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Investigating the results of neuroendoscopic surgery in the treatment of pituitary adenoma and leptin gene expression

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ABSTRACT

Neuroendoscopic surgery of pituitary adenoma has been one of the technologies with rapid progress in neurosurgery in this decade. This method has known advantages and limitations. This study aims to investigate the results of pituitary adenoma treatment using the neuroendoscopy technique in a group of patients. Also, the level of leptin gene expression (LEP), which is produced exclusively in the pituitary gland, was measured for further evaluation. For this purpose, 26 patients who were diagnosed with pituitary adenoma and underwent endoscopic surgery in the hospital between 2018-2022, in terms of age, gender, disease symptoms, functional and non-functional tumor, and neurological examination findings before and after the procedure, complications, and the length of stay in the hospital were investigated. Also, before and 6 months after the operation, blood samples were prepared from patients to evaluate LEP gene expression by real-time PCR technique. The results illustrated that of the 26 patients studied, 14 were men, and 12 were women. Most of the patients were in their third to sixth decades of life. The tumors were non-functioning adenoma in 11 cases, somatotroph adenoma in 9 patients, corticotroph adenoma in 3 cases, and prolactinoma in 3 cases. Seven patients suffered postoperative complications, including 6 cases of reversible complications and one case of patient death. In the 2-year follow-up, 6 cases of tumor recurrence were observed. Also, the evaluation of LEP gene expression showed no significant difference between pre-operative and post-operative expressions. In general, neuroendoscopic surgery in treating pituitary adenoma is a method worthy of attention, considering factors such as fewer complications and a shorter stay in the hospital increase the acceptability of this method.

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Introduction

In general, endoscopic diagnosis and treatment methods are included in the group of minimally invasive surgical techniques (1). The endonasal sphenoid terrace surgery was used for the first time in 1909 in Vienna, Austria, to treat pituitary adenoma (2). This method was abandoned very soon due to the high risk of infection and creating a direct connection between the infected nasal cavity and the intracranial space. But it was revived again in 1987 by Griffith, and Cooke presented the first reports of good results of microscopic endonasal surgery (3).

As a pioneer of transnasal endoscopic surgery, Jho (4) used non-flexible endoscopy without the need to make an incision in the nose or use a nasal speculum. This method was first in the combined use of endoscope and microscope in endonasal trans-sphenoidal surgery, and finally, it has evolved as an exclusively endoscopic method (5). Nowadays, most authors consider the endoscopic method preferable to the microscopic process and suggest its use as the method of choice in pituitary adenoma surgery. This method has known advantages and limitations that will be discussed further (5, 6).

Leptin protein is made by fat tissue in the human body and released into the blood, which is expressed in various adult brain tissues, in the pituitary gland, and in a brain-derived cell line (7-9). The LEP gene provides instructions for making this protein (8). In this study we investigate the results of neuroendoscopy surgery in the treatment of pituitary adenoma and its effect on leptin gene expression.

Materials and Methods

Evaluations of patients

This case study includes 26 patients with pituitary adenoma who underwent neuroendoscopic surgery in the hospital between 2018 and 2022. The following findings were recorded for all patients: age, sex, disease symptoms, functional or non-functional tumor (according to the results of hormonal tests), and neurological examination findings before and after the operation. The level of hormones secreted from the pituitary gland was measured before and after surgery.

Also, the size of the tumor in MRI, the possible complications of the patient during and after surgery, the surgeon's quality assessment of the operation, the length of the patient's stay in the hospital, and the recurrence of the disease (based on MRI and hormone tests) were evaluated. The surgical indication was determined in consultation with the endocrinologist and neurosurgeon, and the surgi-

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cal method used (open or endoscopic surgery) was chosen based on the joint opinion of the neurosurgeon and the ear, throat, and nose specialist. The patient cleaned the nasal cavity with tetracycline ointment from the day before the operation. First-generation cephalosporin (intravenous cefazolin) was used for antibiotic prophylaxis. After general anesthesia, the patient's head was turned 15 degrees to the right, and the neck was placed in an extension of 10 degrees relative to the body.

The endoscope was connected to a 3D camera, the resulting images of which were displayed on the screen in front of the surgeon. A non-flexible 4mm Olympus endoscope with zero and 30-degree lenses was used for surgery. The location of the middle and upper nasal prongs was identified, and then the sphenoid sinus opening was reached through the lower margin of the middle nasal prong. Cutting and dissection in mucoperiosteum were necessary only at the junction of cartilaginous septum and vomer bone. After pushing the end of the cartilage and bone to the opposite side, the opening of the sphenoid sinus could be accessed.

Then the opening of the sphenoid sinus was dilated by Cresson forceps on both sides. By this means, it entered the sphenoid sinus. After removing the septum or septums inside it, it was possible to identify the elements of the Turkish saddle area and its adjacent ones. Then, by the neurosurgeon, the surrounding elements were searched in the following order: in the posterior wall of the sinus in the craniocaudal direction, the sphenoid planum and clivus were identified, and in the lateral walls of the sinus in the rostrocaudal order, the optic prominence, optic-carotid junction, and Carotid prominences were identifiable. The floor of the Turkish saddle cavity was opened with a drill and dilated with Cresson forceps, and then the dura mater was opened on the bottom of the hole.

The tumor was slowly removed with slow rotating movements of the curt and suction. At the end of the operation, a 30-degree lens was used to see the blind spots inside the Turkish saddle cavity. If the tumor had a firm and sticky consistency, the mass was avoided from being strongly stretched, and the tumor was evacuated as much as possible using angled lenses. In the end, if needed, the dead space created by tumor removal was filled by removing fat tissue from the patient's abdominal wall. If there is a need to reconstruct the seller, this work was done with pieces of the sphenoid septum bone or the vomer bone. Then, if needed, the middle cavity and nasal septum were packed using 60 cm sterile gauze impregnated with sterile tetracycline eye ointment. This sterile gauze was released from the nose 24 to 48 hours after the operation. The neurosurgery clinic followed up with all patients in the initial period after the operation and after discharge (for two years).

If the patients had no complications and had good general condition, they were usually discharged on the fourth day after the operation. The first visit was six weeks after the operation when the person came for hormonal evaluation and examination. Patient control was done with gadolinium MRI, usually three months after the operation. Also, a visual field examination was done in the third month of the visit, and it was essential for patients who had visual field defects before the operation. The resulting information was recorded in the data collection form during each visit and analyzed using statistical tests.

Evaluations of LEP gene

Blood samples were collected from patients before and six months after surgery. After collecting blood samples in tubes containing anticoagulants, RNA extraction, and cDNA cis-synthesis were performed. For this purpose, an RNA extraction kit (RNX) and cDNA synthesis kit) were manufactured by Bioneer Company were used. The forward and reverse primer sequences for LEP gene were 5'-GGATGACACCAAAACCCTCA-3' and 5'-CATGAGCTATCTGCAGCACG-3', respectively. The β-actin gene was used as the housekeeping gene which its forward and reverse primer sequences were 5'-GCCAACAGAGAGAAGATGACAC-3' and 5'-GTAACACCATCACCAGAGTCCA-3', respectively.

After RNA extraction and cDNA synthesis, PCR reaction was performed in 23 μL. First, Mix2x Master Primer and DEPC were brought to ambient temperature, and after a centrifuge, they were ready to be used. The strips (100 μL tubes connected to the Time-Real PCR machine) were placed on ice under the hood, and 5 μL (2x Mix Master), 3 μL of water containing DEPC, and 0.5 μL of the primer were added to them. The cDNA was melted on ice, and after short centrifugation, 1 μL was added to each strip. Finally, the desired sample was placed in the PCR machine, and 45 cycles of amplification were performed. The resulting data were collected and recorded. To quantify the desired gene expression values, first, the optical absorption coefficient obtained in the Corbett device was converted to numerical data by the Gene Rotor Mode Virtual-series 6000 software designed by Corbett, Germany. Then 2- ΔΔCT was used in Excel software. Gene expression levels and fold change values were measured relative to the β-actin control gene.

Results

Of the 26 patients studied, 14 (8.53%) were male, and 12 (1.46%) were female. The age distribution of the studied patients and the symptoms of the patients are given in Table 1. It should be mentioned that the symptoms of increased intracranial pressure, high blood pressure, diabetes insipidus, cranial nerve entrapment, convulsions, and psychological symptoms that were considered in the questionnaire were not observed in any of the patients. Out of 26 patients studied, 13 had macro-adenoma, 10 had micro-adenoma, and 3 had invasive pituitary adenoma. Prolactinoma tumor was reported in three patients (12%), ACTH-secreting tumor in three patients (12%), and growth hormone-secreting tumor in 9 patients (34%) and in 11 cases (42%), the cancer was non-functional. Among 26 patients, 6 (23.07%) had tumor recurrence, including four patients with non-functioning adenoma, one with prolactinoma, and one with corticotroph adenoma. It is worth mentioning that in 4 patients, the recurrence symptoms were not similar to the initial signs of the patient. Seven patients had postoperative complications, and one died due to venous thrombosis and pulmonary embolism.

Five cases had diabetes insipidus, which was controlled with drug treatment. One patient had bleeding in the operation area, which was treated conservatively. The surgeon's qualitative assessment of the surgery is given in Table 2.

The evaluation of LEP gene expression showed that there was no significant difference between pre-operative and post-operative expressions (Figure1).

Table 1. Frequency distribution of evaluated treatments among patients.

Treatment	Frequency	Percent
Age Decade		
Second Decade	2	7.69
Third Decade	5	19.23
Fourth Decade	4	15.38
Fifth Decade	10	38.46
Sixth Decade	5	19.23
Disease Sign		
Headache	14	53.84
Visual field disorder	15	57.69
Weight Gain	3	11.53
Galactorrhea	4	15.38
Amenorrhea	6	23.07
Libido Decreasing	5	19.23
Hirsutism	1	3.84
Musculoskeletal Symptoms	1	3.84
Length of Stay in Hospital		
Less than 5 days	19	73.09
5-10 days	6	23.07
More than 10 days	1	3.84
Symptoms of Relapse		
Visual Field Disorder	1	3.84
Galactorrhea	1	3.84
Amenorrhea	1	3.84
Libido Decreasing	2	7.68
Hypothyroidism	1	3.84

Table 2. Frequency distribution of surgeon quality assessment.

	Low	Medium	High
Tumor removal rate	2	11	13
Appropriate endoscopic vision	1	9	16
Risk of Surgery	20	5	1

Discussion

It is difficult to estimate the actual prevalence and occurrence of pituitary adenomas, but in epidemiological studies, the majority is about 0.0002, and the incidence is about 0.0006 per year (10). Non-selective biopsy studies show that 20 to 25% of all people have a pituitary microadenoma with no clinical symptoms (11). Pituitary adenomas occur at all ages, but most affected people are in the 3rd to 6th decade of life. Based on its function, pituitary adenoma is divided into types with hormonal function (according to the type of hormone secreted by the tumor) and non-hormonal process (12). Surgery is the treatment of choice for these tumors, except for pro-lactinoma (11, 12).

There are various surgical methods used for pituitary lesions, and the choice of surgical procedure depends on several factors, including the degree of mineralization, the size and presence of air in the sphenoid sinus, the position and curvature of the carotid arteries, and the extent and spread of the tumor (13). Sometimes it is necessary to use a combination of transcranial and trans-sphenoidal methods. Today, it is believed that 95% of pituitary adenomas can be treated with the trans-sphenoidal process. The trans-sphenoidal procedure can be performed in two ways: endonasal and sub-labial (14). The endonasal method is often used, and the sub-labial method is reserved for large lesions. In recent studies, the superiority of the endonasal endoscopic method has been proposed in terms of otolaryngological, hormonal, and neurological complications (13).

Endoscopic pituitary surgery is performed through the natural nasal passage without any incision (14). The surgery has three stages: nasal, sphenoidal, and seller (15). In this method, a 4 mm endoscope is placed in front of the tumor in the sphenoid sinus, and the tumor is removed. In this method, discomfort after the operation is minimal; in most cases, there is no need to pack and close the nose (16). Among its advantages are a wider field of view, less tissue invasion, direct vision of vital elements in the side walls of the sinus, and minimal trauma to the patient's nostrils (17). Among the limitations mentioned for this method are the lack of familiarity of several neurosurgeons with the endoscopic way and the existence of a long-term learning curve compared to microscopic operations, the need to use special tools and equipment for this type of surgery, and create less three-dimensional vision than the microscopic method (18).

Typically, the inside of the middle or nasal cavity is not packed at the end of the surgery because the bleeding is minimal (19). The patient is asked not to exercise during the first 48 hours after the surgery, to avoid physical activity (such as lifting heavy objects) for a few days, and to avoid all activities that increase blood pressure (13). In some cases, oral broad-spectrum antibiotics are prescribed for a week. Currently, the trans-sphenoidal method is safe in the treatment of pituitary adenoma (14). The percentage of mortality and complications in this method is 0.5 to 2.2% (16). Death is usually caused by intracerebral hemorrhage, hypothalamus injury, or meningitis associated with cerebrospinal fluid fistula. Another cause of death in the trans-sphenoidal method is vascular complications and arterial damage. In previous studies, researchers mentioned the maximum age range of the disease from the third to the sixth decade of life, which was almost the same in our patients (18, 19).

Some studies have mentioned 10 to 15% of primary brain tumors as pituitary adenoma, among which prolactinoma constitutes about 30% of adenoma cases and is the most common type of pituitary adenoma (20). While in

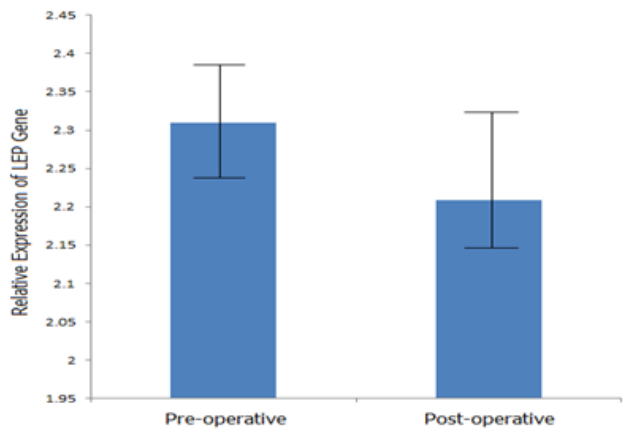

Figure 1. The relative expression of the LEP gene before and after neuroendoscopy surgery.

this study, 12% of the patients had a prolactinoma. The reason for the above difference could be because the information of this study was collected from the surgical departments and related to the cases that indicated surgical treatment.

This study's results show that most patients were hospitalized for a short time for treatment. It has been mentioned in previous studies that the patients who are treated with this method were discharged from the hospital about 4 to 6 days after the operation (20). The study of the surgeon's qualitative assessment of this method shows that, in most cases, this method is considered appropriate in terms of technique and tumor removal power. The results of this study are almost similar to other studies in terms of the effectiveness of the treatment method, and some of them seem to be somewhat better in terms of complications (21). Of course, due to the small sample size, the results cannot be judged definitively and need to be confirmed in the continuation of the study. Besides, the results of LEP gene expression illustrated no significant difference between pre-operative and post-operative expressions of this gene, which showed that neuroendoscopy surgery had not hurt the pituitary gland, as the LEP gene is expressed in this gland. Therefore, the pituitary gland has kept its function as before the operation.

All in all, neuroendoscopic surgery is a method worthy of attention. The comfort of the patients during and after the surgery, the short duration of hospitalization and the low cost of hospitalization, and the quality assessment of the surgeons in terms of the risk of the operation and the surgeon's view and the amount of tumor removal add to the acceptability of this method. Although this method costs more in terms of providing the tools and technology used in the short time, if we consider factors such as the length of the patient's stay in the hospital and the costs caused by the complications of the patients, this method is appropriate in the long term.

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Availability of data and materials
The data used to support the findings of this study are available from the corresponding author upon request.

Statements and Declarations
The author declares that no conflict of interest is associated with this study.

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