**EXPERIMENTAL STUDY**

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# Permanent and temporary epiphysiodesis: an experimental study in a rabbit model

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**Objective:** The aim of this study was to compare the Phemister technique (permanent epiphysiodesis) with temporary epiphysiodesis with stapling and evaluate the degree of epiphyseal plate activity following staple removal using radiographic, scintigraphic and histopathologic methods and to investigate whether bone scintigraphy could provide clues on expected physis activity.

**Methods:** This study included 24 New Zealand rabbits divided into three groups of 8. Group 1 underwent Phemister's epiphysiodesis; Group 2, epiphysiodesis with stapling; and Group 3, epiphysiodesis stapling group with staples removed in the 3rd week. Radiological and scintigraphic data were recorded in the 3rd and 6th weeks. All animals were sacrificed for histopathological evaluation at the 6th week. Limb length was calculated using radiological assessment and their growth plate activation recorded from scintigraphic region of interest (ROI) assessment in the 3rd and 6th weeks. Histopathological findings of the growth plate were measured using qualitative and quantitative methods. All data were compared to the non-operated right side.

**Results:** Group 1 had effective scintigraphic, radiologic and histopathological findings than the other groups ($p<0.05$). In Group 3, growth plate activation was detected to be higher than the regular physis activity ($p<0.05$).

**Conclusion:** Preoperative and postoperative scintigraphy combined with radiological assessment can provide an idea on the reversibility of growth plate activation.

**Key words:** Epiphysiodesis; growth plate; Phemister technique; stapler; scintigraphy.

Lower limb length discrepancy is a common problem in children that requires treatment. Length discrepancies that lead to disruption of biomechanics and do not respond to conservative treatment necessitate surgical intervention to inhibit the growth of the longer extremity or to lengthen the shorter extremity.[1] Epiphysiodesis is a surgical technique used to correct lower limb length discrepancy during childhood, first described by Phemister.[2] Today, temporary epiphysiodesis with stapling is the most preferred technique.[3]

Several studies have been published in the literature comparing epiphysiodesis techniques using direct X-rays, magnetic resonance imaging and histopathological methods. However, to our knowledge, a study com-

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paring epiphysiodesis techniques using scintigraphic methods has not yet been performed.

The first objective of this study was to radiographically, scintigraphically and histopathologically compare the Phemister technique (also defined as permanent epiphysiodesis) with temporary epiphysiodesis with stapling. The second objective was to evaluate the degree of epiphyseal plate activity following staple removal through radiographic, scintigraphic and histopathological assessments. The third objective was to investigate whether bone scintigraphy would give clues about expected physis activity during temporary epiphysiodesis when staples were removed.

## Materials and methods

Approval from the Animal Ethics Committee was obtained. Thirty New Zealand rabbits (age: 6 to 12 weeks; weight: 1,500 to 2,000 grams) were used in this study. Based on the size of the epiphyseal plate of rabbits, 0.8-mm-thick K-wires were prepared to serve as 6-mm-long staples and a specially designed staple holder for this size was arranged to apply epiphysiodesis with a stapler.

Animals were randomly divided into three groups of 10 each. The first group underwent the Phemister technique, the second group epiphysiodesis with stapling in which the staplers were kept, and the third group epiphysiodesis with stapling in which the staples were later removed. A dose of 35 mg/kg ketamine (10% injectable Alfamine; Alfasan International BV, Woerden, the Netherlands) and a dose of 8 mg/kg xylazine (Ksilazol; Provet, İstanbul, Turkey) were mixed and intramuscularly administrated as anesthesia to the gluteal muscles for 45 minutes. Rabbits were placed in the supine position and their right knees shaved. Surgical sterility was established with a solution of polyvinyl iodine (Batticon; Adeka Corp., İstanbul, Turkey) and the surgical site was draped. A longitudinal skin incision to the anterior of the right knee was made. The quadriceps muscle was identified and a parallel incision to the muscle fibers was performed to access the right knee joint with a parapatellar medial approach. Retractors were placed to visualize the medial and lateral proximal epiphyseal plates of the right tibia and the exact localization of the epiphyseal plate was checked with the tip of an injector. In the first group, the medial and lateral epiphyseal plate was removed rectangularly, rotated 180 degrees and sutured to the periosteum, as defined in the Phemister technique for permanent epiphysiodesis (Fig. 1). For the other two groups, after visualization of the medial and lateral epiphyseal plates, the localization of the epiphyseal plate was checked. Staples were applied for epiphysiodesis as two pieces for each medial and lateral epiphyseal plate (Fig. 2). After washing with saline, the muscles and skin were closed using 2/0 Vicryl and

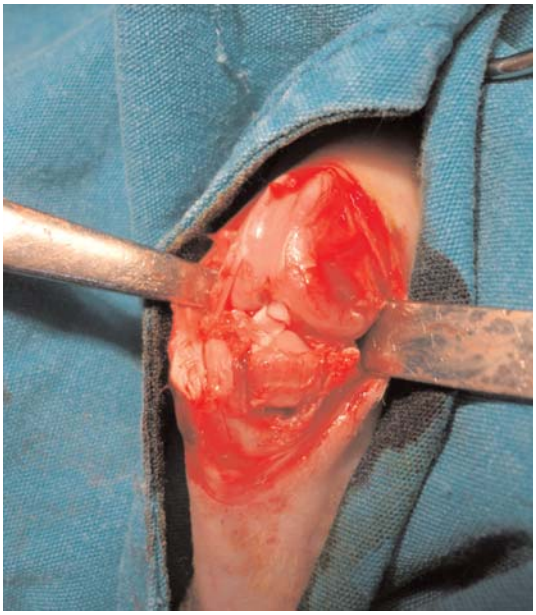

**Fig. 1.** Application of the Phemister technique. [Color figure can be viewed in the online issue, which is available at www.aott.org.tr]

**Fig. 2.** Staples were applied for epiphysiodesis as two pieces for each medial and lateral epiphyseal plate. [Color figure can be viewed in the online issue, which is available at www.aott.org.tr]

4/0 Nylon sutures, respectively. Intramuscular 50 mg/kg of cefotaxime was administered to all animals at the postoperative 48th hour for prophylaxis. The proximal epiphyses of left tibias were spared for comparison purposes in all rabbits.

All rabbits returned to their normal cage activities. At the postoperative 3rd week, rabbits were examined by direct X-ray and scintigraphy. Staples of rabbits in the third group were removed from the right knee using the previous incision and layers were closed as previously described. All rabbits were left to normal cage activities for an additional 3 weeks. During this period, a total of 6 rabbits, 4 rabbits from the first and second group and 2 rabbits from the third group were excluded from the study due to infection and implant failure, respectively. The remaining 24 rabbits, with 8 rabbits in each group, were examined using radiography and scintigraphy at 6 weeks. Consequently, all rabbits were sacrificed and evaluated histopathologically.

At the postoperative 3rd and 6th weeks, 1 mCi/kg of Tc-99m MDP (technetium-99m-labeled methylene diphosphonate) was intravenously administrated under anesthesia. Early-phase scintigraphies (first pass and blood pool) and, after one hour, late-phase scintigraphies were obtained. The early phase of scintigraphic examination provides information on the arterial blood supply and the late phase of scintigraphic examination provides information on osteoblastic activity. All scintigraphic imaging procedures were performed using a low-energy general-purpose collimator, Toshiba GCA-602A device. A 64×64 dynamic imaging matrix and 256×256 static imaging matrix were selected as the imaging parameters.

All animals' operated and non-operated tibial proximal epiphyses were selected as a region of interest (ROI). ROI was drawn for the operated and non-operated knees (Fig. 3).

Average ROI count values of the operated and non-operated tibia were calculated by subtracting the background activity to obtain late phase parameters that show osteoblastic activity.

At the 3rd and 6th weeks, radiographs of both lower limbs from the knee to the ankle were obtained with computed radiographic technique (phosphor plate computed radiography). A Siemens Multix CP device was used as the X-ray source and Kodak Direct View CR 975 device was used to scan the phosphor plate. The length of the operated and non-operated tibia were measured quantitatively using a Kodak Direct View MX Workstation (version 5.2.1 SA.SP1) (Fig. 4). Measurements of the non-operated left tibias were evaluated as the control group. The operated and non-operated tibia lengths and their differences were measured at the 3rd and 6th weeks.

The proximal portion of each tibia was excised including the operated epiphyseal line and diaphysis in the axial plane. Specimens were fixed for approximate-

**Fig. 3.** Region of interest drawings.

**Fig. 4.** Measuring the tibia length using a Kodak Direct View MX Workstation.

ly 24 hours in 10% buffered neutral formalin and decalcified in 20% formic acid for 48 to 96 hours at room temperature. Five µm of sagittal sections were obtained and stained with hematoxylin-eosin.

Quantitative pathological examination was performed in two ways:

a) Epiphyseal plate area (EPA) measurement: Sections of the operated and non-operated tibia were digitally photographed with a x10 lens (Olympus 5660CZ camera). Photographs were evaluated using AxioVision LE, Rel. 4.6 morphometry software (Carl Zeiss Microimaging Inc., Thornwood, NY, USA). The software was calibrated with a micrometer for standardization of the measurements. With ×10 magnification, size of the photographs was 2.98 mm × 2.27 mm = 6.76 $mm^2$. Attention was paid to centering the epiphyseal plate in the photograph while parallel to the longer axis. EPA was measured in $mm^2$ for each tibia.
b) Epiphyseal plate thickness (EPT) measurement: After the EPA of the operated and non-operated knees of all animals was measured in $mm^2$, the length of the epiphyseal plate in the photograph was measured with AxioVision LE, Rel. 4.6. EPT was calculated by dividing the EPA to the length of the epiphysis (Figs. 5 and 6).

To formulate; mean thickness of the epiphysis (EPT) = epiphyseal plate area (EPA) / epiphyseal plate length

Statistical analyses were performed using SPSS software (version 10.0). The mean, standard deviation, minimum and maximum values were calculated to analyze descriptive data. The Wilcoxon signed-rank test was used for intergroup comparison of parameters based on quantitative measurements (tibial lengths, late phase scintigraphy findings, histopathological assessments of EPA and EPT). One-way ANOVA (analysis of variance) was performed for the intergroup assessments. A p value of $<0.05$ was considered statistically significant.

## Results

Radiographic and scintigraphic late-phase data were obtained for each tibia at the 3rd and 6th weeks. At 6 weeks, in addition to radiographic and scintigraphic data, EPA and EPT were compared histopathologically. Results of all three groups at 3 and 6 weeks are shown in Tables 1-3.

The operated and non-operated tibias were compared within intergroup assessments. Tibia length difference was calculated using X-ray and statistically analyzed at the 3rd and 6th weeks. For the first and second groups the difference between the tibia lengths was found to have decreased significantly at 3 weeks ($p<0.05$). For the second group, this difference decreased significantly at 6 weeks ($p<0.05$). For the third group, the difference decreased significantly at 3 weeks ($p<0.05$) whereas the difference was statistically insignificant by 6 weeks.

Late-phase values for the first group decreased with a borderline significance at Week 3 and significantly at Week 6 ($p=0.05$ and $p<0.05$, respectively). For the second group, late-phase values were not significantly dif-

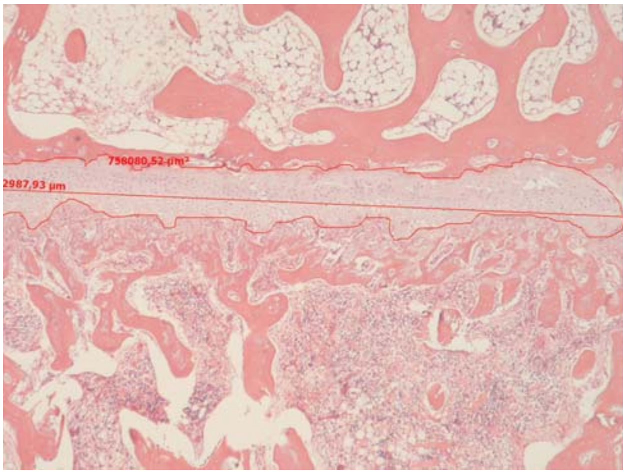


**Fig. 5.** Histopathological section and measurement of the epiphyseal plate that underwent epiphysiodesis with stapling. [Color figure can be viewed in the online issue, which is available at www.aott.org.tr]

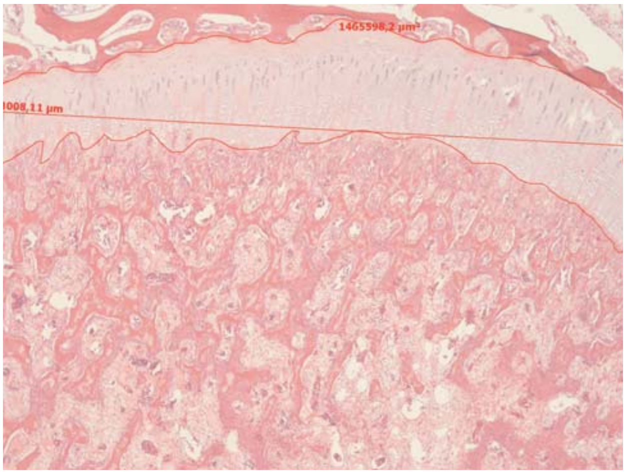


**Fig. 6.** Histopathological section and measurement of the epiphyseal plate following staple removal. [Color figure can be viewed in the online issue, which is available at www.aott.org.tr]

**Table 1.** Radiologic, scintigraphic and EPT assessments of Group 1.

| Group 1 means | Radiologic limb length (mm) (3rd week) | Scintigraphic ROI ratio (3rd week) | Radiologic limb length (mm) (6th week) | Scintigraphic ROI ratio (6th week) | Epiphyseal plate area ($mm^2$) | Epiphyseal plate thickness (µm) |
|---|---|---|---|---|---|---|
| Non-operated knee | 85.6 | 40.5 | 93 | 18.9 | 1.03 | 356.3 |
| Operated knee | 80.2 | 32.8 | 81.8 | 14.6 | 0.68 | 305.2 |

ferent at 3 weeks (p>0.05). However, these values were found to have decreased significantly in scintigraphic assessment at Week 6 (p<0.05). Late-phase values for the third group were not significantly different at Week 3 (p>0.05) whereas the Week 6 results showed a significant increase (p<0.05). Scintigraphic intergroup assessment results are shown in Table 4.

EPA and EPT of the first group decreased significantly (p<0.05). EPA and EPT of the second group also decreased significantly (p<0.05). In the third group, EPT increased significantly (p<0.05) and EPA insignificantly (p>0.05).

Operated tibia lengths were measured by radiography at 3 and 6 weeks and their differences were calculated and compared. The first group had the highest difference with 11.2 mm, the second group had a moderate difference (3.7 mm) and the third group had a minimum difference (1.7 mm). Differences between all three groups were statistically significant (p<0.05).

Late-phase scintigraphic values of operated tibias were compared between groups. No significant difference was observed between the groups at 3 weeks (p>0.05). At 6 weeks, the difference in the third group was significantly higher than the other two groups (p<0.05). Scintigraphic results between groups are shown in Table 5.

In the histopathologic intergroup assessment, the first group had the lowest values and the third group the highest values for both EPA and EPT. The first group showed a significant decrease compared to the second group (p<0.05). The increase in the third group was also significant when compared with the other groups (p<0.05).

## Discussion

The epiphyseal plate in rabbits has been shown to be active until the 28th week of development.[4] As all animals in this study were followed postoperatively for 6 weeks, evaluation was completed while the proximal tibial epiphyseal plate was active. In a study dated 1997, Ross and Zionts[5] compared the Phemister technique with epiphysiodesis with stapling and percutaneous epiphysiodesis using histopathological and radiographic methods with 2-week intervals. A significant decrease in thickness of physis and significant losses of hypertrophy zone were observed in the stapling group at the 2nd postoperative week. Chondrocytes showing columnar arrangement in the 2/3 of the center of the epiphyseal and metaphyseal region but with no growth pattern was observed at Week 4. At 6 weeks, the growth plate was found to have almost stopped. They found no significant histopathological difference between the 6th and 10th weeks. Similar histopathological findings were found for the first group in our study. All animals in our study were histopathologically evaluated 6 weeks after surgery. In the histopathological examination of the second group, which underwent epiphysiodesis with stapling, histopathological changes similar to those in the literature were seen. To our knowledge, there is no study in the literature investigating the histopathological changes after staple removal.

In our study, third group late-phase values of the operated tibias were increased compared to the non-operated side at 6 weeks. This finding was interpreted as an increase in the osteoblastic activity in this group. This late-phase increase was considered to be a scintigraphic reflection of maturation and cellular proliferation at the zone of hypertrophy in the histopathologic examination.

**Table 2.** Radiologic, scintigraphic and EPT assessments of Group 2.

| Group 2 means | Radiologic limb length (mm) (3rd week) | Scintigraphic ROI ratio (3rd week) | Radiologic limb length (mm) (6th week) | Scintigraphic ROI ratio (6th week) | Epiphyseal plate area ($mm^2$) | Epiphyseal plate thickness (µm) |
|---|---|---|---|---|---|---|
| Non-operated knee | 89.1 | 31.1 | 95.6 | 15.3 | 0.96 | 396.8 |
| Operated knee | 86.4 | 29.9 | 90.1 | 13 | 0.67 | 361.5 |

**Table 3.** Radiologic, scintigraphic and EPT assessments of Group 3.

| Group 3 means | Radiologic limb length (mm) (3rd week) | Scintigraphic ROI ratio (3rd week) | Radiologic limb length (mm) (6th week) | Scintigraphic ROI ratio (6th week) | Epiphyseal plate area ($mm^2$) | Epiphyseal plate thickness (μm) |
|---|---|---|---|---|---|---|
| Non-operated knee | 85.5 | 40.8 | 92.3 | 26.1 | 0.91 | 332.3 |
| Operated knee | 82.8 | 25.5 | 90.6 | 33.4 | 0.94 | 417.2 |

In the literature, few studies investigating age-related scintigraphic activity of the growth plate have been reported.[6,7] New epiphysiodesis techniques have been defined and publications comparing new epiphysiodesis techniques have emerged in recent years as well.[8,9] However, despite publications comparing epiphysiodesis techniques using radiographic, magnetic resonance imaging and histopathological methods, to our knowledge, no study comparing the epiphysiodesis techniques using scintigraphic methods exists in the literature.

Canale and Christian[10] examined the percutaneous epiphysiodesis technique in their study in 1990. In this study, measuring the limb length using radiographs to determine epiphyseal plate activity yielded better results than measuring the limb length by computed tomography in long-term follow-ups. In our study, we also utilized radiographs to measure leg length in order to evaluate the success of epiphysiodesis technique.

Epiphyseal plate growth inhibition caused by increased bone bridge formation in the classical technique was shown histopathologically in our study. Similarly, bone bridge formation was observed in the stapling group. More bone bridge formation was observed in the classical technique than with stapling, which was consistent with the literature.[5] Bone bridge formation in the group with removed staples was less than in the other two groups. The increase of EPT in this group was statistically significant. At the same time, the uptake of Tc-99m involvement in the late-phase due to osteoblastic proliferation was statistically significant in this group. In 1993, Harcke and Mandell[11] reported that bone scintigraphy is important in evaluating the growth plate. In this study, they showed that scintigraphic comparison of the epiphyseal plate of the affected and normal side may give information about total or segmental closing. Van Roermund et al.[12] investigated the epiphyseal activity of the rabbit proximal tibia and distal femur scintigraphically in 1994. As in our study, they used Tc-99m as the radioisotope. In a study dated 1993, Çelen et al.[13] examined the activity of the distal femoral epiphyseal plate in 334 children aged between 2 and 20 years. Activity of the distal femoral growth plate was proportioned to the ipsilateral femoral diaphysis activity. The highest elongation was observed when this proportion was also at its highest. In a 2006 study including 81 men and 46 women, Yang and Yang[14] followed epiphyseal plate activation from the second postnatal week up to age 24 to present the scintigraphic assessment of the normal activity of epiphyseal plate. This study was the first in the literature to assess the scintigraphic activation of the epiphyseal plate in humans. The study demonstrated that scintigraphy is important in evaluating the growth plate activation.

Recent literature suggests that epiphysiodesis may be performed by electrical stimulation.[15] However, to provide temporary epiphysiodesis, epiphysiodesis with stapling is still valid.

Histomorphometric measurement of the epiphyseal plate thickness varies in the literature. In Mobarakeh et al.'s 2005 study, epiphyseal plate thickness was measured by the average of five different regions.[16] In Atabek et al.'s study in 2006, the mean of the measurements from three different regions was considered as epiphyseal plate thickness.[17] In 1981, Seinsheimer and Sledge measured the epiphyseal plate thickness from the shortest vertical line of the plate and the region parallel to the columnar arrangement.[18] In our study, EPA and EPT was utilized as the histomorphometric method. Measurements were not calculated separately for each zone. EPT measure-

**Table 4.** Scintigraphic intergroup assessment results.

| Intergroup scintigraphic assessment | Group 1 | Group 2 | Group 3 |
|---|---|---|---|
| Scintigraphy at week 3 | p=0.05* | p>0.05 | p>0.05 |
| Scintigraphy at week 6 | p<0.05† | p<0.05† | p<0.05‡ |

*Borderline significance, †Significant decrease, ‡Significant increase

**Table 5.** Scintigraphic results between groups.

| Scintigraphic assessment between groups | Group 1 | Group 2 | Group 3 |
|---|---|---|---|
| Scintigraphy at week 3 | p>0.05 | p>0.05 | p>0.05 |
| Scintigraphy at week 6 | p>0.05 | p>0.05 | p<0.05* |

*Significant increase

ments were performed by dividing the histomorphometrically measured area by the length of the epiphysis. Our study is compatible with the literature in terms of measurement methods and a pioneer work in terms of EPT measurement method. In 2001, Snyder et al.[19] showed bone bridge formation of the epiphyseal plate occurs at the fourth week. They reported no significant difference after 6 weeks. As follow-up in our study was 6 weeks, we were able to fully observe the impact of epiphysiodesis.

For the classical epiphysiodesis group, EPA decreased significantly when compared to other groups. The group in which staples were removed group showed significantly increased EPT compared to the other groups. Histopathologic appearance was interpreted as an increase in chondrocyte proliferation at the proliferative and maturation zone in EPA, following removal of the staples.

After the staples were removed, periosteum may be partially responsible for the epiphyseal plate activity as a result of the secondary surgery. Excluding this periosteal reaction is the main weakness of our study.

Based on these results, in addition to pre- and post-operative radiographic assessments in children planned to receive permanent epiphysiodesis with the Phemister technique, evaluation of a three-phase bone scintigraphy may give an idea about growth inhibition. Epiphysiodesis with stapling is an effective method of epiphysiodesis and its reversibility provides an advantage. However, this reversible activity is unpredictable. In clinical practice, scintigraphy may provide information about the activity of the physis before and after the removal of staples in temporary epiphysiodesis with stapling. In this regard, while this group of patients undergo radiological assessments before surgery, the addition of a three-phase bone scintigraphy and follow-up after surgery may give an idea about return activity of the growth plate after staple removal. In recent years, scintigraphic studies aiming to show the normal activation of the growth plate have been conducted. A similar standardization can be applied to children who have undergone epiphysiodesis.

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**Conflicts of Interest:** No conflicts declared.

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