Experimental Study / Deneysel Çalışma

Seeking an innocent method for pediatric septoplasty: *in vivo* comparison of Cottle's method, radiofrequency and laser in rabbits

Pediyatrik septoplasti için masum bir yöntem arayışı: Tavşanlarda Cottle yöntemi, radyofrekans ve lazerin *in vivo* karşılaştırması

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Objectives: In this study, in vivo histopathological and radiological findings in rabbit septum through laser, radiofrequency (RF) and Cottle's method were investigated.

Materials and Methods: This study was conducted between November 2007 and February 2008 on 36 New Zealand rabbits aged four-to-six months and weighing 1.5 to 2 kg. Subjects were divided into six equal groups. The first group was defined as the control group. Next four groups consisted of subjects where RF or laser was either applied transmucosal or directly to the cartilage. Cottle's method was used in the sixth group. Histopathological and radiological changes were investigated in each group.

Results: Histopathological changes in mucosa were not significantly different from those of control group. However, post-intervention changes in cartilage were significantly different, compared to the control group. The highest mucosal and submucosal reaction and damage in cartilage with ossification was found in Cottle group. It was found that radiofrequency was less damaging to mucosa, creating an equal degree of degeneration as laser in cartilage.

Conclusion: Study results suggest that Cottle method is not so innocent with a considerable reaction rate, whereas RF and laser do not cause irreparable damage in cartilage and surrounding tissues. Radiofrequency seems superior to laser, as it causes more degeneration in cartilage, but no loss in epithelium even transmucosally. The major problem is the unpredictability of the damage.

Key Words: Carbondioxide laser; cartilage reshaping; laser surgery; nasal septum; pediatric septoplasty; radiofrequency.

Amaç: Bu çalışmada lazer, radyofrekans (RF) ve Cottle yöntemi ile dejenerasyonun derecesini belirlemek amacıyla tavşan septumunda in vivo histopatolojik ve radyolojik değişiklikler incelendi.

Gereç ve Yöntemler: Kasım 2007 - Şubat 2008 tarihleri arasında yapılan bu çalışmada ağırlıkları 1.5-2 kg arasında değişen dört-altı aylık 36 Yeni Zelanda tavşanı kullanıldı. Denekler altı eşit gruba ayrıldı. Birinci grup kontrol grubu olarak belirlendi. Diğer dört grup RF veya lazerin mukoza üzerinden veya mukoperikondriyum elevasyonundan sonra doğrudan septal kıkırdağa uygulandığı deneklerden oluşuyordu. Altıncı grupta Cottle yöntemi kullanıldı. Histopatolojik ve radyolojik değişiklikler herbir grupta araştırıldı.

Bulgular: Mukozadaki histopatolojik değişiklikler, kontrol grubuna kıyasla, anlamlı olarak farklı değildi. Ancak girişim sonrasında kıkırdakta oluşan değişiklikler, kontrol grubundan anlamlı olarak farklıydı. Ossifikasyonlu kıkırdakta en yüksek hasar ve mukozal ve submukozal reaksiyon Cottle grubunda tespit edildi. Radyofrekansın mukozaya daha az hasar verdiği ve kıkırdakta oluşturduğu dejenerasyonun lazerin oluşturduğu dejenerasyona eşit olduğu bulundu.

Sonuç: Çalışma bulguları, Cottle yönteminin çok da masum olmadığını, önemli ölçüde reaksiyona yol açtığını, RF ve lazerin kıkırdak ve çevre dokularda onarılamaz hasara yol açmadığını düşündürmektedir. Transmukozal uygulamada dahi epitel kaybı olmaksızın kıkırdakta daha çok dejenerasyon oluşturması nedeniyle RF, lazere üstün görünmektedir. En büyük sorun hasarın önceden tahmin edilememesidir.

Anahtar Sözcükler: Karbondioksid lazer; kıkırdak şekillendirme; lazer cerrahisi; nazal septum; pediyatrik septoplasti; radyofrekans.

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Deviation of the nasal septum is the one of the most frequently seen causes of nasal obstruction, which can be corrected by various surgical interventions. Contemporary septum surgery was started by Goldman and Cottle who stressed the disadvantages of radical septal surgery. In the present day, conservative septoplasty with limited tissue excision and reconstruction of the supportive septal parts has gained popularity.[1-2] The objective of septal deviation surgery is to restore the anatomic deformities in the nasal septum by conserving the cartilaginous and bony framework as much as possible. Decades after Cottle put forth the drawbacks of radical septal surgery, conservative concepts like limited tissue excision and preservation and reconstruction of the supportive areas, described by the term "septoplasty" is now the preferred method.[3,4] However, there is still a search for newer techniques which may be more cost-effective, provide more patient comfort and minimize the postoperative healing process. On the other hand, cautionary statements on effects of nasal surgery on the growing nose have been made since the 1950s, when Gilbert and Segal[5] published against resection of quadrilateral cartilage. Farrior and Connolly[6] made a consensus statement after a review of the literature in 1970, that nasal surgery in children should be delayed, if possible, until growth is complete.

It may be sometimes rather difficult to restore the deviated cartilage without surgically removing it. Furthermore, inability of cartilage to sustain its new form due to internal tensile forces still remains a major problem. In recent years, with various in vitro and in vivo studies, efforts at reshaping of cartilage by thermal energy have emerged and positive results have been obtained.[7-10] In the light of the studies made, we aimed to examine the change in the shape of rabbit nasal septum in vivo using CO_2 laser or radiofrequency (RF) transmucosally or directly on the cartilage and cold knife (Cottle method) to examine the histopathological and radiological changes occurring in vivo in layers of the septum. No other in vivo studies were found in the literature comparing the three methods. The possibility for application of less invasive techniques for pediatric septum surgery is discussed.

MATERIALS AND METHODS

This study was conducted in a tertiary referral center from November 2007 to February 2008. The surgeries and the follow-up care of the subject animals were performed in the local university Medical School's Animal Experiment Research Laboratory with the approval of the ethical committee of the same university (Approval no: 2007-28). Thirty-six New Zealand rabbits ranging from four to six months of age, weighing 1.5-2 kg were used. Rabbits were separated into six groups: The first was the control group. Group 2 (muc-lz) had CO_2 laser applied transmucosally to the septum and group 3 (car-lz) had laser treatment directly to the cartilage after elevation of mucoperichondrium. Likewise, RF was applied transmucosally to septum in group 4 (muc-rf) and directly to cartilage in group 5 (car-rf). Group 6 rabbits (Cottle group) underwent cold knife septoplasty. Before the operation, axial computed tomography (CT) images of septums of the rabbits were taken in the Radiology Department.

Animals were anesthetized with 40 mg/kg ketamine (100 mg/ml) and 5 mg/kg xylazine (20 mg/ml) intramuscular injection by the anesthesiologist. A pediatric nasal speculum, a 2.7 mm 0-degree rigid telescope and/or a surgical loupe with 2.5 magnification was used for a better view of the small surgical site. One percent lidocaine hydrochloride plus 1/200000 epinephrine (Jetokain® 2 ml) were administered to the columellar zone and submucoperichondrial space on both sides. Open technique through a transcolumellar incision was preferred. In the muc-lz group mucosa was treated over the quadrangular cartilage at a point 4 mm from caudal and ventral margins and 1 mm from the dorsal margin by direct tip of a carbon dioxide laser (model 1040; Sharplan Laser Industries, Ltd, Tel Aviv, Israel) with a wavelength of 10.6 μm. The laser beam was focused to a spot size of 2 mm, transparent at 10.6 μm, and with a focal length of 400 mm. Pulsed exposures were used; the exposure time was 0.5 seconds, and the output power was 5W. The laser beam was used directly over cartilage after unilateral elevation of the mucoperichondrium in the car-lz group, with 3W energy output in pulsed mode for 0.5 seconds. The energy acquired in each shot was 48 Joule x cm^2. Twenty to thirty shots were made to reach 60-90 Joule of energy, which is the amount required to remodel the cartilage. These laser energy output variables were based on the results of previous experiments by Helidonis et al.[7]

Radiofrequency was applied over the mucosa to the muc-rf group rabbits by leaving a robust part of cartilage in dorsal and caudal sections of the quadrangular cartilage (Vesalius® LX 80 model Molecular Resonance Generator, Telea, Italy) producing 60 Watt RF energy at 4-8-12-16 mHz with power adjustment according to tissue resistance to reach a maximum of 62.9 °C, which is the coagulation temperature. In six rabbits in the car-rf group RF was applied directly to the cartilage with the same energy parameters. In the Cottle group, after unilateral elevation of the mucoperichondrium, cartilage was incised in a checkerboard pattern down to the opposite perichondrium. With this method, independent cartilage islets supplied from the contralateral perichondrium were formed. For closure, septal mucosa was approximated, and skin incisions sutured together. All surgeries were performed by the same surgeon.

After the operation, the rabbits were followed up in laboratory and were sacrificed after three months. Two rabbits each in the muc-lz and car-lz groups and one rabbit in the muc-rf group were lost during follow-up. The axial CT scans of the postmortem septums were taken. In the Pathology Department, the septums were first evaluated macroscopically by elevation of the overlying skin and deep soft tissues before the nasal cavity and paranasal sinuses were exposed. All septa were cut into 6-7 slices in full layer and embedded in paraffin blocks by a blinded pathologist. Epithelium integrity and degree of proliferation was investigated, together with the subepithelium regarding the inflammatory response and the condition of the mucosal glands. "Mild inflammation" (+) was used to define the presence of edema and inflammatory elements in dispersed condition. "Severe inflammation" (++) was used to define the presence of intensive groups of inflammatory elements. The fibrosis in submucosal glands and retentions in glands' ducta were recorded. In rabbits with cartilage exposure, perichondrium reaction, degeneration and regeneration findings in cartilage were qualitatively evaluated as present/absent. For statistical analyses, Statistical Package for Social Sciences (SPSS) version 10.0 software was used. For comparison of qualitative data, Fisher's exact probability test was used. The results were evaluated in the reliability interval of 95% and $p<0.05$ was accepted as significant.

RESULTS

Histopathological findings

All changes observed in epithelium, subepithelium, cartilage and perichondrium are listed in Table 1. The control group epithelium, cartilage

Table 1. Histopathological findings in all groups

	Group 1 (control) (n=5)	Group 2 (muc-lz) (n=4)	Group 3 (car-lz) (n=4)	Group 4 (muc-rf) (n=5)	Group 5 (car-rf) (n=5)	Group 6 (Cottle) (n=5)
Epithelium						
Normal	5	3	2	4	5	–
Loss	–	1	1	–	–	–
Proliferation	–	–	1	1	–	5
Inflammation						
Normal	3	1	1	1	–	–
Inflammation (+, ++)	2	3	3	4	5	5
Salivary gland fibrosis	–	–	–	–	1	1
Cartilage						
Normal	5	–	–	–	–	–
Degeneration	–	4	4	5	5	5
Thinning	–	1	–	–	–	1
Total loss	–	–	1	1	–	2
Perichondrium						
Normal	5	–	–	–	–	–
Thickened	–	4	4	5	5	5

muc-lz: Laser applied transmucosally; car-lz: Laser applied directly to cartilage; muc-rf: Radiofrequency applied transmucosally; car-rf: Radiofrequency applied directly to cartilage.

and perichondrium were observed to be normal in all animals. Likewise, no epithelial abnormalities were found in the car-rf group where RF was applied directly to the cartilage. Epithelial proliferation obtained in all rabbits of the Cottle group was found to be significant at the limit level with muc-rf and car-rf groups (p=0.048) and at strong level with the control group. The car-rf and muc-rf groups were significantly different at border level (p=0.048). No statistically significant difference between the groups was obtained with regard to epithelium loss observed in the muc-lz and car-lz groups. Inflammation or salivary gland fibrosis in the subepithelium was observed in all groups. None of the rabbits had normal subepithelium in the Cottle group and car-rf group. In intergroup comparison of normal subepithelium appearance, no statistically significant difference was obtained. Similarly, no statistically significant difference was obtained in intergroup comparison of subepithelium mild inflammation (+) finding, nor in the intergroup comparison regarding subepithelium severe inflammation (++) finding.

All cartilage specimens were evaluated as normal in the control group, whereas there was a total loss or degeneration-regeneration in cartilage of all animals in all groups other than the controls. These degenerative and regenerative alterations was found to be statistically significant when compared to the control group (p<0.05). In the Cottle group, there was loss of septal cartilage in one rabbit and ossification was observed in four. Thinning or total loss of cartilage was observed in all rabbits except the car-rf group (Figure 1). Similarly, perichondrium was thickened in all animals in intervention groups. These changes in perichondrium were found to be different significantly from the control group (p<0.05).

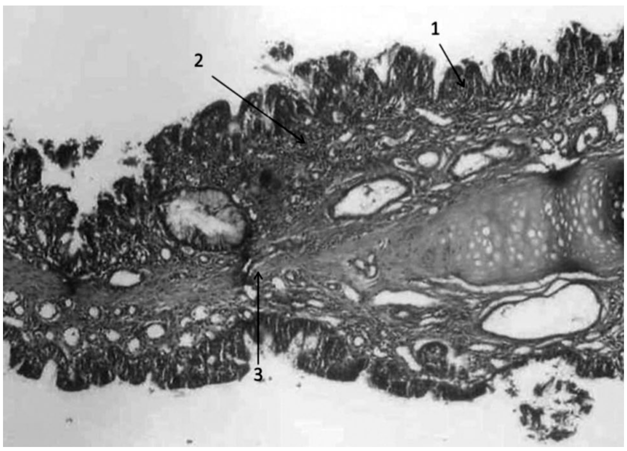

Figure 1. *Epithelial proliferation (1), submucosal inflammation (2), thinning and loss of cartilage (3) (H-E x 40).*

Preoperative and postmortem CT scans were evaluated by the same radiologist. Changes were recorded as septum being deviated to the right or left of the midline. Presence of deviation was compared both pre- and postoperatively with the control group and among all groups. No statistically significant difference was found between groups.

DISCUSSION

Several techniques were either put forward or studied in pediatric septal surgery. The use of crushed cartilage has proved its value in reconstructing the nasal septum of adult patients; but experimental data have clearly demonstrated the limitations for application in the growing nose.[11,12] There is also no data pertinent to effects of scoring on the mechanical strength/weakening of the septal cartilage, nor the effects on later mid-facial development. At the same time, inability of the formed cartilage to maintain its shape permanently is another challenge, due to the inner tensile strength of the cartilage.[13]

Choosing the efficient method which does not compromise growth centers of the mid-face is still a challenge. These patients are prone to hearing loss, facial deformity, cardiac or pulmonary disturbances due to hypoxia, growth retardation and low academic success. In recent years, efforts to reshape the cartilage permanently by thermal energy have been increasing and positive results have been obtained with various in vitro and in vivo studies. Laser reshaping of the cartilage was first suggested in 1993 by Helidonis, where he shaped rabbit ear cartilage with CO_2 laser in vitro and determined that the thin cartilages maintained their new shapes.[7] In 1994, Sobol et al.[14] presented another publication supporting this study. The fact that thermal heating by laser decreased the tensile strengths inside the cartilage ensuring the cartilage to preserve its new shape has been defined by many other experimental studies.[15-17] The temperature can be non-uniform in a laser-irradiated cartilage specimen. At least half of the cartilage thickness must be heated over critical temperature (Tc=70±5 °C), for effective stress relaxation.[18] But, non-occurrence of a significant damage on the surrounding tissues due to the thermal communication between the area to which laser is applied and the normal tissue ensures laser to be applied to a desired limited area.

Radiofrequency is another heat source, which creates a very small and extremely controlled necrosis. The healing of the necrosis causes scar formation and tension in the tissue. Over 47 °C, irreversible damage as a result of protein denaturation occurs.[19,20] The surgical effects of RF depend on energy waveforms, determined power rate, electrode dimensions, manipulation, application time and tissue properties. In 2007, Hatayama et al.[21] examined the histopathological changes which occurred in the repair of rabbit joint cartilage after administration of RF energy, putting forward that RF energy triggers cell division. Only one in vitro experimental study on reshaping of nasal septum cartilage by RF has been found in the literature. In this study by Keefe et al.,[22] RF heating reshaped porcine septal cartilages while maintaining cellular viability. They noted that with optimization of the instrument variables, more uniform and rapid heating of the cartilage specimen might be possible. As the RF settings in this study are arbitrary units based on the power knob of the device used, the results are not comparable to those of our study.

In our study, the statistically significant alterations in septum epithelium of all subjects in the Cottle group compared to the other groups can be explained by the fact that epithelium responds to elevation trauma with inflammatory reaction and proliferation. It suggests that related with the damage level which occurred in cartilage in this group, fibroblast migration and increase in number of fibroblasts were higher. Verwoerd-Verhoef et al.[23] have determined that new cartilage formation originating from perichondrial stem cell in young rabbits two weeks after submucosal resection depends on perichondrial mesenchymal cells triggering epithelial proliferation in response to the damage. Although elevation of the mucoperichondrium seemed not to affect nasal growth, it appeared to result in short-term effects at tissue level with swelling of the lamina propria and exudate, and a more prolonged proliferative effect of cell production. In reciprocal evaluation of groups treated either with laser or RF in our study, there was no significant difference between them with respect to epithelial proliferation. Unlike Hatayama et al.[21] epithelial proliferation was observed in only one subject with RF application and this was not statistically significant.

In our study, severe (++) inflammation was seen mostly in the Cottle and car-rf groups. Although in RF groups, particularly in the car-rf group, severe inflammation occurred in a greater number of subjects in comparison to laser-treated groups, there was no statistically significant difference between the groups. Severe inflammation (++) finding is expected to bring proliferation along with it (Figure 2). This might be due to elevation of perichondrium, because the proliferation finding in the Cottle group was different at statistically significant levels from the control group (p=0.008). Overall, this finding suggests that epithelial damage was more prominent in the Cottle group and may reflect higher degree of reaction in cartilage tissue. This data is consistent with the literature.

When the cartilage integrity is disturbed, chondroblasts form new cartilage by migrating to the defect area. Ultimately, when perichondrium touches the matured cartilage, new cartilage formation is inhibited.[24] In our study, thickening in the perichondrium was observed in all groups other than the control group. No difference was found between RF, laser and Cottle method with respect to perichondrium proliferation and all these methods lead to proliferation in perichondrium of all subjects. This is thought to be perichondrium's reaction to repair the damage of surgical intervention by regenerative response. Wong et al.[25] reported data providing evidence that laser irradiation, along with other thermal and mechanical treatments, causes a proliferative response in chondrocytes, and that this was observed ex vivo in the absence of cellular and humoral repair mechanisms. He stated that some critical temperature threshold exists, and that the

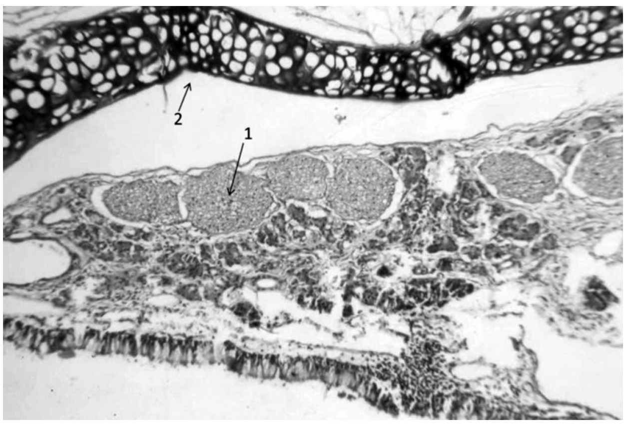

Figure 2. *Severe inflammation with presence of intensive groups of inflammatory elements (1) and change in shape of cartilage (2) (H-E x 100).*

proliferation response is observed in a transition zone between necrotic cells at the center of the laser-treated spot and the periphery of the cartilage specimen, where temperature elevations occur only via heat conduction. Similar to all these studies, we also have observed that perichondrium responds by proliferation to every kind of intervention where cartilage integrity is disturbed.

In our study, when compared with the control group, the cartilage alterations were found to be statistically significantly different for each intervention group ($p<0.05$). In all subjects, degenerative and regenerative changes were observed at various degrees (Figure 3). Perichondrium reaction accompanied all these changes. No thinning of cartilage or total loss was encountered in the car-rf group. There was an only degeneration-regeneration finding, which may suggest that perichondrium had fulfilled the renewal process. When the RF and laser groups were compared with respect to cartilage thinning and cartilage loss, no statistically significant difference was found. Based on these findings, it can be concluded that CO_2 laser and RF energy do not affect cartilage life significantly and they do not create much damage to lessen regeneration. This observation is consistent with the study of Holden et al.[26] that showed when rabbit cartilage is exposed to laser, an intact collagen matrix is left, which likely allows chondrocyte recovery on an intact scaffold. In this also *ex vivo* study, he concluded that the cellular response to laser irradiation was distinct from that observed in conventional wound healing. Besides, Protsenko et al.[27] showed that modification of mechanical properties of septal cartilage by laser heating can be mimicked by heating tissue samples with RF electric current with the added advantage of a uniform temperature profile. He recommended that generalization of this methodology may aid in optimizing clinical laser cartilage reshaping procedures.

In our study, thinning of cartilage was found in one and total loss in two rabbits in the Cottle group. This loss may be indirectly related that the renewal process of the organism remaining insufficient because of degeneration being wider or more severe. After the developmental age period, the damaged cartilage is renewed with difficulty and often insufficiently.[25] Although the subjects selected in our study were at developmental age, regeneration insufficiency might be related to extensive degeneration.

The finding of ossification in septum cartilage was seen only in the Cottle group and in four of five rabbits (Figure 4). In this group, there was progressive enchondral bone formation in cartilage tissue. A statistically significant difference from the other groups was found regarding ossification ($p<0.05$). This is in accordance with the study of Haberal et al.,[28] who reported that ossification in traumatized cartilage is evident and this may lead to thickening in cartilage and revision surgery in advance. The fact that no ossification was observed in laser or RF groups might suggest that both techniques create less histological damage than the Cottle's method.

Although the effect of all above-mentioned energy types on perichondrium and cartilage is evident, there is no consensus on either the amount of energy required to reshape the cartilage by laser

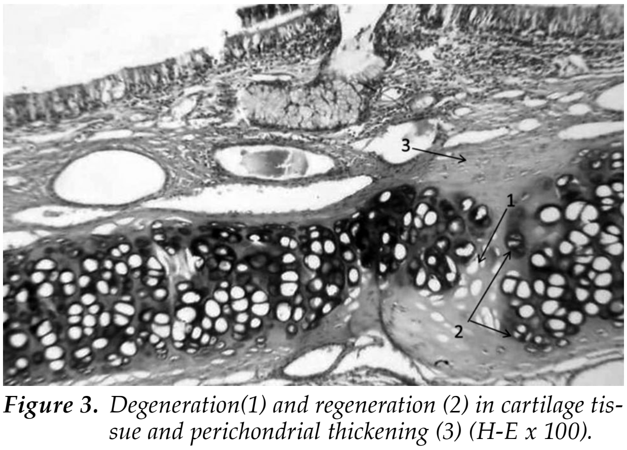

Figure 3. *Degeneration(1) and regeneration (2) in cartilage tissue and perichondrial thickening (3) (H-E x 100).*

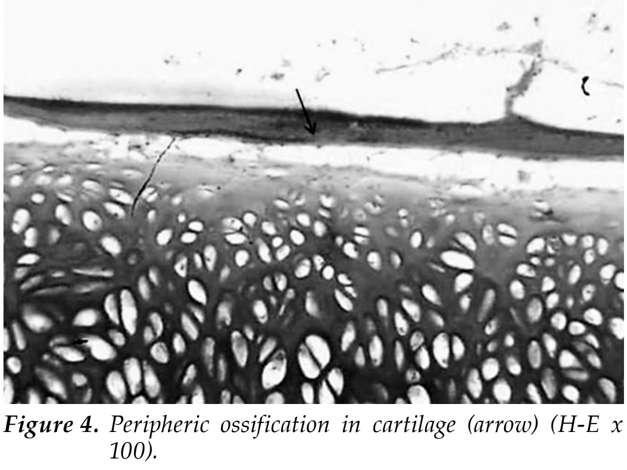

Figure 4. *Peripheric ossification in cartilage (arrow) (H-E x 100).*

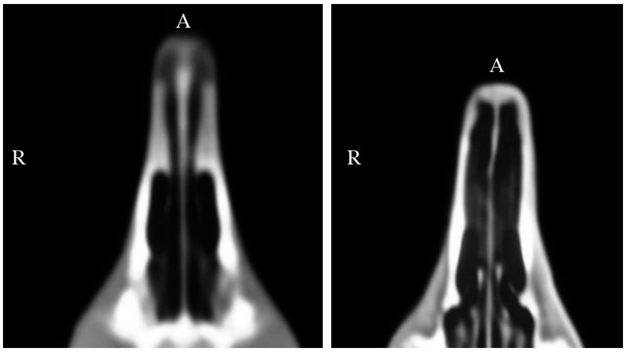

Figure 5. *Preoperative and postmortem computed tomography scans of a rabbit in car-lz group.*

or RF, nor on absolute amount of change in shape to predict when applied to human nasal septum. Although we have observed no ossification either after RF or laser application, and both methods cause cartilage degeneration which is repairable, in our opinion, preservation of epithelium, less inflammation in subepithelial tissues, and uniform temperature profile which can be adjusted based on tissue type are the issues which seem to render RF energy slightly superior to laser in nasal septal reshaping.

In our study, the radiological changes occurred in the septum of rabbits were evaluated by preoperative and postmortem paranasal sinus CT. While the preoperative and postmortem CT images could not be accurately overlapped and no difference could be angularly calculated, and the rabbits were in the growing process, the value of these statistically non-significant visual data is questionable (Figure 5).

In conclusion, it was observed that transmucosally or submucosally applied laser and RF energy did not lead to degeneration in nasal septal cartilage and surrounding tissues to an unrepairable extent and the cartilage vitality persisted after the application. Radiofrequency application seems to be somehow superior to the laser due to the fact that it does not cause any loss of epithelium but more cartilage degeneration even with transmucosal application.

Cottle's septoplasty is not an innocent method causing highest reaction. When laser and RF energies are compared with Cottle's method, they are alternatives that may be used in septoplasty in the future for pediatric septum surgery because they create less inflammatory response and do not lead to ossification in cartilage. However, the major reason restricting the use of both laser and RF in septoplasty is that the transformation cannot be predicted. Although it can be presumed that focal heating of septal tissues can prevent damage to growth centers of the pediatric septum, there is need for controlled in vivo studies which will focus on the objective measurement of transformations in the shape of cartilage created by laser and RF application, in order that these alternative techniques can be used safely in pediatric septal surgery.

Declaration of conflicting interests

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