The free neural grafting for recurrent nerve laceration

Experimental study in rabbit

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AIM: *The most dreaded complication of thyroidectomy is recurrent laryngeal nerve damage, which is most of the time hardly irreversible. In our experimental study we researched the use of free nerve grafts in the treatment of laryngeal nerve damage in rabbit.*
MATERIAL AND METHODS: *There were three groups in our study. In the first group, the recurrent laryngeal nerve was severed and then a free nerve graft was interposed between the phrenic nerve and distal end of recurrent laryngeal nerve. In the second group, a defect in the continuity of the laryngeal nerve was created. The two ends of the nerve were joined together later by an interposed free nerve graft. In the third group, only a defect in the recurrent nerve was created without any attempt at uniting the ends together so that these latter subjects could be assigned as control group. In the evaluation process we performed laryngeal endoscopy, laryngeal EMG and histopathologic examination.*
RESULTS: *On the 21. day of trial, in the first and second group vocal cord movements were detected on the laryngoscopy along with regeneration waves on EMG. In the third group there was no vocal cord movements on the side where a neural damage was created intentionally. On EMG there was degeneration waves as opposed to regeneration waves seen in the first and second groups. Histopathologic findings were similar.*
CONCLUSIONS: *Recurrent laryngeal nerve paralysis is an unwanted complication because it causes permenant sequela. Studies which intend to find a cure for this complication are increasing in number. We aim to find new approaches to cure patients suffering from this devastating complication as well. In our exprerimental study, vocal cord movements were reproduced without causing diaphragmatic paralysis. We believe the results of our study promise to relieve the suffering of patients. The results are encouraging.*

KEY WORDS: Muscle, Rat model, Reinnervation, Surgery

Introduction

Thyroidectomy is one of the most common operations performed by general surgeons. Various complications might be encountered following thyroidectomy. These complications are worrysome for both patients and surgeons. Of these complications some might be permanent while others might be temporary. Sometimes reintervention is inevitable to alleviate symptoms caused by some of these complications.

Recurrent laryngeal nerve paralysis is one of the complications. The frequency of temporary and permanent recurrent nerve paralysis is 5,2% and 1,4% respectively. These complication rates increase even more in the oper-

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ations performed for malignancy and recurrent substernal thyroidectomy. Nerve damage could be unilateral or bilateral. This kind of nerve damage could cause a variety of complications ranging from hoarseness to airway obstruction which needs tracheostomy [1-3].
There are a variety of methods to repair nerve damage. In our research, the topic of recurrent laryngeal nerve defect repairing with free nerve graft was studied, showing that there were signs of healing by videoendoscopy, EMG and histopathologic examination.

Material and Method

The permission for our experimental study was given by Ethical Committee of Ege University. The study was conducted in Experimental Surgery Deparment of Ege University. The animals used in this experiment were New Zealand white rabbits because anatomy of these animals was the most suitable for the experiment. Regarding ethical issues, the ethical commitee allowed us to use minimum number of animals, which was eight rabbits for each group.
The operation was performed under general anaesthesia(ketamine 90 mg/kg and Xsilasine 3mg/kg). After preoperative surgical field preparation, a Kocher collar incision was placed. The subcutanous tissue and muscle layers were dissected. Pretracheal muscles were retracted laterally gaining exposure to the right recurrent laryngeal nerve. The study was designed to involve three groups. In each group, the right recurrent laryngeal nerve was severed at the point that is one centimeter proximal to where it enters the larynx and approximately one- centimeter –long segment of the nerve was removed. The reason for removal of a segment of the nerve is to prevent spontenous healing of the severed nerve because such a process could invalidate the research results. The free nerve grafts were prepared from sciatic nerve because of its thickness. The nerve anastomosis was made by using 10/0 polypropylen suture.
First group: Right recurrent laryngeal nerve and right phrenic nerve were identified and suspended by silk sutures (Fig. 1)
The lateral wall of phrenic nerve and the free nerve graft (Fig. 2) were anastomosed together in an end-to-side configuration (Fig. 3).

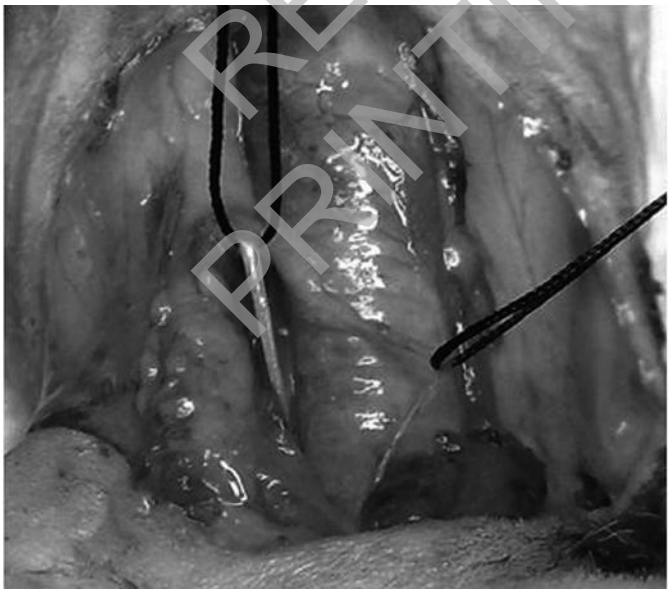

Fig. 1: Recurrent laryngeal nerve and phrenic nerve.

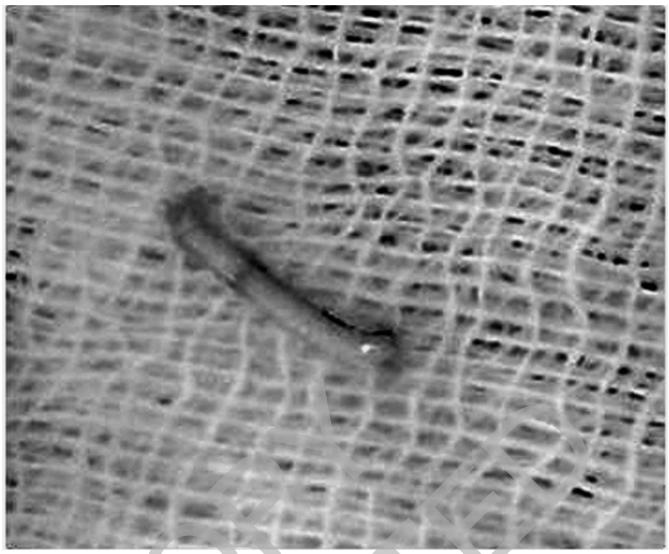

Fig. 2: Free nerve graft prepared from sciatic nerve branches.

Fig. 3: End-to-side anastomosis between lateral wall of the phrenic nerve and free nerve graft.

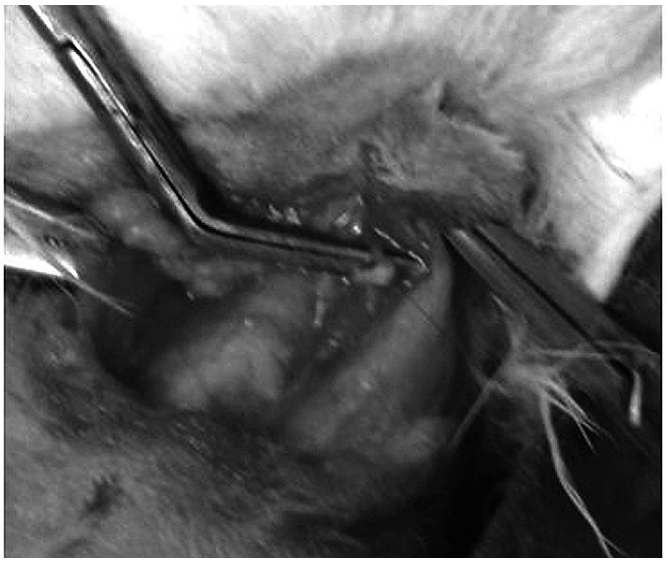

Fig. 4: The complete end-to-end anastomosis between the end of distal segment of recurrent laryngeal nerve end.

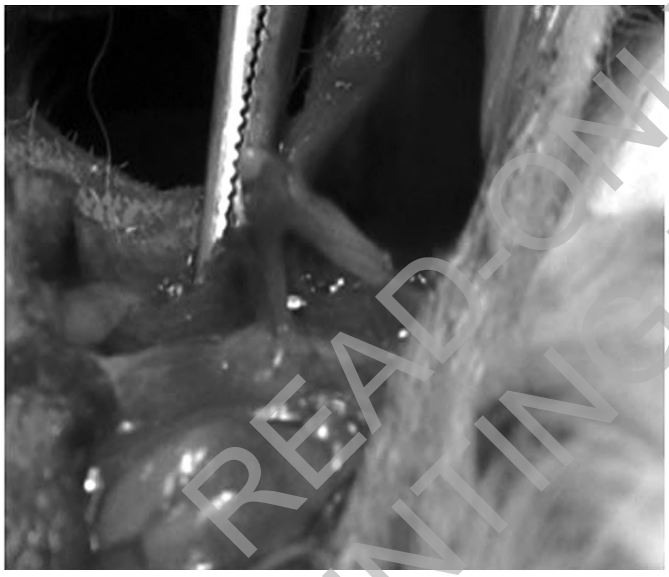

Fig. 5: The complete anastomosis in end-to –end configuration between two ends of severed recurrent laryngeal nerve.

The epineurium layer of phrenic nerve might jeopardize the success of anastomosis; therefore, it was eroded by the help of scalpel at the anastomosis site allowing exposure of nerve fibers. An end-to-end anastomosis was created between the other end of free nerve graft and distal end of recurrent laryngeal nerve entering the larynx (Fig. 4). The sciatic nerves were used as donor site for nerve grafts because the size of research animals was small.

Second group: A one-centimeter-long defect in the recurrent laryngeal nerve was created and then, free nerve graft was implanted by the help of end –to-end anastomosis between two ends of severed recurrent laryngeal nerve (Fig. 5).

Third group: Again, in the same manner as other two groups, a defect in the recurrent laryngeal nerve was created in this group, too. No anastomosis was created so that this last group could be used as control group.

In the postoperative period, controls were performed by a general surgeon, ENT specialist, neurologist and pathologist. Antibiotic treatment was given untill third postoperative day.

General surgery examination: Rabbits were closely monitored from the day of operation to the day of sacrification.

Videoendoscopic examination: Baseline videoendoscopic evaluation was performed preoperatively and then on the first postoperative day. On the third postoperative week, rabbits were examined by laryngoscopy to evaluate the research results.

EMG examinations: On the 21st postoperative day, EMG examinations were performed by the help of EMG Medelec Sapphire 4 ME device using concentric intramuscular neddle.

All videoendoscopic laryngoscopy and intramuscular EMG studies were conducted under general anesthesia (ketamine 90 mg/kg).

Fig. 6: Macroscopic view of larynx removed from sacrificed rabbit.

Histopathological examinations: After EMG study, the larynxes of sacrified rabbits were removed and kept in formaline solution to be examined later in the pathology department. For histological examinations, larynx was dissected by a posterior approach, divided into right hemilarynx, left hemilarynx, supraglottic and infraglottic larynx. All specimens were examined. After routine follow up of formaline fixated tissues, paraphine slices were examined under light microscope and the features of muscle tissues were noted.

Results

In our experimental study there were three different groups as described above.

General surgery examinations: During postoperative period, surgical wounds were checked daily. All wounds seemed to be unproblematic. On the twenty first postoperative day rabbits looked healthy. They were in no apparent breathing stress in group one and two. There was no wound infection.

Videoendoscopic laryngoscopy examinations: preoperatively all rabbits were examined by videoendoscopic laryngoscopy. Both right and left vocal cord movements were present in all rabbits.

On the first postoperative day all rabbits were evaluated by videoendoscopic laryngoscopy. left vocal cord movements were present in all rabbits. right vocal cord movements on the other hand, were absent.

On the twenty first postoperative day, videoendoscopic vocal cord examinations were performed again. In the first and second group, left vocal cord movements were present, right vocal cord movements were partial. In the third group left vocal cord movements were present, however, right vocal cord movements were absent.

Electromyography examination: The results of EMG examinations which were performed on the 21. postoperative day are given below.

In the first group left vocal cord was normal. Right vocal cord showed regeneration waves which is considered to be a sign of healing (Fig. 7). Right hemidiaphragm was normal on EMG (Fig. 8). Laparatomy was performed and diaphragm was directly observed. Diaphragm was functioning normal (Fig. 9)and there was no sign of damage to phrenic nerve.

In the second group, on EMG examination, left vocal cords were found to be normal and right vocal cords showed signs of regeneration (Fig. 10).

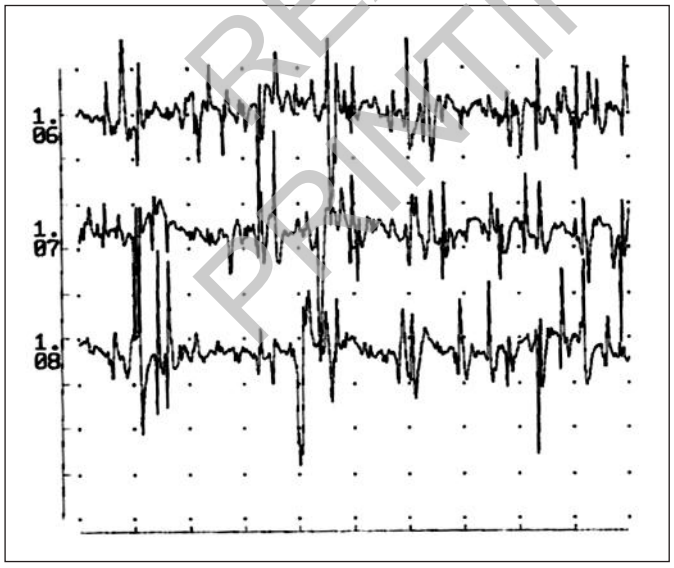

Fig. 7: First group. Regeneration waves can be observed in right cricoarytenoid muscle.

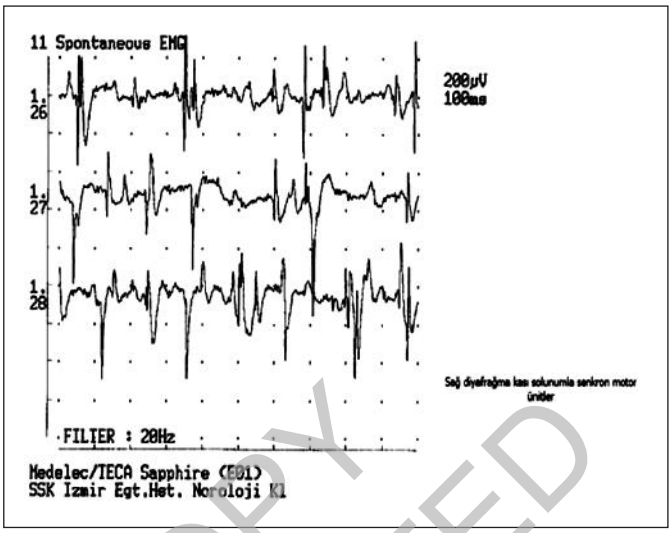

Fig. 8: Motor units were seen in right diaphragm muscle.

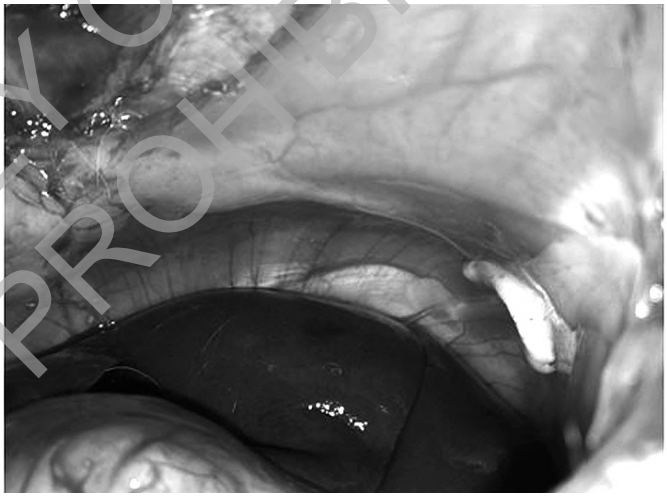

Fig. 9: In the first group, the right diaphragm of the rabbits which were subjected to phrenic recurrent nerve transposition by free nerve grafts showed normal function. The view of right diaphragm during contraction. There was no diaphragm paralysis.

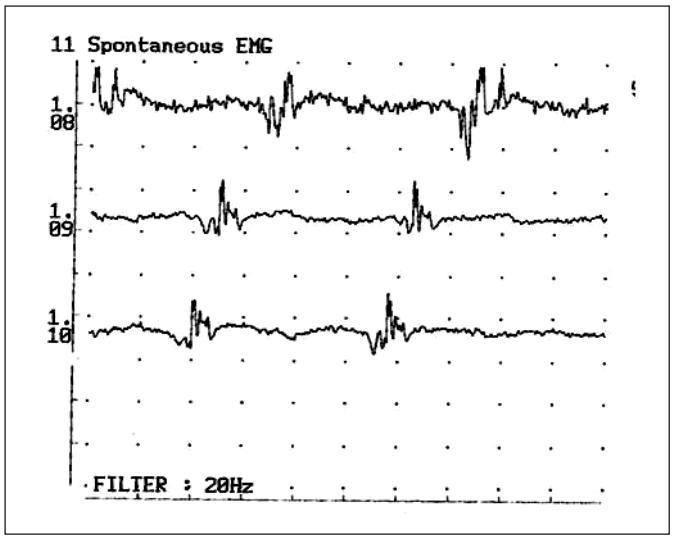

Fig. 10: Right cricoarytenoid muscle regeneration potentials in the second group.

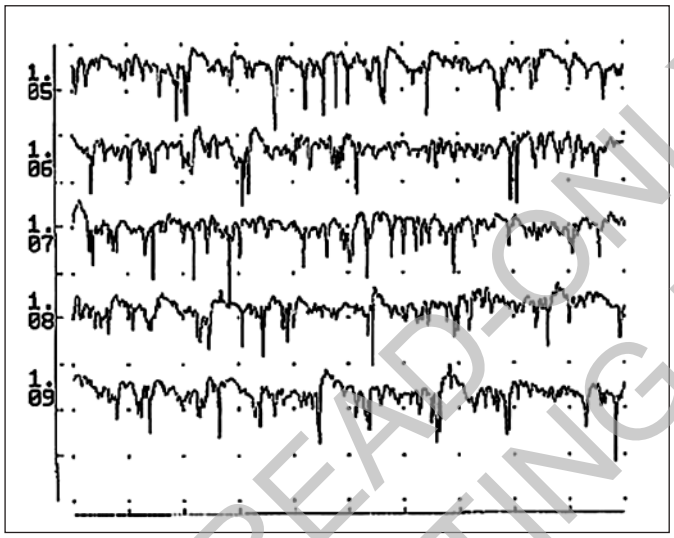

Fig. 11:Total denervation of right vocal cord in the control group.

In the third group, left vocal cords were normal, right vocal cords showed extensive degeneration waves and there was no regeneration waves observed on EMG examination(Fig. 11).

Histopathologic examination: On histological examination under light microscopy the first and second groups showed no sign of atrophy of vocal cord muscles (Figs. 12, 13). In the third group, on the other hand, atrophy in the right vocal cord was observed (Fig. 14).

Conclusions

In the larynx there are two groups of muscles. Extrinsic muscles are responsible for all movements of larynx.

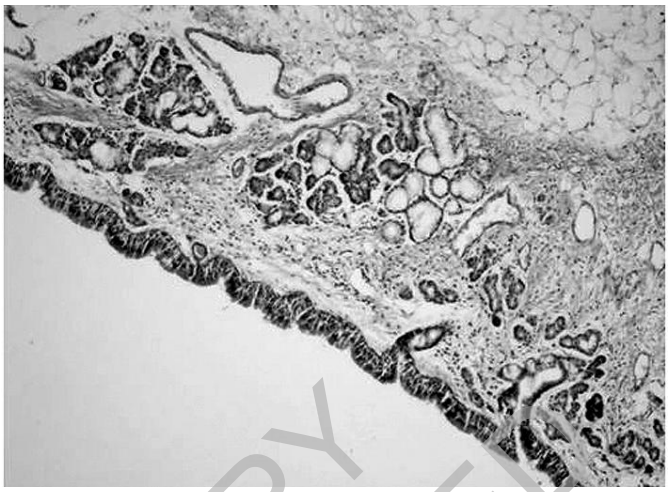

Fig. 12:.In the first group, no atrophy was seen.

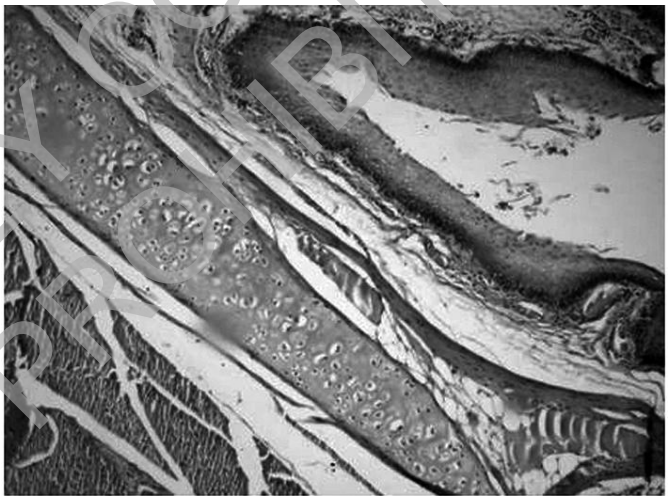

Fig. 13: A sample from second group.

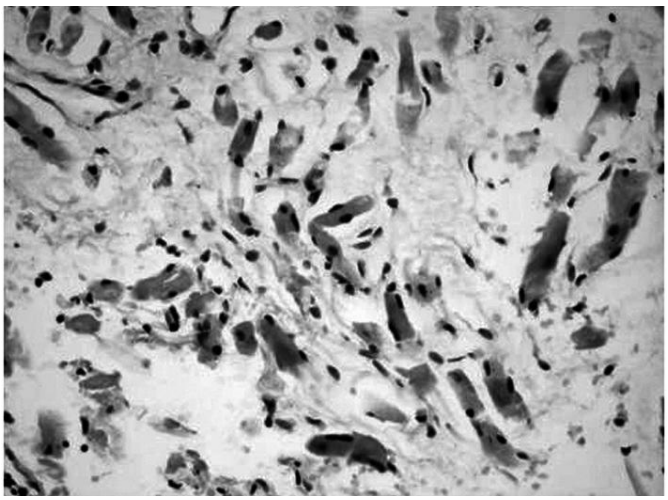

Fig. 14: Edema, inflammation and findings of degeneration can be observed among the muscle fibers. HEx400.

Vocal cord movements are carried out by intrinsic muscles. Extrinsic muscles are also called as strap muscles. Some of the extrinsic muscles are elevators while others are deppressors. The intrinsic muscles of larynx are the cricothyroid, posterior cricoarytenoid, lateral cricoarytenoid, thyroarytenoid and arytenoid with its transverse and oblique fibers. Posterior cricoarytenoid muscle is the only true abductor muscle of vocal cords. Recurrent laryngeal nerve innervates all the intrinsic muscles except for the cricothyroid muscle [4-6].

Thyroidectomy can be performed either unilaterally or bilaterally. The whole gland can be removed or it can be removed partially as performed in subtotal thyroidectomy. Complications regarding recurrent laryngeal nerve can be encountered after total or subtotal thyroidectomy. Recurrent laryngeal nerve damage might cause dyspnea and stridor and for the patients who have prominent airway obstruction, trachoestomy might be inevitable [7,8].

In our study problems due to recurrent laryngeal nerve laceration were investigated. The ideal treatment option for recurrent nerve lacerations is end-to-end primary saturation [9]. This type of anastomosis is the most succesful of all anastomosis varieties [10]. However, what if there is a huge defect between the two ends of the nerve which precludes primary anastomosis? Or what to do if only the distal end of the severed nerve that enters the larynx could be identified and the proximal end is missing? Our experimental study aims to solve these problems.

In the past, many experiments regarding recurrent laryngeal nerve damage were conducted. However, only primary suturation was generally accepted. We are only giving a list of articles [11-18].

Since we have used free nerve graft in our study, we would like to give a little information about the subject. Neuronal extensions are bundled together into groups known as fascicles. Several fascicles may be in turn bundled together to form peripheral nerve fibers. There are three different sheaths in a peripheral nerve fiber. In other words, each nerve is covered by three layers, the inner layer is called endoneurium; the middle layer is called perineurium and the outer layer is called epineurium. Epineurium is the outermost connective tissue layer. Perineurium is the sheath which surrounds fascicles. Endoneurium consists of a thin layer of connective tissue which is dispersed throughout the interstitial tissue within the fascicle. Re-vascularisation of the nerve graft starts on the third postoperative day. If the diameter of free nerve graft exceeds 5 mm, revascularisation of it could be difficult and central necrosis could develop. Therefore, ideal graft diameter shoul be 2-3 mm. Sural nerve, lateral femoral cutanous nerve, superficial radial nerve, lateral antebrachial cutanous nerve can be used as donor nerve in humans [10,19]. Shorter nerve grafts yield better outcomes. However, free nerve grafts can be as long as 25 centimeters [20].

Our study and its importance

Recurrent laryngeal nerve damages could be noticed intraoperatively or postoperatively. However, intraoperative diagnosis is rare. It is usually diagnosed during postoperative period either in the operation room when the patient could not produce any voice at all immediately after recovering from anesthesia or several days after the operation. If the patient is reoperated, surgeon could look for nerve ends and actually find them.

Our experimental study was designed to repair recurrent laryngeal nerve lacerations after thyroid surgery. If there is a laryngeal nerve damage and the two ends can be joined together without tension, primary suturation is a common and accepted practice.

If the two ends could be found but an anostomosis without tension was impossible, that condition was treated by free nerve grafting in our study. The outcome of this procedure was found to be very succesfull in our series. This graft technique was performed in end-to-end configuration and all the anastomosis created by this technique were succesfull. On vocal cord endoscopic examination, vocal cord movements were seen on the side where graft was used. On EMG examination regeneration waves were detected, which proves nerve anastomosis is succesfull. On histological examination there was no atrophy of muscles which is an indirect finding of abnormal nerve conduction.

If the proximal end of the severed nerve could not be found and only the distal end which enters the larynx can be detected, free nerve graft still can be used. In our study, the graft was interposed between phrenic nerve and recurrent laryngeal nerve and neuronal signals were transmitted from phrenic nerve to the laryngeal nerve through the neural graft. Since the graft was anastomosed in end-to-side configuration there was no loss of function of phrenic nerve. In our study we proved that diaphragm did not loose any of its functions by directly observing diaphragmatic contractions and by the abscence of degeneration waves and presence of synchronized motor units. Moreover, vocal cord movements observed directly by endoscopy, EMG evidence of healing represented by presence of regeneration waves and histologic findings indicating absence of atrophy indicate a succesfull anastomosis.

To the literature knowledge; denervation potentials and re-innervation potentials begin to be seen on laryngeal EMG two weeks after the operation. This is why we have conducted EMG studies on the third postoperative week [21].

Some researchers have tried to use other nerves instead of phrenic nerve. However, they have cut the nerve and muscles innervated by that nerve have atrophied. Since in our experiment none of the repiratory muscles were denervated and none of the anatomical structures were disrupted, we can classsify our method of surgery as the most appropriate surgery that preserves normal function and anatomy. We have chosen the phrenic nerve because

it innervates such a big respiratory muscle as diaphragm and allows an end-to-side anastomosis without causing any harm to the nerve.

Riassunto

Quando viene rilevato un danno al nervo ricorrente riteniamo che sia meglio procedere ad una correzione chirurgica che non danneggi la fisiologia respiratoria piuttosto che non fare nulla. Pertanto, dopo la sperimentazione da noi condotta, raccomandiamo questo metodo chirurgico che rispetta la normale anatomia e la fisiologia.

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