

**CLINICAL RESEARCH**

**HISTOPATHOLOGICAL CHANGES AT THE SURGICAL SITE IN  
EXPERIMENTAL ANIMALS FOLLOWING PARTIAL CRICOID RESECTION  
AND THYROTACHEAL ANASTOMOSIS**

**E. MORERA<sup>1</sup>, H. ÁLVAREZ<sup>2</sup>, L. FONTES<sup>3</sup>, M. GOROSPE<sup>4</sup>, R. BERNÁLDEZ<sup>5</sup>, J. GAVILÁN<sup>5</sup>**

<sup>1</sup>MÉDICO ESPECIALISTA EN ORL. INSTITUTO ANTOLÍ-CANDELA.

<sup>2</sup>SERVICIO DE ORL, HOSPITAL INFANTIL DE MÉXICO.

<sup>3</sup>ESPECIALISTA EN ORL, HOSPITAL EDSON-RAMALHO, JOAO PESSOA, BRASIL.

<sup>4</sup>SERVICIO DE ANATOMÍA PATOLÓGICA, HOSPITAL UNIVERSITARIO LA PAZ. MADRID.

<sup>5</sup>SERVICIO DE ORL, HOSPITAL UNIVERSITARIO LA PAZ. MADRID.

**ABSTRACT**

**O**bjective: To study in an animal model the local healing process after partial cricoid resection with thyrotacheal anastomosis. *Material and methods:* Partial cricoid resection with thyrotacheal anastomosis was performed in 17 New Zealand rabbits. Experimental animals were gradually sacrificed and the laryngotracheal complex was removed to proceed to a histopathological study. *Results:* An acute inflammatory response followed by a chronic inflammatory process was found in the surgical site.

Normal tissue architecture was established after an eight week period. Anteroposterior and transverse diameters at all levels of the upper respiratory tract were not significantly altered at any time along the healing process. Experimental animals remained free from symptoms during the follow-up period. *Conclusions:* These results support that partial cricoid resection with thyrotacheal anastomosis is a safe procedure for the treatment of severe laryngotracheal stenosis.

**KEY WORDS:** Laryngeal stenosis. Partial cricoid resection. Upper respiratory tract.

**Correspondencia:** Eduardo Morera Serna. C/ Argensola 24, 4º izq. 28004 Madrid. E-mail: morera@hotmail.com

**Fecha de recepción:** 2-12-2003

**Fecha de aceptación:** 29-1-2004

## INTRODUCTION

Subglottic stenosis continues to be one of the most difficult pathologies to treat for otorhinolaryngologists and, more specifically for specialists of the upper respiratory tract<sup>1</sup>.

Prior to the introduction of endotracheal intubation and mechanical ventilation, the majority of cases were of idiopathic, congenital or traumatic origin. This panorama changed at the end of the sixties and beginning of the seventies with the starting of intensive care units and the widespread usage of prolonged orotracheal intubation with assisted ventilation as an alternative to tracheotomy; since then the most frequent cause of laryngotracheal stenosis has been the sequelae from long term intubation<sup>2,3</sup>.

Initial attempts to solve the problem were directed to increase the airway in the stenosed segment. In the fifties Rethi described a surgical procedure by incision of the cricoid lamina and the interposition of a graft of costal cartilage in such incision, in this way increasing the glottic and subglottic diameter<sup>4</sup>.

Cotton and Fearon subsequently contributed with the use of anterior grafts after cutting the cricoid ring and the first tracheal rings at the level of the stenosis<sup>5</sup>, since then, and with small modifications, numerous surgical techniques to expand the diameter have emerged<sup>6-14</sup>.

In parallel with the development of expansion techniques other authors have described new procedures based on the surgical resection of the affected area by means of end to end anastomosis<sup>15</sup>. In 1974 Pearson published a technique consisting of an oblique partial cricoid resection respecting recurrent nerves and the posterior thyrotracheal union, which standardized this procedure rationally and produced excellent results<sup>16-17</sup>. These techniques of resection and end to end anastomosis were not applied in paediatric medicine until the nineties when Monnier improved the procedure and used it to treat children with severe subglottic stenosis, achieving the same results that in adults<sup>18</sup>. The application of this procedure has since become widespread, as well as have its successes<sup>19,20</sup>.

The histopathological processes that take place in the adaptation of cartilage grafts in laryngotracheal reconstruction<sup>21-26</sup> have been widely studied. There are, however, few studies to investigate the sequence of events that happen during the healing of the surgical

thyrotracheal reconstruction in resection and termino-anastomosis<sup>27-29</sup>.

The objective of this study is to describe the processes of histopathological adaptation at the surgical site during healing and the alterations in the calibre of the lumen in laboratory animals which have undergone partial cricoid resection and thyrotracheal anastomosis or Pearson's laryngotracheoplastia.

## MATERIAL AND METHODS

This study was carried out in the Otorhinolaryngology and Cervico-facial Pathology and Experimental Surgery departments of the University Hospital La Paz between the months of March 1999 and February 2000. We designed an experimental, longitudinal and prospective study in which partial cricoid resection and thyrotracheal anastomosis was performed on 15 adult white New Zealand rabbits of 4-4.5 kg in weight. The animals were divided into five groups of three each, and sacrificed at 0, 1, 2, 4 and 8 weeks. Two more rabbits were used for control purposes. When sacrificed the larynx and the upper tracheal area of the animals was removed for further histopathological examination.

### Procedure

**Anaesthesia:** Atropine (0.05 mg/kg), diazepam (2 mg/kg) and Ketamine (20 mg/kg) were introduced intravenously to induce unconsciousness. A mixture of oxygen and sevoflurane at 3% was used to keep the animals anaesthetized during the surgical procedure. The animal's respiratory automatism was at first maintained spontaneously by means of a facial mask and later by means of a tracheostomal cannula. A dose of dexamethasone (1 mg/kg) was administered to all animals as an anti-inflammatory, a dose of buprenorphine (10 mcg/kg) as an analgesic and a dose of cefazoline (30mg/kg) as a primary prophylaxis to prevent surgical infection. The rabbit was monitored throughout the surgery by means of patches which registered electrocardiographical signals, a rectal thermometer and a pulsometer placed on the tail. Throughout the procedure a line was maintained in one of the dorsal veins of the ear lobe through which mixtures of serum solutions were passed.



**Figure 1. Larynx and trachea exposition.**

**Surgical technique:** Once the animal was asleep the cervical area was shaved. A horizontal incision of six centimetres in diameter was made at the level of the cricothyroid membrane. The upper and lower flaps were lifted at a subplatysmal level to the edge of the upper thyroid, cranially, and the sternum caudally. The tissue in the midline, the prelaryngeal fatt and the infrahyoid muscles were cut longitudinally along the midline; the thyroid gland was divided at istmus and pulled back laterally thus exposing the larynx and the cervical portion of the trachea (Figure 1).

The cricothyroid muscle was divided along the midline and dissected together with the external perichondrium of the cricoid ring. Both were retracted laterally to the level of the cricothyroid joint, thus respecting the recurrent laryngeal nerves, the identification of which was not attempted. Following these, a tracheotomy was performed by a horizontal incision at the level of the fifth intercartilaginous membrane, a cannula of 2.5 cm in diameter was then set in place to allow continuous anaesthesia.

Resection of the cricoid ring was then performed using the cricothyroid joint as lateral limits avoiding entering trachea (figure 2). Then, an incision was made into the cricothyroid membrane from the lower edge of the thyrioid



**Figure 2. Resection of the cricoid ring.**

cartilage and resection of the first two tracheal rings was carried out (figure 3).

Closing was then carried out. Three posterior sutures with Vicryl 5-0 were done followed by five lateral ones with the same material, thus effectuating an adequate closing of the airway (figure 4); it was not necessary to use any techniques of liberation of laryngotracheal tension. Once the airway had been closed ventilation and anaesthesia were reiniciated by means of a facial mask, and then the tracheotomy was closed with the same Vicryl 5-0. The animal was then sutured in layers using absorbable suture so that it would not be necessary to remove stitches from a conscious animal a few days later.

**Post-operative care:** The animals remained in a paediatric incubator for the first 12-24 hours after the operation with environmental oxygen at 40%. They received antibiotic and analgesia twelve hours after the operation. After this period they were put into special individual cages in which they remained prior to being sacrificed.

**Sacrifice:** The rabbits were first sedated with halothane using a facial mask and then humanely sacrificed with an intravenous overdose of sodic pentobarbital. After the sacrifice a complete laryngectomy was performed for histological study.



**Figure 3. Cricotracheal resection.**



**Figure 4. Tirotracheal suture.**

### **Histopathological study**

Immediately after sacrifice, the surgical pieces were taken to the Pathology Department for their preparation. They were fixed with phormol at 10% and preserved in paraffin. Serial cuts were performed at the glottic, supraglottic and subglottic levels, and the longitudinal and transverse diameters were measured at each of these levels. The cuts were stained with hematoxylin-eosin and viewed under an optical microscope, examining the degree of inflammation in the mucosa and submucosa, fibrosis, chondrogenesis, foreign body reaction and epithelial metaplasia at the different levels.

## **RESULTS**

### **Histopathological changes**

The laryngeal cuts of the rabbits sacrificed immediately, and one week, after surgery showed an acute inflammatory reaction with foci of submucosal hemorrhage and areas of detachment from respiratory mucosa (figure 5). Two weeks after surgery we could see persistency of the inflammatory infiltration was

seen, albeit less exuberant, with oedema of the lamina propia and foci of foreign body reaction to the suture material (figure 6).

The histopathological findings at four weeks after surgery showed progressive reduction in the inflammatory reaction with persistence, in some pieces, of areas of epithelial detachment; a foreign body reaction around the stitches was still in evidence.

At eight weeks we were able to discern a reestablishment of the histopathological architecture of the airway, with focal areas of fibrosis and foreign body reaction but with epithelial continuity reestablished (figure 7). No changes in the cartilage were evident in any of the pieces studied.

### **Measurement of the calibre of the airway**

The average measurement of the calibre of the trachea of the rabbits in each group sacrificed was calculated, measuring the longitudinal and transverse diameters at the glottic, supraglottic and subglottic levels. The measurements obtained are shown in Table 1. There were no significant differences in the diameters measured at the different levels; it is



**Figure 5. Submucosal hemorrhage and epithelial detachment.**

only worth noting that there was a small reduction in the anteroposterior diameter at the subglottic level until the fourth week after intervention, a decrease attributable to the inflammatory reaction and the submucosal haemorrhage. After this period of time the diameter of the airway of the animals which had been operated on was equal to that of the control ones.

## DISCUSSION

Laryngotracheal stenosis continues to represent a challenge to airway specialists. There is a widespread opinion that there is no one procedure that can be used to treat all cases and that the surgeon, on choosing a course of treatment, must take into account, in each patient, several factors such as the type of stenosis (congenital or acquired) its localization (supraglottic, subglottic, glottic, tracheal or combined) its grade of obstruction and its length,



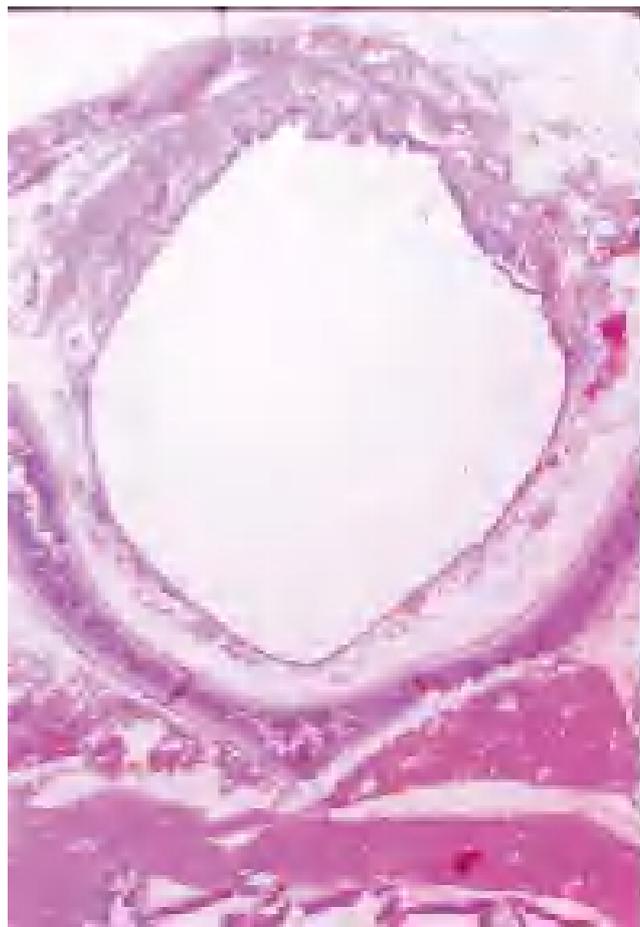
**Figure 6. Chronic inflammation and oedema of the lamina propria.**

and finally its association with defects of the motility of the vocal chords<sup>18</sup>.

Severe subglottic stenoses, grades III and IV in the Cotton classification<sup>30</sup>, have been treated by laser resection, techniques of laryngotracheoplastia and resections of the stenosed area with end to end anastomosis.

The use of laser has shown a certain efficacy only with thin stenoses, but not circumferential stenoses nor those greater than 1cm in length or higher than grade I in the Cotton classification. Some studies extend the application of endoscopic laser resection to higher grade stenoses<sup>31</sup> or even to circumferential stenoses<sup>32</sup> but the results in these cases are not outstanding and a high percentage of patients need further surgical procedures. Even advocates of laser treatment speak of thermic tissular damage, the risk of chondritis and exuberant granulation, and frequent restenosis after treatment<sup>31</sup>.

Laryngotracheoplastia techniques have as a common denominator the incision in the middle



**Figure 7. Reestablishment of the airway and of the mucose continuity.**

anterior laryngotracheal line with the interposition of an anterior or posterior graft.

This implies two problems. The first is the risk of a secondary collapse of the airway after the surgical resection in the case of graft displacement. The second is the slow integration of the graft in the laryngeal skeleton. The graft, usually cartilaginous, remains exposed to the airway, without epithelial cover with the consequent risk of bacterial infection, granulation tissue formation and restenosis<sup>18</sup>.

Various studies have been carried out on animals to evaluate the histopathological changes of the graft in the receptor bed<sup>24,26,33</sup>. However, these studies all share the same defect in that they used healthy animals which did not suffer from problems of the upper airway. For this reason the information regarding scarring and the integration of the graft is not wholly applicable to patients with laryngotracheal stenosis and pathological scar tissue in that area.

**Table 1. Result of the of the measure of the longitudinal and transversal diameters of the airway (average arithmetic of the rabbits from each group)**

	Glottic area	Supraglottic area	Subglottic area
Controls	8mm x 2mm	7mm x 4mm	6mm x 4mm
0 weeks	7mm x 2mm	6mm x 4mm	5mm x 4.5mm
1 week	9mm x 2mm	6.5mm x 4mm	5mm x 4mm
2 weeks	8mm x 2mm	7mm x 3mm	5mm x 4mm
4 weeks	8mm x 2mm	8mm x 3mm	6mm x 4mm
8 weeks	8mm x 2.5mm	7mm x 4mm	6mm x 3.5mm

In contrast to the many studies about the feasibility of using grafts in expansion techniques, the techniques of partial cricoid resection and thyrotracheal anastomosis have barely been the subject of investigation. In 1985, Fearon and McMillan carried out an experimental study on three nine-month old Rhesus monkeys. One of Pearson's techniques was performed on the monkeys who were then sacrificed 18 weeks after the operation and the laryngotracheal changes were analysed histologically<sup>27</sup>. The authors concluded that partial cricoid resection with laryngotracheal anastomosis did not interfere with normal growth of the larynx and the trachea, despite the fact that one of the monkeys developed exuberant granulation tissue and needed to be sacrificed ahead of schedule. This study shows various defects which the authors themselves confess to, such as the low number of animals used (three), the absence of a control group and the simultaneous sacrifice of all the animals, which prevented the observation of a sequence of the histological phenomena that takes place at the surgical site<sup>29</sup>.

Ward and Triglia recently evaluated the effect of said procedure on the growth of the airway in 29 rabbits between 8 and 11 weeks old. The animals underwent cricothyroid resection with thyrotracheal anastomosis and were sacrificed four months later. The results of this study do not show any differences in the diameter of the air way when comparing those that had been operated on and with the control group. However, the absence of staggered sacrifice prevents us from observing a

sequence of the histological events that take place at the surgical site.

The methodology employed in this study complements previous investigations. Unlike other studies the animals were sacrificed at different intervals with a view to observing a sequence of events at a histological level at the site of the anastomosis, which allowed us to study the changes that had not been previously reported. The results of this study, unlike those carried out with laryngotracheal expansion and cartilaginous graft techniques can be applied to real clinical situations. The procedure involves the resection of the affected area and the anastomosis between two ends of healthy and epithelized tissue, so that there are no great differences between patients with stenosis and healthy animals. We chose rabbits for the similarity in calibre of their airway to those of children.

The results of the experiment show us, above all, the conservation of the normal dimensions of the airway, estimated from the longitudinal and transversal diameters at the glottic, subglottic and supraglottic levels of the rabbits that were operated on. This resulted in the absence of clinical symptomatology during the immediate and long-term post-operative period. No case needed to maintain the tracheotomy.

The histological findings reflect an initial inflammatory reaction that progressively lessened until it disappeared by the eighth week, leaving only localized foci of fibrosis and foreign body reaction around some of the stitches. It is possible that the eventual absorption of the stitches goes hand in hand with the disappearance of the inflammatory reaction; it is important to note that at no time did the phenomena occurring at the thyrotracheal site provoke a significant reduction of the tracheal diameter.

Our hypothesis is that the continuity of the epithelial covering in the surgical site, as well as the solidity of the cartilaginous skeleton formed previously by the thyrotracheal anastomosis and

subsequently by the cricoid seal are the factors which determine the excellent results of partial cricoid resection with thyrotracheal anastomosis. The complete resection of the diseased tissue and the reconstruction performed exclusively with healthy airway, unlike expansion techniques, favour a more efficient scar formation process. This also leads to a moderate, self-limited inflammatory reaction and allows for early decannulation in experiment animals.

In view of the results obtained from this study and comparing them with the series of patients, both adults and children, who have also undergone this procedure, we can conclude that partial cricoid resection with thyrotracheal anastomosis is a safe technique for the treatment of severe subglottic stenosis. However, we would recommend more prospective studies to be carried out, with a greater number of animals in each group and the introduction of variables such as endoscopic evaluation of the post-operative laryngeal mobility.

## CONCLUSIONS

- Partial cricoid resection with thyrotracheal anastomosis is a safe technique for the treatment of subglottic stenosis.
- Post-operative inflammatory reaction at the surgical site is only mild or moderate in degree, possibly due to the covering of respiratory mucosa of the whole anastomotic area. It is also self-limited with an approximate duration of eight weeks.
- The dimensions of the airway are not significantly affected by this procedure. Thyrotracheal anastomosis provides an adequate solidity to the cartilaginous skeleton at the area of the surgical union.
- Post-operative clinical symptomatology is rare, this results in the absence of dyspnea in the post-operative period and allows for early decannulation of the experimental animals.

## REFERENCES

- 1.- Gavilan J, Cerdeira MA, Toledano A. Surgical treatment of laryngotracheal stenosis: a review of 60 cases. *Ann Otol Rhinol Laryngol* 1998; 107: 588-592.
- 2.- Fearon B. Laryngeal problems in children. *Ann Otol Rhinol Laryngol* 1987; 96: 124-126.
- 3.- Kenna MA, Reilly JS, Stool SE. Tracheotomy in the preterm infant. *Ann Otol Rhinol Laryngol* 1997; 96: 68-71.
- 4.- Rethi A. An operation for cicatricial stenosis of the larynx.

**E. MORERA ET AL. HISTOPATHOLOGICAL CHANGES AT THE SURGICAL SITE**

- J Laryngol Otol 1956; 70: 283-293.
- 5.-** Fearon B, Cotton R. Surgical correction of subglottic stenosis of the larynx. Preliminary report of an experimental surgical technique. *Ann Otol Rhinol Laryngol* 1972; 81: 508-513.
- 6.-** Cotton RT, Gray SD, Miller RP. Update of the Cincinnati Experience in pediatric laryngotracheal reconstruction. *Laryngoscope* 1989; 99: 1111-1116.
- 7.-** Lusk RP, Gray S, Muntz HR. Single - stage laryngotracheal reconstruction. *Arch Otolaryngol Head Neck Surg* 1991; 117: 171-173.
- 8.-** Seid AB, Pransky SM, Kearns DB. One-stage laryngotracheoplasty. *Arch Otolaryngol Head Neck Surg* 1991; 117: 408-410.
- 9.-** Zalzal GH. Treatment of laryngotracheal stenosis with anterior and posterior cartilage grafts. *Arch Otolaryngol Head Neck Surg* 1993; 119: 82-86.
- 10.-** Lusk RP, Kang DR, Muntz HR. Auricular cartilage grafts in laryngotracheal reconstruction. *Ann Otol Rhinol Laryngol* 1993; 102: 247-254.
- 11.-** Cotton RT, Myer III CM, O'Connor DM, Smith ME. Pediatric laryngotracheal reconstruction with cartilage grafts and endotracheal tube stenting: the single- stage approach. *Laryngoscope* 1995; 105: 818-821.
- 12.-** Weisberger EC, Nguyen CT. Laryngotracheal reconstruction using a titanium alloy miniplate. *Ann Otol Rhinol Laryngol* 1996; 105: 363-366.
- 13.-** Mc Guirt WF, Little JP, Healy GB. Anterior cricoid split. Use of hyoid as autologous grafting material. *Arch Otolaryngol Head Neck Surg* 1997; 123: 1277-1280.
- 14.-** Indiyae I, Van Den Abbeele T, François M, Viala P, Tanon-Anonh, Narcy P. Traitement chirurgical des sténoses laryngées de l'enfant. *Ann Otolaryngol Chir Cervicofac* 1999; 116: 143-148.
- 15.-** Gerwat J, Bryce DP. The management of subglottic laryngeal stenosis by resection and direct anastomosis. *Laryngoscope* 1974; 84: 940-945.
- 16.-** Pearson FG, Cooper JD, Nelems JM, van Nostrand AWP. Primary tracheal anastomosis after resection of the cricoid cartilage with preservation of recurrent laryngeal nerves. *J Thorac Cardiovasc Surg* 1975; 70: 806-816.
- 17.-** Pearson FG, Brito-Filomeno L, Cooper JD. Experience with partial cricoid resection and thyrotacheal anastomosis. *Ann Otol Rhinol Laryngol* 1986; 95: 582-585.
- 18.-** Monnier P, Savary M, Chapuis G. Partial cricoid resection with primary tracheal anastomosis for subglottic stenosis in infants and children. *Laryngoscope* 1993; 103: 1273-1283.
- 19.-** Stern Y, Gerber ME, Walner DL, Cotton RT. Partial cricotracheal resection with primary anastomosis in the pediatric age group. *Ann Otol Rhinol Laryngol* 1997; 106: 891-896.
- 20.-** Monnier P, Lang F, Savary M. Partial cricotracheal resection for severe pediatric subglottic stenosis-update of the Lausanne experience. *Ann Otol Rhinol Laryngol* 1998; 107: 961-968.
- 21.-** Pashley NR, Jaskunas JM, Waldestein G. Laryngotracheoplasty with costo-chondral grafts. A clinical correlate of graft survival. *Laryngoscope* 1984; 94: 1493-1496.
- 22.-** Zalzal GH, Cotton RT, Mc Adams AJ. The survival of the costal cartilage graft in laryngotracheal reconstruction. *Otolaryngol Head Neck Surg* 1986; 94: 204-211.
- 23.-** Cotton RT. The problem of pediatric laryngotracheal stenosis: a clinical and experimental study on the efficacy of autogenous cartilage grafts placed between the vertically divided halves of the posterior lamina of the cricoid cartilage. *Laryngoscope* 1991; 101: 101 (suppl 56).
- 24.-** Wiatrak BJ, Albert DM, Holmes DK, Cotton RT. Cartilage graft epithelization: A preliminary study using a goat model. *Arch Otolaryngol Head Neck Surg* 1993; 119: 777-781.
- 25.-** Park HA, Forte V. Effect of harvesting autogenous laryngeal cartilage for laryngotracheal reconstruction on laryngeal growth and support. *Laryngoscope* 1999; 109: 307-311.
- 26.-** Jacobs IN, Podrebarac P, Boden SD, Chen M. Graft healing in laryngotracheal reconstruction: an experimental rabbit model. *Ann Otol Rhinol Laryngol* 1999; 108(6): 599-605.
- 27.-** Fearon B, McMillin BD. Cricoid resection and thyrotacheal anastomosis in the growing primate. *Ann Otol Rhinol Laryngol* 1985; 94: 631-633.
- 28.-** Sullivan MJ, McClatchey KD, Passamani PP. Airway growth following cricotracheal resections in puppies. *Arch Otolaryngol Head Neck Surg* 1987; 113: 606-611.
- 29.-** Ward RF, Triglia JM. Airway growth after cricotracheal resection in a rabbit model and clinical application to the treatment of subglottic stenosis in children. *Laryngoscope* 2000; 110: 835-844.
- 30.-** Cotton RT. Pediatric laryngotracheal stenosis. *J Pediatr Surg*, 1984; 699-704.
- 31.-** Bagwell ChE. CO2 laser excision of pediatric airway lesions. *J Pediatr Surg* 1990; 25: 1152-56.
- 32.-** Werkhaven JA, Weed DT, Ossoff RH. Carbon dioxide laser serial microtrapdoor flap excision of subglottic stenosis. *Arch Otolaryngol Head Neck Surg* 1993; 119: 676-79.
- 33.-** Lofgren L, Lindholm CE, Jansson B. Experimental reconstruction of the airway with buccal mucosa and cortical bone in a single-stage procedure. *Acta Otolaryngol* 1984; 98: 560-72.