the normality using the Shapiro-Wilk test was conducted in the axial length both in the control and treatment groups. It was conducted using the axial length differences from week four and week zero. The minimum and maximum axial length of the rabbit (Oryctolagus cuniculus).

The results of the normality test in the control group are p=0.365, and the treatment group is p=0.071. After both groups were done by the normality test, a significance test was conducted between week four and week 0 using the paired t-test.

TABLE 2: Paired T-Test to see the significant changes in axial length in the control treatment group week four and week 0.

Groups	N	The confidence interval of axial length difference (mm)	mean	*p*
Control	8	0.03697 – 0.72553	0.38125	0.034
Treatment	8	0.34371 – 0.95629	0.65000	0.002

The p-value <0.05. It is shown that there are significant differences in axial length in both the control group and treatment group between week four and week 0 (p = 0.034). It is also shown by the mean value between the treatment group and the control group. The mean value of the treatment group is bigger than the mean value of the control group (mean treatment group =0,65000 > mean control group =0.38125), meaning the treatment group's increasing axial length results are higher than the result from the control group.

REFRACTION STATUS

Initially, both the control and the treatment group showed no difference in status refraction in week 0, and some changes in the refraction status showed in both groups in week four, as described in Table 3. The normality test was conducted in both groups to see the differences in status refraction from week 4 to week 0. The result of the normality test in the control group shows a significance of p=0.018 that will be further examined by the Wilcoxon Test and the treatment group's normality test p=0.202 and will be examined by the Paired Sample T-test.

TABLE 3: Status refraction Median, Mean, and Standard Deviation in week 0 and week 4.

Groups	N	Refraction (Diopter) week 0	Refraction (Diopter) week 4
		Median; Mean ± SD	Median; Mean ± SD
Control	8	2;2,00 ± 0,00	1,5;0,88 ± 1,36
Treatment	8	2;2,00 ± 0,00	-1,00;-0,94 ± 0,78

Wilcoxon test was in group control due to the result was not normally distributed. It was shown that the significant result for the comparison test (p) was p=0.066. It shows that it was bigger than 0.05, which means that there is no significant difference in the control group. Paired sample t-test was conducted for the treatment group. The p= 0.000, it is shown that there are huge differences in status refraction changes in the treatment group from week 4 to week 0.

TABLE 4: Paired Sample T Test treatment group and significant result in the changes between week four and week 0.

Groups	N	Mean		*p*
		Week 4	Week 0	
Control	8	-0.9375	2.0000	0.000

DISCUSSION

One of the most common eye diseases that occurs globally is myopia (near-sightedness), which is a sign of blurry vision because of the images that lie in front of the retina [8]. This happens because of the mismatch between the lens and the eyeball length that grows rapidly during the development [9-10]. Many factors affect the emergence of myopia, such as genetics, lifestyle, heredity, and environment [8]. However, the precise molecular pathways that underlie both the development and the management of myopia remain a mystery [11]. The past few years have shown that the increment in myopia prevalence makes myopia an epidemic, and it occurs mostly in Asia countries [8,12]. Form deprivation in animal models is an important method for further myopia study. Some human visual conditions, including ptosis, corneal opacity, congenital cataracts, and eyelid hemangioma, have been linked to form-deprived myopia due to their similarities in the eye changes of dimension [13]. It is also because of similar anatomical structures and characteristics of refraction [4]. Deprivation has been conducted in various species of animals, such as chickens, guinea pigs, monkeys, mice, and rabbits [12,14].

The deprivation method was performed with diffusers that were attached monocularly to *Oryctolagus cuniculus* with adhesive tape [14]. The reason for choosing rabbits as the animal model for this experiment is consistent with statements from previous research showing that rabbits are easy to handle, price-wise it was not expensive, and no need to put effort into manipulating the rabbits. It made the rabbit become the common animal model for experimental ocular research [15]. This research uses the rabbit's eye. Besides, it has large eyes, anatomically, the rabbit's eye has many similarities with the human eye [16].

Several aspects that can be seen in myopic eyes vary. Both axial elongation and refraction status are two parameters that are common and have been studied by several researchers [15, 4, 12].

However, there are other parameters that we can see from myopic eye conditions in animal models after the deprivation. Wang et al. previous research stated in myopic conditions, elongation of the eye has an association with the changing of the molecular characteristics from the sclera as a major determinant for ocular growth and size. Myopic conditions result from mechanical properties of the sclera, such as tissue degradation and the thinner layer of the sclera. It also experiences hypoxic conditions. Hence, it will increase the axial length [5,11]. The study conducted by Kusumawhardany et al. in 2019 also showed that the FDM applied in rabbits causes the layer from the sclera to experience thinning and lose its rigidity by showing the extracellular matrix accumulation level of the sclera decreasing. Another previous study by Zhou et al. in 2021 examined the choroidal blood perfusions of the eye in guinea pigs. The choroid is an important eye organ located between the sclera and the retina. This functions as an oxygen and nutrient supplier to both the retina and the sclera. When the deprivation was applied, it was found that the choroid layer and the choroid blood supply significantly decreased.

Axial length was defined and measured from the front of the cornea until the posterior part of the sclera. Deprivation of the eye that will induce the axial length growth will cause myopia because of the elongation of the vitreous chamber in the posterior part of the eye, the study by Howlett and McFadden stated that the anterior chamber of the eye was rarely affected by the diffusers [17]. Form deprivation myopia (FDM) can show the elongation of axial length when it is conducted in several species of animal that are still at an early age [12]. We chose to use the rabbits (Oryctolagus cuniculus) at the age of 3-4 months. As shown in Table 2 gives the result of $p<0.005$, which shows there are significant differences in axial length growth from week 0 to week 4. This study is consistent with the Indonesian study that stated FDM will induce the axial length growth that gives results as myopia, the occlusion to the rabbit eye was also conducted for four weeks. [14].

Another study of experimental myopia deprivation was conducted by Xiao et al. in another mammal called a guinea pig. The procedure remains the same, using the opaque eyeshade monocularly. It shows that the FDM group gives a significant result in the axial length growth compared to the control group or fellow control eyes [4]. Nie et al. previous study in 10-day-old rabbits that were monocularly deprived for 30 days, the axial length of *Oryctolagus cuniculus* gained 0.51mm ± 0.09mm. From Table 1, we can observe that the treatment group experienced the final result of mean with a standard deviation of 16,23mm ± 0,49mm from 15,58 ± 0,48, this result corresponds with the previous research by Xiao et al. that gives the same axial elongation from 7.63mm ± 0.04mm and change to 7.93mm ± 0.03mm.

The axial length elongation was examined by the biometer immersion, the same as in the previous study [14,4]. However, the other tool that can be used to examine the axial length is called ultrasonography [12]. The elongation of axial length can be discovered due to the thinning of the scleral layer through the light microscope [4]. This can cause the eyeball rigidity to decrease, hence causing the eyeball to grow [14]. Using the independent sample T-test, we examine both the control and FDM groups each in the difference in week four and week 0, and it shows that both groups' axial length increased more in week four compared to week 0. The result of the significance p=0.190 (p>0.05) was regarded as having no significance for the two groups. This result is linearly accurate with the previous study by Howlett and McFadden that applied the diffusers in the guinea pig and stated that the group not given the diffusers also experienced an increment of the axial length. However, the treatment group provides a bigger increment than the control group [17].

Xiao et al. stated that besides axial length, refraction error will also gain after deprivation in the rabbits' eye. Diffuser-wearing eyes quickly turned myopic, while all other eyes remained mildly hyperopic [17]. It was also shown that after 30 days of monocular deprivation, the refraction status gained -1.00D ± 0.52D. Thus, the addition from the length of the eye caused the refraction status described as a myopic condition [4].

Not only can the axial length and status refraction be gained in myopic eyes, but the vascular part in the eye, such as retinal vascularization, can lessen [18]. Animal models of myopia imply that the systems controlling ocular growth may adapt to different visual experiences, including the effects of induced defocus. The nature and size of naturally existing optical aberrations, which in turn impact the quality of the retinal image and the depth of the eye's focus, have a role in the retina's capacity to detect such changes. Therefore, the impact of focusing mistakes on eye growth for a diffraction-limited eye vs a highly aberrated eye will be significantly different [19].

In our study, the FDM group was tested with the Paired Sample T Test for the result after four weeks of deprivation; it gives a result of the significant value of comparison (p=0.000) showing that there are huge differences for the status refraction changes between weeks 0 and week 4. This result was also supported by the previous study by Nie et al. in 2014 that after deprivation monocularly in guinea pigs for 30 days, the status refraction progressed until -1.00±0.78D. For examining the refraction status, we used tools called streak retinoscopy. The examination was conducted before the deprivation in week 0 and after a week of deprivation. In the control group, the significant result was bigger than 0.05 (p=0.066), this interprets that there is no significant result for the control group result in status refraction changes from week 0 to week 4. There are no differences in terms of refraction status between the control group and the FDM group in week 0; it was all +2.00D. In the previous study, the juvenile animal eye always experiences a process called emmetropization, for instance, the guinea pig was born with a hyperopic eye condition [18]. Emmetropization helps defocus the condition in the eye by adjusting the eye growth through the lens [19]. The value of emmetropic eye status refraction gives the result of +2.00 to –0.25D [8]. In another study that was consistent with our experiment result, the final refractive errors from the 14 days of deprived guinea pig's eye were -3.05 ± 0.71D, which is more significant than in the control group result [4]. This data shows consistency with our result shown in Table 3, where a myopic condition is shown by the refraction status after the emmetropic eyes as many as -0,94 + 0,78D, whereas in Table 4, the result of the significance result from comparing the treatment group from week four and week 0 gives result of P=0.000 from the paired sample T-Test the treatment, P<0.05 shows that there are huge differences from the refraction status changes.

CONCLUSION

This research shows a significant difference in the axial length elongation in the treatment group of FDM (Form Deprivation Myopia) after monocular deprivation from week 0 to week 4. It also shows a significant difference in the status refraction changes in the treatment group of FDM (Form Deprivation Myopia) after monocular deprivation from week 0 to week 4.

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