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RESEARCH ARTICLE
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A Novel Method of Hybrid Ossiculoplasty: Ionomer Bone Cement Coated Mastoid Cortical Bone

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ABSTRACT

Objective: The development of otologic surgical techniques has significantly improved the functional results of surgery. However, the issue of the most effective ossiculoplasty material is still controversial. The study aimed to introduce a novel method of autologous ossiculoplasty, using "ionomer bone cement coated mastoid cortical bone" and to assess short-term and long-term results of this method.
Material and Methods: The study presented a retrospective, consecutive case series of twelve patients who underwent revision surgery as type IV tympanoplasty using Mastoid cortical bone coated with ionomer bone cement in our institution between January 2013 and December 2019. Coating with ionomer bone cement was performed to prevent the autograft from sticking to the surrounding bone tissue. The short- and long-term auditory functions and otologic examination findings of the patients were analyzed.
Results: Physical examination findings were satisfactory. Except one, all patients had a significant improvement in hearing outcomes, and the late-term results were also satisfactory. An average long term audiological gain of 19.9 dB (± 8.7 SD) was found.
Conclusion: A novel method of Hybrid Ossiculoplasty: Ionomer Bone Cement Coated Mastoid Cortical Bone is an effective and safe method of ossiculoplasty. It can be easily eliminated one of the major disadvantages of mastoid cortical bone by coating it with ionomer bone cement.

Keywords: Ossiculoplasty, graft material, mastoid cortical bone graft, ionomer bone cement, type IV tympanoplasty

INTRODUCTION

There are a lot of issues that have not yet been overcome despite the technological developments in otologic surgery. Combating cholesteatoma and long-term outcomes of hearing reconstruction are among the most critical issues of otology (1). Total ossicular replacement prostheses (TORP) are usually placed between the stapes footplate and the tympanic membrane graft when middle ear ossicles are absent or inappropriate to use (type IV tympanoplasty according to Wullstein classification) during middle ear surgery (1). Today, various alloplastic materials are used as TORP, among which primarily are the materialstitanium and polycel. However, long-term results may not be satisfactory due to the obstacles related to the biocompatibility of these materials (2, 3). Although the use of mastoid cortical bone (MCB) as an autologous graft material was described before, it did not gain popularity due to the possibility of resorption and /or adhesion to the surrounding bone tissues (4).

We propose that properly shaped autologous MCB is a suitable material for ossicular reconstruction that is comparable to other synthetic materials. We used properly shaped MCB, the medial half of which was coated with ionomer bone cement in the revision surgeries.

The clinical results of the patients who underwent ossiculoplasty using MCB coated with ionomer bone cement were assessed as a preliminary study.

MATERIAL AND METHODS

The medical records oftwelve patients who were diagnosed with chronic otitis media (COM) and underwent type IV tympanoplasty between January 2013 and December 2019

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were retrospectively reviewed. The inclusion criteria were to be between the age of 18 and 65, performed type IV tympanoplasty surgery using MCB coated with ionomer bone cement and to have at least a 12 month follow-up clinical and audiological examination.

Patients' preoperative, postoperative initial period (6-8 weeks) and late (at least 12 months after surgery) audiogram results, otoscopic examination findings, clinical course and complications were recorded. For patients who previously underwent ossiculoplasty and used alloplast prosthetics, but have worsened hearing and/or extrusion, we recommended Type IV tympanoplasty with ionomer cement coated MCB usage as a salvage surgery.

After the patients were informed of their diseases and treatment options, an informed consent form was obtained, and a treatment process was started.

The postoperative late results were assessed based on the most recent audiograms in the postoperative period, which varied between one and seven years. The hearing was assessed using a pure tone audiometry test. The air and bone conduction pure tone thresholds and air-bone gap (ABG) were assessed (500 Hz, 1000 Hz and 2000 Hz frequencies were considered when measuring the averages of pure tone thresholds). Changes over time in audiologic values were calculated. Cases with ABG values less than 20 dB and gain greater than 10 dB were considered functionally successful.

Surgical Technique

A single surgeon performed all surgeries. Surgical access was provided through a retro auricular incision. A Mastoidectomy was performed with the ''inside out'' technique. The middle ear and mastoid cavity were revised in those who had previously received a performed mastoidectomy. Any cholesteatoma, granulation tissue and /or sclerotic plaques were cleaned. The previously placed prosthesis was removed. The status of the oval and round windows was assessed and the stapes footplate was confirmed to be mobile. Underlay tympanoplasty was performed using a temporal fascia. The posterior part of the fascia graft was placed forward and preparation for hearing reconstruction was provided. After the tympanic cavity was made ready, a bone graft preparation was started. Bone graft with a diameter of 1 mm and a length of 8mm from the mastoid cortex was obtained by drilling and using a gouge and hammer (Figure 1a). The length and shape were arranged according to the depth of the oval window (Figure 1b). Half of the bone to be positioned medially was insulated with ionomeric cement (Glasspolyalkenoate [ionomer] cement, Voco/meron corresponds to EN 29917/150/9917/1994[CE 0482]) (Figure 1c). The prepared bone graft was placed between the stapes footplate and the graft membrane. The lateral part of the MCB graft was not coated, as it adheres to the grafted membrane.A sound transmission chain continuity was provided. A Tympanomeatal flap was laid on the fascia graft.

Postoperative Follow up

The patients were discharged the next day after surgery. Stitches were removed after a week. Tampons from the mastoid cavity were removed after seven days and checked every week for four to six weeks until cavity epithelialization occurred. The postoperative follow-up continued and controls were performed monthly in the first year, and annually after the first year. Hearing tests were conducted six to eight weeks after surgery, on the first year and on annual controls. During this period, the patients were requested to report immediately in case of any problems (e.g., hearing deterioration, discharge, pain).

Data Analysis

Data were analyzed using the SPSS 22.0 software program (IBM Corp. In Armonk, NY. The means and medians, ranges, and standard deviations were calculated. Data distributions were analyzed using the Kolmogorov-Smirnov test and the quantitative data were compared using paired t test. The Friedman test was used to analyze time-dependent changes of the hearing test results of the patients. Level of significance was set at $p>0.05$.

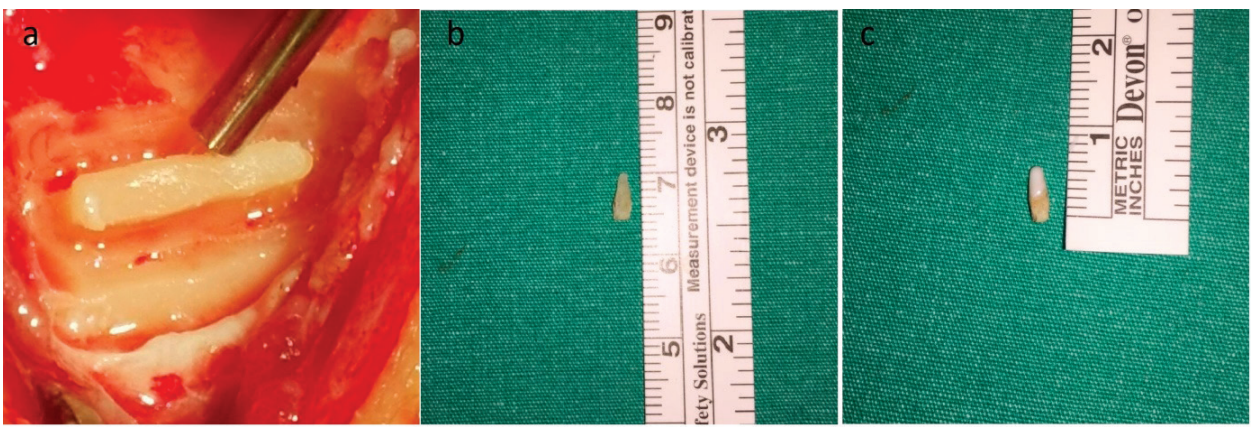

Figure 1: Demonstrates preparation process of Ionomer bone cement coated MCB graft. a: Image of bone extraction by drilling from the mastoid cortex. b: The length and shape of the graft were arranged according to the depth of the oval window. c: medial part of the graft was coated with ionomer bone cement

RESULTS

The mean ages of the cases included in the study at the time of surgical treatment were 43.17 (between the ages of 21 and 72). The demographic characteristics of the patients are given in Table 1.

Table 1: Demographic and paraclinical features of the patients

Patients	12 (100%)
Gender	
Male	7 (58%)
Female	5 (42%)
Average age	43.17 (21-72)
SID	
right	7 (58%)
left	5 (42%)
Postoperative average time	39 month (12-84)

Surgical indications were present in five of the patients due to the extrusion reaction of the prosthesis detected during examination, four of them due to recurred conductive hearing loss and three due to ear discharge and renewed conductive hearing loss. Of the twelve patients, eight (67%) had had surgery in our hospital before, while four (33%) had had prior surgeries in other centers. Three patients were found to have recurrent cholesteatoma in the sinus tympani region and one patient was found to have a displaced prosthesis. All patients had abundant granulation tissue (Table 2). Nine patients had previously had canal wall down tympanoplasty while the posterior canal walls of three patients were intact. In these three patients, thecanal walls needed to be removed by an ''inside out'' technique during the operation. The stapes footplates of all patients were mobile. No complication developed in any of the patients. In the early and late postoperative period after surgery the mastoid cavity epithelialization and middle ear healings were optimal (Figure 2). There was no improvement in the hearing of one patient although there was no problem in the examination and the patient refused revision surgery. Other patients' short- and long-term hearing results improved significantly (Table 3). There was no statistically significant difference between patients' short-term and long-term air conduction pure tone audiometry ($p<0.05$). The patients' long-term bone conduction pure tone audiometry thresholds were slightly elevated but the difference between the results was not statistically significant ($p<0.05$).

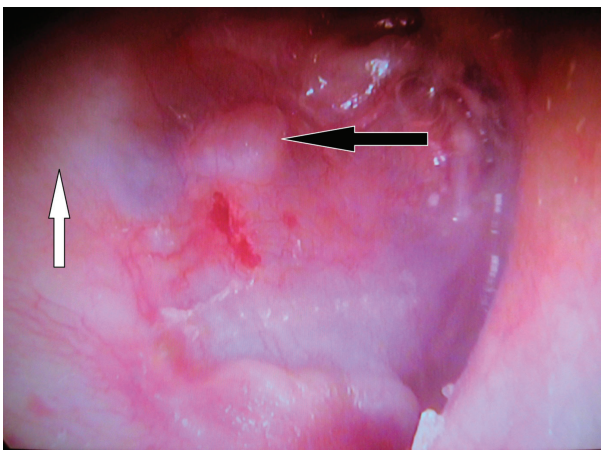

Figure 2: Postoperative 1-year otoendoscopic view (right ear). Black arrow points to the silhouette of the MCB graft. The white arrow points to the facial ridge

Table 2: Clinical findings of the patients

Patient №	Ear discharge	Prosthesis extrusion	Granulation tissue	cholesteatoma	Displacement of the prosthesis	Extracted prosthesis
1	+		+			Titanium PORP
2		+	+			Titanium TORP
3			+	+		Titanium TORP
4		+	+			Titanium PORP
5			+		+	Polycel TORP
6		+	+			Polycel TORP
7	+		+			Titanium TORP
8			+			Polycel PORP
9		+	+			Polycel TORP
10			+			Titanium PORP
11		+	+	+		Titanium TORP
12	+		+	+		Titanium TORP

TORP: total ossicular replacement prostheses; PORP: partial ossicular replacement prostheses.

Table 3: Audiologial examination results of the patients

Values	Pre-op	Post-op early	Post op late	Difference of	p#
	Average±SD	Average±SD	Average±SD	Averages*	
Air conduction	57.5±10.3	35.2±8.6	37.6±8.6	19.9	0.0040
Bone conduction	24.9±6.4	24.8±8.1	26.4±7.1	-1.5	0.905
Air bone gap	32.4±10.9	6.8±8.0	7.2±7.8	20.2	0.0030

#: Paired t test; SD:standart deviation; *: difference between the averages was calculated based on the postoperative late averages.

DISCUSSION

This study presented a novel method of type IV ossiculoplasty using a hybrid material (MCB + ionomer bone cement) to provide continuity of the sound transmission mechanism in the middle ear.Thus, the aim was to eliminate shortcomings of the MCB and ionomer bone cement.

There are many factors affecting the success of hearing reconstruction in middle ear surgery. Some of the most crucial factorsare the condition of the ossicles, extent of the surgical procedure, the condition of the mastoid cavity, the condition of the middle ear mucosa, the condition of the eustachian tube, the technique used during the surgery, the used prosthetic material, and the experience of the surgeon (5-7). The surgical technique and material used in hearing reconstruction have always been the subject of controversy. Chronic otitis media, with and without cholesteatoma, is one of the most important causes of disruption of the integrity of the middle ear ossicular chain (8). In this present case series, the primary diagnosis of all operated patients was COM with cholesteatoma. The posterior canal wall was drilled out in all patients to ensure complete clearance of the cholesteatoma (CWD mastoidectomy). It is a fact that such surgical procedures negatively affect the hearing results of the patients (9). Alaani et al. reported that long-term hearing results of CWD mastoidectomy are worse than canal wall up (CWU) mastoidectomy (10). However, they also emphasized that the functional difference was not statistically significant (10). In some cases, the posterior canal wall and stapes suprastructure were preserved and the MCB was placed between the stapes and the tympanic membrane. However, they were excluded from this study to maintain standardization.

The literature indicates that autologous cartilage, bone, vinyl-acryl, polyethylene, gold, plastipore, hydroxyapatite, bioglass, and titanium may be used to restore the continuity of sound transmission in the middle ear (6). Prosthetic materials used in otologic surgeries are expected to have biocompatibility, durability, good sound transmittance, accessibility and to be easy to use (6, 11). Today, although titanium and synthetic polycel prostheses are mostly used this issue remains controversial and indeed, none of them are considered perfect materials (12). Extrusion rates of allograft materials vary between 3% and 15% in the literature (6, 9, 12-14). However, because bioengineering is developing, the studies in this area are promising. Plastipore is the first alloplastic material that was used and commercialized (15). Although the early short-term result was promising, the long-term hearing results and extrusion rate were not acceptable (12).

One of the most popular alloplast materials today is hydroxyapatite (HA). HA is a natural component of bone tissue and shows high biocompatibility. It is still used in clinical practice and has proven itself in the time test (12, 16). Choi et al. reported the extrusion rate as 6.7% (12).

Polycel is another synthetic material that is widely used today. Moon et al. assessed long-term hearing results and reported that 51.1% of cases showed an ABG of ≤20 dB, and 158 cases (84.0%) had an ABG of ≤30 dB (14). They claimed that extrusion rate to be 3.8% (14).

One of the most interesting allograft materials since 1970s is titanium (17). Nowadays, titanium is widely used in orthopedics and craniofacial surgery (2). As it has been used in otologic surgery for many years, many reports have been published as a long-term result (18, 19). Despite being extremely dependable in terms of biocompatibility, long-term hearing results provide conflicting data in the literature (20). Lahlou et al. recently published a study investigating the anatomical and functional results of the titanium ossicular prosthesis where they reported that ABG ≤ 20dB to be in 65% of cases, displacement rate 6% and extrusion rate 3% (13).

Ulku et al.conducted an animal experimental study with vitallium which is used in orthopedic and orthodontic practice (2). They placed it in the rabbit middle ear and obtained results similar to titanium in terms of biocompatibility. They emphasized higher resistance to corrosion and lower probability of local debris than titanium (2). Studies have shown that both titanium and vitalium are safe in terms of toxicity, mutagenicity, and carcinogenicity (21).

Although homograft materials seem to be more advantageous in suitable cases in terms of both short- and long-term results, they are not preferred due to difficulties such as procurement and infection risks, etc. (2, 6, 18, 22).

Both autologous MCB and bone cement are not new in otology. They have been used in otologic surgery for a long time for a number of purposes and methods. Zeitler et al. stated that the use of autologous ossicles, cartilage, and MCB have advantages such as high biocompatibility, readily available, easy to shape, and low risk of extrusion (18). Malafronte et al. described the double–cartilage block ossiculoplasty method in cases where

stapes suprastructures were preserved and stated that it was a successful and easy method (23). Recently, Kong et al. reported a new technique using an autologous graft, which they describe as a bone cartilage composite graft (BCCG) (6). In their study, they shaped the patient's incus bone or MCB, carved to be a stalk of BCCG. They also harvested a piece of conchal cartilage and shaped it as a 5~6 mm circle to be the cap of the BCCG. Using the cartilage and bone they created a mushroom-shaped graft. Kong et al.compared the anatomical and functional results of these patients with results of patients that used allograft material (polycel and titanium) and suggested that this method had better long-term hearing results and the lowest extrusion rate (0%) (6).

The risk of the development of osteitis, bone resorption and adhesion possibilities to adjacent tissue are the major handicaps of the use of autograft material (24, 25). In this context, the middle ear ossicles seem more advantageous than MCB because they have a periosteum on them (25). However, in this kind of pathologies where CWD mastoidectomy is needed, middle ear ossicles are often absent or not suitable for use. It is also necessary to consider the possibility of re-implantation of cholesteatoma and infectious agents to the middle ear (24).

In fact, Bauer et al. also reported that they used the combination of MCB graft and ionomer cement for ossicular reconstruction, and they argued that this combination was efficient (26). However, they used ionomer cementas a kind of glue toprovide the continuity of the ossicular chain (26). The principle of our technique is completely different.Our purpose of using ionomer cement was to prevent MCB from sticking to the surrounding bone structures. In our clinical practice we have seen that the bone graft may be adhered to the promontorium. Bauer et al. also reported that they encountered this problem in one of their patients (26). To eliminate this problem, we coated the medial part of the bone graft with ionomeric bone cement, which has been used in otological surgery for many years and has been proven to be safe (27). In this way, direct contact of bone tissues was prevented.

In primary surgeries, especially in patients with cholesteatoma and/or infected middle ear, the choice for ossiculoplasty was in favor of alloplast materials. Ionomer Bone Cement Coated MCB was used only in selected clean revision surgeries where the allograft material was extruded. Ionomer Bone Cement seems to be an ideal solution in this sense due to advantages such as: good adhesion to bone, reliability in terms of toxicity, easy access, and easy application (27, 28). The long-term anatomical and functional findings were also favorable (Table 3). The disadvantages of using autograft material are that it prolongs the operation time and it is more difficult to prepare than using the ready-made prosthesis (2). It seems that by using these two materials together in a hybrid way, we can make-up for their disadvantages. We continue to follow up and collect the data of our patients and plan to conduct a study comparing the results of other allograft ossiculoplasty in the future.

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REFERENCES

1. Yu Z, Zhang L, Han D. Long-term outcome of ossiculoplasty using autogenous mastoid cortical bone. J Laryngol Otol 2014;128(10):866-70.
2. Ulku CH, Avunduk MC, Uyar Y, Arbag H. Biocompatibility of vitallium as ossicular reconstruction material in the middle ear: experimental animal study. Acta Otolaryngol 2005;125(1):38-42.
3. Kim HH, Wiet RJ. Preferred technique in ossiculoplasty. Oper Tech Otolaryngol Head Neck Surg 2003;14:243-6.
4. Morris DP, Wong L, van Wijhe RG, Bance ML. Effect of adhesion on the acoustic functioning of partial ossicular replacement prostheses in the cadaveric human ear. J Otolaryngol 2006;35(1):22-5.
5. Faramarzi M, Jahangiri R, Roosta S. Comparison of Titanium vs. Polycel total ossicular replacement prosthesis. Iran J Otorhinolaryngol 2016;28(85):89-97.
6. Kong JS, Jeong CY, Shim MJ, Kim WJ, Yeo SW, Park SN. Comparative study of new autologous material, bone-cartilage composite graft, for ossiculoplasty with Polycel® and Titanium. Clin Otolaryngol 2018;43(2):434-9.
7. Dornhoffer JL, Gardner E. Prognostic factors in ossiculoplasty: A statistical staging system. Otol Neurotol 2001;22(3):299-304.
8. Goyal R, Mourya A, Qureshi S, Sharma S. Modified Radical Mastoidectomy with Type III Tympanoplasty: Revisited. Indian J Otolaryngol Head Neck Surg 2016;68(1):52-5.
9. Lucidi D, De Corso E, Paludetti G, Sergi B. Quality of life and functional results in canal Wall down vs canal Wall up mastoidectomy. ActaOtorhinolaryngol Ital 2019;39(1):53-60.
10. Alaani A, Raut VV. Kurz titanium prosthesis ossiculoplasty--follow-up statistical analysis of factors affecting one year hearing results. Auris Nasus Larynx 2010;37(2):150-4.
11. Truy E, Naiman AN, Pavillon C, Abedipour D, Lina-Granade G, Rabilloud M. Hydroxyapatite versus titanium ossiculoplasty. Otol Neurotol 2007;28(4):492-8.
12. Choi YS, Shin SO. Results of hearing outcome according to the alloplastic ossicular prosthesis materials. Indian J Otolaryngol Head Neck Surg 2018;70(2):184-7.
13. Lahlou G, Sonji G, De Seta D, et al. Anatomical and functional results of ossiculoplasty using titanium prosthesis. Acta Otorhinolaryngol Ital 2018;38(4):377-83.

14. Moon IS, Song MH, Kim HN, Chung MH, Lee WS, Lee HK. Hearing results after ossiculoplasty using Polycel prosthesis. Acta Otolaryngol 2007;127(1):20-4.
15. Shea JJ. Plastipore total ossicular replacement prosthesis. Laryngoscope 1976;86(2):239-40.
16. Grote J. Tympanoplasty with calcium phosphate. *Arch Otolaryngol* 1984;110(3):197-9.
17. Wang X, Song J, Wang H. Results of tympanoplasty with titanium prostheses. Otolaryngol Head Neck Surg 1999;121(5):606-9.
18. Zeitler DM, Lalwani AK. Are postoperative hearing results better with titanium ossicular reconstruction prostheses? Laryngoscope 2010;120(1):2-3.
19. Hess-Erga J, Møller P, Vassbotn FS. Long-term hearing result using Kurz titanium ossicular implants. Eur Arch Otorhinolaryngol 2013;270(6):1817-21.
20. Bance M. Optimizing Ossicular Prosthesis Design and Placement. Adv Otorhinolaryngol 2018;81:14-23.
21. Katzer A, Hockertz S, Buchhorn GH, Loehr JF. Invitro toxicity and mutagenicity of CoCrMo and Ti6Al wear particles. Toxicology 2003;190(39):145-54.
22. Bauer M. Ossiculoplasty: autogenous bone grafts, thirty-fouryears' experience. Clin Otolaryngol Allied Sci 2000;25(4):257-63.
23. Malafronte G, Filosa B, Mercone F. A new double-cartilage block ossiculoplasty: long-termresults. Otol Neurotol 2008;29(4):531-3.
24. el Seifi A, Fouad B. Autograft ossiculoplasty in cholesteatoma. ORL J Otorhinolaryngol Relat Spec 1992;54(6):324-7.
25. Ojala K, Sorri M, Vainio-Mattila J, Sipilä P. Late results of tympanoplasty using ossicle or cortical bone. J Laryngol Otol 1983;97(1):19-25.
26. Bauer M, Pytel J, Vóna I, Gerlinger I. Combination of ionomer cement and bone graft for ossicular reconstruction. Eur Arch Otorhinolaryngol 2007;264(11):1267-73.
27. Chen DA, Arriaga MA. Technical refinements and precautions during ionomeric cement reconstruction of incus erosion during revision stapedectomy. Laryngoscope 2003;113(5):848-52.
28. Aldosari B, Thomassin JM. Audiological results of endoscopic surgical repair of the lengthy process of incus. World J Otorhinolaryngol Head Neck Surg 2017;3(3):148-52.

RESEARCH ARTICLE
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Evaluation of LRIG1 Expression in Larynx Pathologies

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ABSTRACT

Objective: Studies have been performed on many biomolecules to determine the prognosis of LSCC and predict the course of the disease. However, a molecular marker that can be used clinically has not yet been found. Therefore, in this study, we aimed to investigate the expression levels of LRIG 1 in laryngeal cancer.
Materials and Methods: In our study, 219 cases who underwent surgery due to LSCC and 88 randomly selected patients whose pathologic result were benign and premalignant lesions in Marmara University Pendik Education and Research Hospital between 2003 and 2018 were analyzed. Patients' data were obtained from the medical records. The tissue microarray method was used to evaluate specimens.
Results: There was a statistically significant difference between the tumor differentiation, diagnosis, and the expression of LRIG1 (respectively p=0.045, p<0.001). Also, an increase in the degree of dysplasia in premalignant lesions correlates with a decrease in LRIG1 expression (p=0.015).
Conclusion: Our findings suggest that LRIG1 plays a role in the early tumorigenesis of LSCC. Therefore, LRIG1 can be a target molecule for treatment approaches. However, LRIG1 was not correlated with overall survival of the LSCC.

Keywords: LRIG1, Laryngeal carcinoma, tumorigenesis

INTRODUCTION

Squamous cell carcinoma of the larynx (LSCC) accounts for 2.8 % of all cancers and is the second most common head and neck malignancy (1). Smoking and alcohol are the main known etiological factors for LSCC (2). In recent years, increasing exposure to toxic substances and smoking in women has reduced the male-to-female ratio to 6 (3). However, the decline in mortality rates parallels the reduction in the incidence of LSCC is not at the desired level; 5-year survival rates have not improved (1, 3). The expectation of an increase in estimated incidence and mortality rates in developing countries, particularly Turkey, indicates the need to develop more aggressive treatment methods. Therefore, elucidating the pathogenesis of LSCC is critical to this process.

3% of proteins in the human proteome consist of immunoglobulin (Ig)-like domain, and 0.9% contain a leucine-rich repeat (LRR) region (4). LRRs are proteins with repeating segments containing 11 aliphatic amino acids, including leucine. This repeating part is found in many proteins and is thought to be involved in interprotein interactions (5). The LRIG family are extracellular integral membrane proteins with 15 LRR and 3 Ig domains, a single-row transmembrane domain, and a cytoplasmic tail region.

The LRIG1 gene is located on the 3rd chromosome (3p14) and is expressed in many tissues (6). Deletion of the 3p14 region

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is frequently observed in various cancers (7). LRIG1 is a single transmembrane protein involved in growth factor signaling, cell proliferation, and tumor suppression mechanisms (7, 8). In addition, several recent studies suggest that the genes, transcripts, and proteins of leucine-rich repeats and immunoglobulin-like domains (LRIG1) have prognostic significance in various cancers including cutaneous squamous cell carcinoma, prostate cancer, glioma, and nasopharyngeal carcinoma (9-12).

Studies have been performed on many biomolecules to determine the prognosis of LSCC and predict the course of the disease. However, a molecular marker that can be used clinically has not yet been found. When we searched the literature, we did not find any study that investigated the effects of gene expression of LRIG 1 on LSSC. Therefore, in this study, we aimed to investigate the expression levels of LRIG 1 in laryngeal cancer.

MATERIALS AND METHODS

This study was approved by the Clinical Research Ethics Committee dated 07/04/2017, number 09.2017.283. In our study, the data of 219 cases who underwent surgery due to LSCC and 88 randomly selected patient whose pathologic result were benign and premalignant lesions in Marmara University Medical Faculty of Medicine Hospital between 2003 and 2018were obtained from the medical records.

The tissue microarray method, which allows the evaluation of more than one tissue at a time, was used. Hematoxylin-eosin (H&E) stained slides were examined, and areas where the tumor was seen were marked with a glass pen. For each patient, 2-3 tumor areas and one lymph node metastasis area were identified. The paraffin blocks of the marked slides were removed, and tissues were harvested from the regions that matched the marked sites using the 3-mm needle of the "quick-ray device." The removed tissues were embedded in 6x5 receiver blocks of the device. The sections obtained from these blocks were stained with H&E, and the accuracy of tissue removal was verified. The presence of tumor tissue was verified by reexamining the cases whose tumors could not be observed after blocking for technical reasons. Cases with small tumor tissue, premalignant and benign, were evaluated by examining sections taken directly from paraffin blocks.

The entire immunohistochemical staining process, including deparaffinization and antigen exposure, was performed with a fully automated immunohistochemistry stainer (Ventana BenchMark Ultra, Ventana Medical Systems, Tucson, AZ). From formalin-fixed, paraffin-embedded tissues, four µm-thick sections were prepared on positively charged slides. The slides were kept in an oven at 70°C for 1 hour. Antigen recovery was performed with ethylenediaminetetraacetic acid (EDTA) at pH:8. Incubation of the LRIG1 antibody at a dilution of 1/100 was performed for 1 hour. Harris Hematoxylin (Ventana Medical Systems) was used for background staining for 16 minutes. The bluing reagent (Ventana Medical Systems) was used for 4 minutes. The sections were completed with the hematoxylin and bluing solution, dehydrated, cleared with xylene, and covered with a coverslip, and the process was complete. The function of LRIG1 antibodies was confirmed by control staining. All slides were evaluated by the same two pathologists who were unaware of the patients' clinical data. The staining intensity after staining was classified into two different groups as follows; 0: not expressed and 1: expressed (Figure 1). If the scores were different in at least two other tissues of the same patient, the highest score was accepted. By evaluating the pathology reports of the cases, the tumor classifications were revised according to the 2017 revised American Joint Committee on Cancer (AJCC) TNM classification.

SPSS 25.0 program was used for statistical analysis, and $p<0.05$ was considered statistically significant.

RESULTS

Between 2003 and 2018, 219 patients had undergone total or partial laryngectomy and neck dissection for laryngeal pathology, and 88 patients who had undergone direct

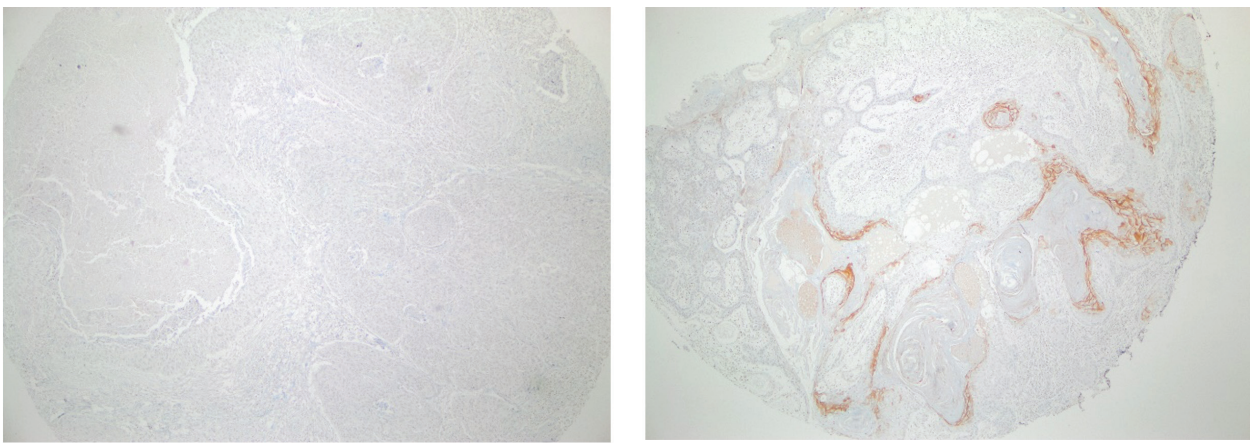

Figure 1: Expression of LRIG1 in tumoral tissue: On the left image, LRIG1 was not expressed, while LRIG1 expression was seen on the right image (X4 magnification)

laryngoscopy in the Department of Otolaryngology, Marmara University were included in the study. Twenty (12%) cases were female, and 283 (92.2%) were male. The mean age of the patients was 59.97±10.697. Of the patients, 258 (84%) had a smoking history, and 47 (15.3%) had an alcohol history. In addition, 91 patients received RT, and 70 patients received CT after primary surgery.

Comparison of LRIG1 expression and clinical and histopathological data

The relationship between the clinical and pathological data from the medical records and the expression of LRIG1 shown in Table 1.

There was a statistically significant difference between the tumor differentiation, diagnosis, and the expression of LRIG1 (respectively p=0.045, p<0.001). While LRIG 1 expression is more expressed in benign pathologies, it is significantly decreased in malignant cases. Furthermore, an increase in the degree of dysplasia in premalignant lesions correlates with a decrease in LRIG1 expression (p=0.015). Also, in the presence of cartilage invasion, the intensity of immunostaining of LRIG1 decreases (p=0.026).

LRIG1 expression was not related to age, sex, stage, smoking, alcohol, tumor localization, perineural invasion, lymphovascular invasion, extranodal spread, postoperative CT, and RT requirement (p>0.05).

Survival Analysis

The clinical and histopathologic prognostic parameters affecting the survival of patients in the study group were evaluated using the Kaplan-Meier method and a Cox regression analysis. The survival of cases expressing LRIG1 in tumor tissue was

Table 1: Tumoral clinical and histopathological features according to LRIG1 expression

		Not expressed		Expressed			
		n	%	n	%	X2	p
Premalignant	**CIS**	12	66.7	6	33.3	8.419	0.015*
	HGD	12	54.5	10	45.5		
	LGD	4	21.1	15	78.9		
Diagnosis	**Malign**	161	80.9	38	19.1	27.58	<0.001**
	Premalign	28	47.5	31	52.5		
	Benign	16	59.3	11	40.7		
Stage	**Early**	45	78.6	12	21.1	0.387	0.547
	Advanced	115	82.7	24	17.3		
Differentiation	**Well**	28	68.3	13	31.7	6.207	0.045*
	Moderately	91	84.3	17	15.7		
	Poorly	40	87.0	6	13.0		
Lymph node metastasis	**no**	106	79.1	28	20.9	1.928	0.235
	yes	55	87.3	8	12.7		
Extranodal extension	**no**	28	84.8	5	15.2	0.376	0.710
	yes	27	90.0	3	10.0		
Cartilage invasion	**no**	102	79.2	27	20.8	0.188	0.026*
	yes	57	86.6	9	13.4		
Perineural invasion	**no**	128	84.2	24	15.8	0.720	0.463
	yes	29	78.4	8	21.6		
Lenfovascular invasion	**no**	107	81.7	24	18.3	0.058	0.998
	yes	54	83.1	11	16.9		
Chemotherapy	**no**	108	81.2	25	18.8	0.103	0.846
	yes	54	83.1	11	16.9		
Radiotherapy	**no**	94	81.0	22	19.0	0.116	0.852
	yes	68	82.9	14	17.9		
Smoking	**no**	21	67.7	10	32.3	0.465	0.522
	yes	175	73.5	63	26.5		

*p<0.05, **p<0.01 CIS: Carcinoma-in-situ, HGD: High grade dysplasia, LGD: Low grade dysplasia

significantly better than that of tumor cases lacking LRIG1. While the mean survival time for the LRIG1+ tumors was 2396 days, it was 1505 days in the LRIG1- group (p=0.01) (Figure 2). Disease stage, cartilage invasion, and lymphovascular invasion were also significantly related to LSCC survival in an univariate Cox regression analysis. In contrast, differentiation, perineural invasion, and extranodal spread were not associated with LSCC (Table 2). However, in a multivariate Cox regression analysis, only the stage of disease was associated with LSCC survival (95%CI, 1.055-28.895, p=0.043).

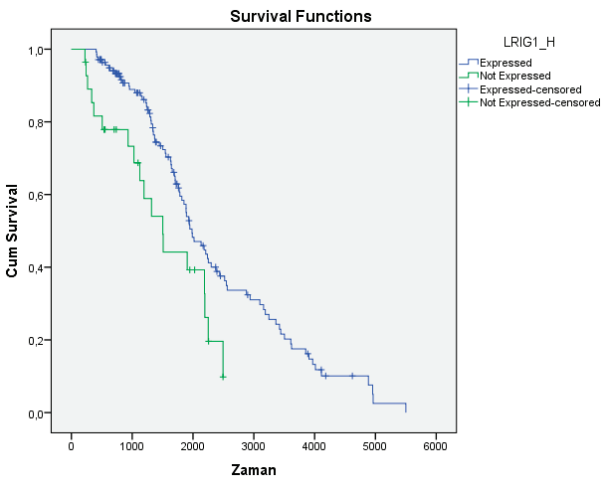

Figure 2: Survival of the patients according to LRIG1 expression

Therefore, LRIG1 is thought to be a tumor suppressor gene. Moreover, the 3p14 locus is deleted in many malignancies, supporting this view (7, 8, 12, 17).

LRIG1 has been shown to regulate contact inhibition in the lung cancer cell line. LRIG1 provides contact inhibition by forming a triple complex with E cadherin and EGFR. Induction of LRIG1 expression in these cells with weak endogenous LRIG1 expression significantly reduced tumor burden. Moreover, the reduced LRIG1 expression in early lesions compared with surrounding tissues suggests it is involved in the early stages of tumorigenesis (18). Also, LRIG1 is expressed in well- and moderately-differentiated tumors, whereas it is either weakly expressed or not expressed in undifferentiated tumors (19). Consistent with the literature, significant expression of LRIG1 was observed in benign pathologies in our study, whereas LRIG1 expression was decreased in premalignant and malignant cases. In addition, it was observed that LRIG expression decreased significantly when the degree of dysplasia increased in premalignant lesions (p=0.015). Also, in the presence of cartilage invasion, the intensity of immunostaining of LRIG1 decreases (p=0.026).

As the severity of dysplasia increases in premalignant lesions, the decrease in LRIG1 expression suggests that LRIG1 plays a role in the early stages of LSCC tumorigenesis. In our study, the severity of LRIG1 expression was found to be significantly lower in malignant pathologies than in premalignant and benign pathologies. Since the severity of dysplasia increases in

Table 2: Univariate cox regression analysis

	Hazard ratio	95% Confidential interval	p
Stage	3.598	1.620-7.993	0.001**
Differentiation	1.375	0.731-2.586	0.340
Perineural invasion	1.472	0.869-2.492	0.151
Cartilage invasion	1.910	1.242-2.936	0.003**
Lymphovascular invasion	1.949	1.263-3.008	0.003**
Extra nodal Spread	1.025	0.506-2.075	0.945
LRIG1	1.918	1.697-2270	0.011*

*p<0.05, **p<0.01

DISCUSSION

LRIG1 interacts with many tyrosine kinase receptors and inhibits EGFR, RET, and MET receptor signaling pathways in different ways. Inhibition of these tyrosine kinases regulate cell proliferation. LRIG1 increases EGFR receptor ubiquitination, leading to receptor degradation via ligand-dependent negative feedback and by exhibiting a paracrine effect (13, 14). Dysregulation of EGFR has been shown to play a role in the pathogenesis of many epithelial malignancies. Also, LRIG1 interacts directly with MET receptors and induces lysosomal degradation of the receptors independently of ubiquitination (15). LRIG1 interacts with the RET receptor and prevents binding and activation of the ligand to the RET receptor (16).

premalignant lesions, the decrease in LRIG1 expression suggests that it plays a role in the early stages of LSCC tumorigenesis.

In our study, the presence of cartilage invasion, the intensity of immunostaining of LRIG1 decreases (p=0.026).

Decreased LRIG1 expression has been shown to be a poor prognostic marker for survival in skin cancer, cervical cancer, breast cancer, and bladder cancer (9, 20, 21). In addition, increased LRIG1 expression in oropharyngeal cancer, vaginal cancer, and cervical adenocarcinoma has been shown to indicate a good prognosis and correlate with prolonged survival (12, 22, 23). In our study, the mean survival time of LRIG+ cases were found to be longer in LSCC cases than in LRIG- cases.

However, it was not detected as an independent variable in multivariate Cox regression analyses.

Once antitumor effects were understood, LRIG1 was tested as a target molecule for therapy. LRIG1 gene transfer was performed with viral agents onto tumor tissue generated from 16 bladder cancer cell lines. It was found that tumor burden was significantly lower in cases who underwent LRIG1 gene transfer than in the control group (24). LRIG1 was transferred to implanted glioblastoma cells in a similar study, and patients with LRIG+ had more prolonged survival (25).

Recent studies have suggested that tumor tissues with increased LRIG1 expression are associated with a better response to platinum-based chemotherapeutic agents (26, 27). The demonstration that cancers showing increased LRIG1 expression respond well to platinum-based chemotherapy, which is commonly used for head and neck tumors such as LSCC, indicates that LRIG1 may be a target for new treatment regimens to be developed.

CONCLUSION

Low LRIG1 expression was significantly associated with prolonged survival in our study. Moreover, the expression of LRIG1 decreases as the degree of differentiation decreases. At the same time, it is more strongly expressed in benign cases, while its expression decreases in malignant cases. Our findings suggest that LRIG1 plays a role in the early tumorigenesis of LSCC. Therefore, LRIG1 can be a target molecule for treatment approaches.

Limitations

We used the tissue microarray method to evaluate malignant tissue, therefore the malignant tissue could not be evaluated as a whole. The relationship between the tumor and the surrounding tissue could not be evaluated. The limitations of our study are that it is retrospective and was only assessed at the protein level. Since RT has become more important in the treatment of early stage patients of LC in recent years, the number of early stage cases of LSCC was limited.

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Ethics Committee Approval: This study was approved by the Clinical Research Ethics Committee dated 07/04/2017, number 09.2017.283.

Informed Consent: Written informed consent was obtained.

Peer-Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- Y.G., A.G., A.C.Y.; Data Acquisition- Y.G., A.G., S.Y.E., Z.L.C., T.M.; Data Analysis/ Interpretation- Y.G., O.A., A.C.Y.; Drafting Manuscript- Y.G., A.G., S.Y.E., T.M.; Critical Revision of Manuscript- Y.G., O.A., A.C.Y., Z.L.C.; Final Approval and Accountability- Y.G., A.G., O.A., A.C.Y., S.Y.E., T.M.

Conflict of Interest: Authors declared no conflict of interest.

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REFERENCES

1. Howlader N, Noone A, Krapcho M, Miller D, Brest A, Yu M, et al. SEER Cancer Statistics Review, 1975–2018, National Cancer Institute. Bethesda, MD. 2018. https://seer.cancer.gov/statfacts/html/laryn.html (Accession date: 25.04.2022)
2. Nocini R, Molteni G, Mattiuzzi C, Lippi G. Updates on larynx cancer epidemiology. Chin J Cancer Res 2020;32(1):18.
3. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021;71(3):209-49.
4. Pruess M, Fleischmann W, Kanapin A, Karavidopoulou Y, Kersey P, Kriventseva E, et al. The Proteome Analysis database: a tool for the in silico analysis of whole proteomes. Nucleic Acids Research 2003;31(1):414-7.
5. Kobe B, Kajava AV. The leucine-rich repeat as a protein recognition motif. Curr Opin Struct Biol 2001;11(6):725-32.
6. Nilsson J, Vallbo C, Guo D, Golovleva I, Hallberg B, Henriksson R, et al. Cloning, characterization, and expression of human LIG1. Biochem Biophys Res Commun 2001;284(5):1155-61.
7. Hedman Hk, Nilsson J, Guo D, Henriksson R. Is LRIG1 a tumour suppressor gene at chromosome 3p14.3? Acta Oncol 2002;41(4):352-4.
8. Jensen KB, Watt FM. Single-cell expression profiling of human epidermal stem and transit-amplifying cells: Lrig1 is a regulator of stem cell quiescence. PNAS 2006;103(32):11958-63.
9. Tanemura A, Nagasawa T, Inui S, Itami S. LRIG-1 provides a novel prognostic predictor in squamous cell carcinoma of the skin: immunohistochemical analysis for 38 cases. Dermatol Surg 2005;31(4):423-30.
10. Thomasson M, Wang B, Hammarsten P, Dahlman A, Persson JL, Josefsson A, et al. LRIG1 and the liar paradox in prostate cancer: a study of the expression and clinical significance of LRIG1 in prostate cancer. Int J Cancer 2011;128(12):2843-52.
11. Guo D, Nilsson J, Haapasalo H, Raheem O, Bergenheim T, Hedman H, et al. Perinuclear leucine-rich repeats and immunoglobulin-like domain proteins (LRIG1-3) as prognostic indicators in astrocytic tumors. Acta Neuropathol 2006;111(3):238-46.
12. Lindquist D, Kvarnbrink S, Henriksson R, Hedman H. LRIG and cancer prognosis. Acta Oncol 2014;53(9):1135-42.
13. Suzuki Y, Sato N, Tohyama M, Wanaka A, Takagi T. cDNA cloning of a novel membrane glycoprotein that is expressed specifically in glial cells in the mouse brain: LIG-1, a protein with leucine-rich repeats and immunoglobulin-like domains. J Biol Chem 1996;271(37):22522-7.
14. Yi W, Holmlund C, Nilsson J, Inui S, Lei T, Itami S, et al. Paracrine regulation of growth factor signaling by shed leucine-rich repeats and immunoglobulin-like domains 1. Exp Cell Res 2011;317(4):504-12.

15. Shattuck DL, Miller JK, Laederich M, Funes M, Petersen H, Carraway III KL, et al. LRIG1 is a novel negative regulator of the Met receptor and opposes Met and Her2 synergy. Mol Cell Biol 2007;27(5):1934-46.
16. Ledda F, Bieraugel O, Fard SS, Vilar M, Paratcha G. Lrig1 is an endogenous inhibitor of Ret receptor tyrosine kinase activation, downstream signaling, and biological responses to GDNF. J Neurosci 2008;28(1):39-49.
17. Jensen KB, Collins CA, Nascimento E, Tan DW, Frye M, Itami S, et al. Lrig1 expression defines a distinct multipotent stem cell population in mammalian epidermis. Cell Stem Cell 2009;4(5):427-39.
18. Lu L, Teixeira VH, Yuan Z, Graham TA, Endesfelder D, Kolluri K, et al. LRIG1 regulates cadherin-dependent contact inhibition directing epithelial homeostasis and pre-invasive squamous cell carcinoma development. J Pathol 2013;229(4):608-20.
19. Jensen KB, Jones J, Watt FM. A stem cell gene expression profile of human squamous cell carcinomas. Cancer Letters 2008;272(1):23-31.
20. Muller S, Lindquist D, Kanter L, Flores-Staino C, Henriksson R, Hedman H, et al. Expression of LRIG1 and LRIG3 correlates with human papillomavirus status and patient survival in cervical adenocarcinoma. Int J Oncol 2013;42(1):247-52.
21. Krig SR, Frietze S, Simion C, Miller JK, Fry WH, Rafidi H, et al. Lrig1 is an estrogen-regulated growth suppressor and correlates with longer relapse-free survival in ERα-positive breast cancer. Mol Cancer Res 2011;9(10):1406-17.
22. Lindquist D, Näsman A, Tarjan M, Henriksson R, Tot T, Dalianis T, et al. Expression of LRIG1 is associated with good prognosis and human papillomavirus status in oropharyngeal cancer. Br J Cancer 2014;110(7):1793-800.
23. Ranhem C, Lillsunde Larsson G, Hedman H, Lindquist D, Karlsson MG, Hellström A-C, et al. Expression of LRIG proteins as possible prognostic factors in primary vaginal carcinoma. PLoS One 2017;12(8):e0183816. doi: 10.1371/journal.pone.0183816
24. Li F, Ye Z-Q, Guo D-S, Yang W-M. Suppression of bladder cancer cell tumorigenicity in an athymic mouse model by adenoviral vector-mediated transfer of LRIG1. Oncol Rep 2011;26(2):439-46.
25. Johansson M, Oudin A, Tiemann K, Bernard A, Golebiewska A, Keunen O, et al. The soluble form of the tumor suppressor Lrig1 potently inhibits in vivo glioma growth irrespective of EGF receptor status. Neuro Oncol 2013;15(9):1200-11.
26. Stutz MA, Shattuck D, Laederich M, Carraway KL, Sweeney C. LRIG1 negatively regulates the oncogenic EGF receptor mutant EGFRvIII. Oncogene 2008;27(43):5741-52.
27. Li F, Yang W, Guo D, Hu Z, Xu H, Ye Z. LRIG1 combined with cisplatin enhances bladder cancer lesions via a novel pathway. Oncol Rep 2011;25(6):1629-37.

RESEARCH ARTICLE
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A Comparison of Fas-Fas Ligand Mediated Apoptosis with Clinical and Pathological Parameters in Larynx Cancers; Twenty Years After Laryngectomy

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ABSTRACT

Objective: Larynx cancer constitutes 2% of all cancers in adults with 96% of larynx malignancies being squamous cell carcinoma (SHC). Apoptosis is a cell death mechanism that is quite different from necrosis. It is also known as programmed cell death, physiological cell death, or cell suicide. Physiological mediators that activate programmed cell death (apoptosis) are Fas and Fas Ligand (FasL).
Materials and Methods: In a thesis study conducted by our authors in 2004, we investigated the relationship of Fas-Fas ligand-mediated apoptosis with survival in laryngeal cancer, prognostic factors (age, localization, histological grade, tumor size, lymph, blood vessel invasion), stage, and inflammatory response of the tumor. In this study, we investigated the relationship between survival and death rates after 20 years and Fas-FasL.
Results: When FAS was evaluated 20 years later, a statistically significant difference was found between mortality rates depending on stage ($p=0.023$; $p<0.05$). While the survival rate is higher in stage 1 cases, the rate of death is higher in stage 3 cases. No statistically significant difference was found between mortality rates according to stages for FAS Ligand 20 years later ($p>0.05$).
Conclusion: In conclusion, we found that the Fas/Fas-L system was not associated with clinical parameters in laryngeal cancers in our short-term follow-up. However, when we repeated our follow-up 20 years later, we found that Fas system deficiency, although not in FAS Ligand, adversely affected survival in the long term in laryngeal cancer patients.

Keywords: Fas, Fas ligand, laryngectomy, larynx cancer, squamous cell tumor

INTRODUCTION

Larynx cancer constitutes 2% of all cancers in adults. This rate is 2.2% in men and 0.4% in women. 96% of larynx malignancies are squamous cell carcinoma (SHC), and 26% of this type of malignancy in the head and neck region is located in the larynx (1).

Apoptosis is a cell death mechanism that is quite different from necrosis, which is known as the classical form of cell death, in terms of many features. It has also been found to be implicated in many other pathological conditions including autoimmune disorders, AIDS, certain major neurodegenerative disorders including Alzheimer's disease, and even malignancies. Apoptosis is also known as programmed cell death, physiological cell death, or cell suicide (2).

Physiological mediators that activate programmed cell death (apoptosis) are Fas and Fas Ligand (FasL). Fas is found in lymphoid cells, hepatocytes, some tumor cells, lungs, and even the myocardium. Its ligand of interest is called Fas ligand (FasL). FasL, tumor necrosis. It is a member of the factor (TNF) family. FasLis found in cells of cytotoxic T lymphocytes and "natural killers" (3).

Fas-FasL interaction with the cell membrane surface has a very important role in killing tumor cells by cytotoxic T lymphocytes and natural killer (NK) cells. Expression of Fas Ligand in tumor

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cells helps tumor cells to evade the immune system by developing resistance to Fas-related apoptosis (4).

In a thesis study conducted by our authors in 2004, we investigated the relationship of Fas-Fas ligand-mediated apoptosis with survival in laryngeal cancer, prognostic factors (age, localization, histological grade, tumor size, lymph, blood vessel invasion), stage, and inflammatory response of the tumor. In this study, we investigated the relationship between survival and death rates after 20 years and Fas-FasL.

MATERIALS AND METHODS

In the first phase of our study, 20 years ago, between December 2000 and October 2003, the cases that involved operations for laryngeal carcinoma in the Haseki Training and Research Hospital Ear Nose and Throat Clinic were scanned in the Pathology laboratory. Of these cases, 60 (59 males, 1 female) were selected for inclusion in the study. The patients in these cases were between the ages of 35-82 and the mean age was 58.3±9.71 years. All specimens from the cases were reviewed and evaluated. All individuals in this study signed a written informed consent form for participation. This work was done under the principles of the Declaration of Helsinki. Because this was a retrospective study and there was no experimental intervention involved, ethical committee approval was not needed.

The most suitable tumor blocks were selected for immunohistochemical examination. Clinical data of the cases were obtained from archive records. Age at the time of diagnosis was divided into 2 groups – those under 60 years old and those above. In terms of the largest diameter of the primary tumor, it was evaluated in two groups as being greater than 4 cm and less than 4 cm. The tumor was divided into 3 groups - supraglottic, glottic, and transglottic according to the location in the larynx. Histological grade was classified as good, moderate, and poorly differentiated (grade 1,2,3) considering the degree of keratinization, pearl formation, and the presence of intercellular bridges between tumor cells. Inflammatory and desmoplastic responses around the tumor were subjectively defined as weak, moderate, and severe. Lymphovascular, perineural, and any cartilage invasion were evaluated. The primary tumor (T), the status of lymph nodes (N), presence or absence of distant metastases (M) were evaluated. It was grouped as early-stage (stage 1-2) and advanced stage (stage 3-4) according to AJCC (American Joint Committee on Cancer) criteria.

Immunohistochemical Examination

2-3 µm paraffin sections of the tumors were placed on slides pretreated with Histogrip (Zymed) and kept in an oven at 37°C overnight. Sections were clarified in xylene and rehydrated in alcohol. Sections were heated in a microwave oven for 3x5 minutes in 10 mM citrate buffer (pH 6) to reveal antigen. It was allowed to cool at room temperature for 20-30 minutes in the same buffer. To eliminate the endogenous peroxidase activity in the tissues, a 3% hydrogen peroxide solution was dripped onto the sections and left for 10 minutes and then into a protein block solution (TA-125-UB, Lab Vision Corp. Fremont, CA, USA, 10 min), primary antibody, secondary antibody (TA-125-BN, Lab Vision Corp. Fremont, CA, USA, 10 min) and treated with streptavidin-HRP (TA-125-HR, Lab Vision Corp. Fremont, CA, USA, 10 min). Aminoethyl carbazole (TA-125-HA, Lab Vision Corp. Fremont, CA, USA, 20 min) was used as the chromogen. Sections were rinsed with PBS after each treatment. Finally, it was counterstained with Mayer's hematoxylin and closed. negative controls. This was done by skipping the primary antibody step. Positive controls: Small intestinal mucosa and lymph node sections were used for Fas, prostate and testis sections were used for FasL.

Early assessment

The following antibodies were used in immunohistochemistry: CD95 (FAS) Ab-3 (Clone 95 C03, Mouse monoclonal antibodies Neomarkers, Fremont, CA, USA, 1/20 dilution 120 min), Moroccan Ligand Ab-1 (Clone FSL01, Mouse monoclonal antibodies, Neomarkers, Fremont, CA, USA, 1/15 dilution 180 min). Fas immunohistochemistry showed a positive reaction as intracytoplasmic granular staining. Staining was observed in tumor cells and stromal lymphoplasmacytic cells. Fas Ligand (FasL) immunohistochemistry revealed a positive reaction as intracytoplasmic granular staining. Staining was observed in tumor cells and stromal lymphoplasmacytic cells. A total of 1000 cells were counted in the area where staining was most prevalent in the tumor. Cells that showed positive reactions were identified and their percentages were calculated. Staining intensity was not taken into account. Evaluations were made under the Olympus B×50 light microscope at 400× (40× objective lens, 10× ocular lens, 0.151 mm^2) magnification. Statistical analysis of the data was performed using the chi-square test, Fisher's exact test, Kruskal Wallis analysis of variance, and Mann-Whitney-U test in the SSPS/PC program. p values below 0.05 were considered significant.

Evaluation after 20 years

We reached out to our patients or their relatives from the contact information in the archive to find out if they were still alive. If they were alive, we questioned whether there was any sign of tumor recurrence. Since we could not obtain clear information about the cause of death in the deceased patients we reached, we could not learn whether the deaths were due to tumor recurrence.

RESULTS

Early Results

In the early period, no statistically significant relationship could be demonstrated between Fas and age, diameter, grade, localization, blood vessel invasion, perineural invasion, cartilage invasion, lymph node involvement, and survival. Fas Ligand (FasL) staining showed a positive reaction with immunohistochemistry as intracytoplasmic granular staining. Staining was observed in tumor cells and stromal lymphoplasmacytic cells. Staining was not observed in 3 (5%) cases (Stage I), 1-10% (Stage II) in 41 (68.3%) cases, 11-25% (Stage III) in 14 (23.3%) cases, 2 (3%) cases. 3) staining was

observed at a rate of 26-50% (Stage IV) in one case. There was no correlation between the other parameters compared with FasL in the cases. There was no correlation between Fas and FasL ratios in tumor cells.

Results after 20 years

We designed our study 20 years later to find out the survival status of patients by accessing their file information. We were able to reach 36 of 60 (9 Ex) cases.

Since we could not meet face-to-face with our patients due to covid restrictions, we reached out to our patients or their relatives via telephone. We asked our questions from a standard questionnaire we had prepared. According to the information given, we were able to obtain information on current survival and tumor recurrence status. While our questionnaire had more questions, due to the large number of deceased patients, the answers to most questions did not yield a statistically significant result. We had to be content with just assessing survival rates.

There was no history of tumor recurrence or metastasis in the healthy patients. We found and compared the fas and fas ligand staining rates of right and ex-patients from their files. 24 of 36 cases had died. Three of the 12 survivors had received radiotherapy within 5 years of surgery. The survivors had no morbidity other than permanent laryngeal tracheostomy. Nine of the 12 surviving patients were retired and not working elsewhere (Table 1).

Fas evaluations

Initially, in FAS cases, no statistically significant difference was found between stages and mortality rates (p>0.05).

When FAS was evaluated 20 years later, a statistically significant difference was found between mortality rates according to the stage (p=0.023; p<0.05). While the survival rate was higher in stage 1 cases, the rate of death is higher in stage 3 cases (Table 2).

In stage I cases, 22.2% of the cases died in the initial period, and only 3 cases could be questioned after 20 years, and since all of them lived, no significant difference was found between the mortality rates of the two periods (p>0.05).

In stage II cases, 11.9% of the cases died in the initial period, and this rate was 66.7% after 20 years; a significant difference was found between the mortality rates of the two periods (p<0.01).

In stage III cases, 11.1% of the cases died in the initial period, and this rate was 88.9% after 20 years; a significant difference was found between the mortality rates of the two periods (p<0.01) (Figure 1) (Table 2).

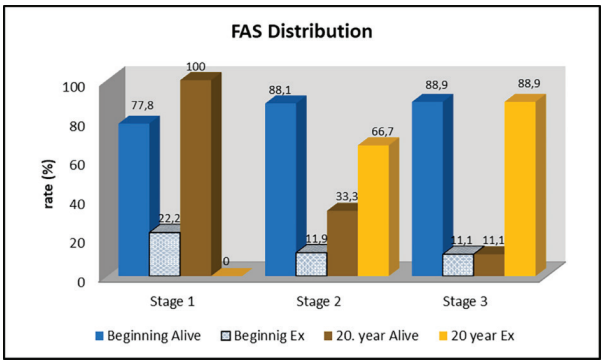

Figure 1: Fas Distribution

Table 1: Questionnaire for follow up of long-term survival

1) Is the patient alive?
2) If he is dead, what is the cause of death?
3) If alive, is there any recurrence?
4) If alive, is there any metastasis?
5) If alive, is there lymph node involvement?
6) Is there any morbidity related to the surgery?
7) Is there any morbidity related to the disease?
8) Has he received chemotherapy or radiotherapy in the long term after the operation?
9) If he has received chemotherapy or radiotherapy, is there any associated morbidity?
10) Has laryngeal cancer adversely affected your work life?
11) Has laryngeal cancer adversely affected family life?
12) If so, how many years have passed since the patient's death?

Table 2: FAS Evaluations

	FAS Beginning (n=60)		FAS 20 years later (n=36)		[b]*p*
	Alive	**Ex**	**Alive**	**Ex**	
Stage 1	7 (77.8)	2 (22.2)	3 (100)	0 (0)	**1.000**
Stage 2	37 (88.1)	5 (11.9)	8 (33.3)	16 (66.7)	**0.001****
Stage 3	16 (88.9)	2 (11.1)	1 (11.1)	8 (88.9)	**0.001****
p	[a]**0.763**		[a]**0.023***		

[a]Fisher Freeman Halton Test, [b]Fisher Exact test, *p<0.05, **p<0.01

Fas Ligand evaluations

In the initial evaluations of FAS Ligand, no statistically significant difference was found between death rates according to the stage (p>0.05).

No statistically significant difference was found between mortality rates according to stages for FAS Ligand 20 years later (p>0.05) (Table 2).

In the stage I cases, 40% of the cases died in the initial period, and this rate was found to be 25% after 20 years. There was no significant difference between the mortality rates of the two periods (p>0.05).

In stage II cases, 10.9% of the cases died in the initial period, and this rate was 71.4% after 20 years. A significant difference was found between the mortality rates of the two periods (p<0.01).

In stage III cases, 12.5% of the cases died in the initial period, and this rate was 72.7% after 20 years. A significant difference was found between the mortality rates of the two periods (p<0.01).

In stage IV cases, 100% of the cases died in the initial period and no cases could be questioned after 20 years, so the difference between them could not be examined (Figure 2) (Table 3).

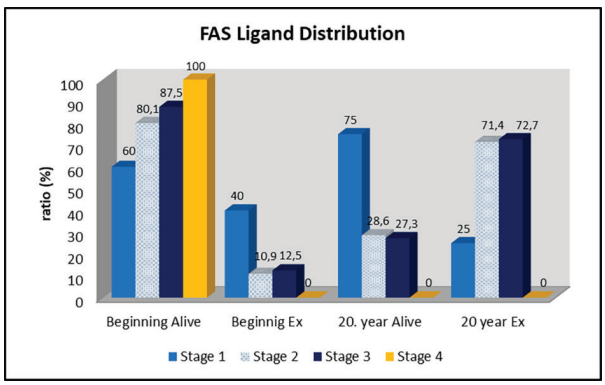

Figure 2: Fas Ligand Distribution

DISCUSSION

Tumors developed because the balance between spread and death was disrupted. Tumor antigens can cause an immune reaction that can destroy tumor cells. In both steps, defects in the Fas system contribute to tumorigenesis (5).

In our retrospective study, we investigated Fas and Fas-ligand expression (release) in laryngeal squamous cell cancer and analyzed its relationship with clinical-pathological parameters. We evaluated the association of Fas and Fas-ligand expression (release) with survival (6).

As an important result, we observed that Fas immunoreactivity decreased in advanced-stage tumors as the T stage of the tumor increased. This showed us that down-regulation of cell-surface Fas receptors is accompanied by an increase in tumor stage and T-stage. The clinical significance of Fas release in head and neck cancers is still not fully understood. Previous studies of Fas expression in these organ tumors included limited specimens and gave conflicting results. Although high levels of Fas expression were found in tongue cancer (6), significant suppression of Fas receptors was observed in other series of oral squamous cell cancers compared with normal epithelium (7). There is a proven association between the functional FAS and FASL and the risk of developing laryngeal and hypopharyngeal squamous cell carcinomas (8).

While Muraki et al found a relationship between Fas release and clinical staging in well and moderately differentiated oral carcinomas, they did not observe such a relationship in poorly differentiated oral carcinomas (9). Similar contradictory results were obtained from esophageal squamous cell carcinomas. (10, 11) It seems that downregulation of Fas is exhibited as a feature of some tumors. This hypothesis is in line with our study, which had broadly distributed results obtained as a result of Fas staining in laryngeal carcinomas. However, it is not an essential step in the malignant transformation of the squamous epithelium (12). Another important result we obtained was the positive correlation between Fas immunoreactivity and lymphoplasmacytic stroma reaction. This result showed us that down-regulation of Fas receptors on the cell surface may be accompanied by the absence of inflammatory cells infiltrating the tumor stroma. The same results were obtained from gene transfer experiments on murine tumor cells (13).

Table 3: FAS Ligand Evaluation

	FAS Ligand Beginning (n=60)		FAS Ligand 20 years later (n=36)		[b]p
	Alive	Ex	Alive	Ex	
Stage 1	3 (60.0)	2 (40.0)	3 (75)	1 (25)	**1.000**
Stage 2	41 (89.1)	5 (10.9)	6 (28.6)	15 (71.4)	**0.001****
Stage 3	14 (87.5)	2 (12.5)	3 (27.3)	8 (72.7)	**0.003****
Stage 4	2 (100)	0 (0)	0 (0)	0 (0)	-
p	[a]**0.366**		[a]**0.198**		

[a]Fisher Freeman Halton Test, [b]Fisher Exact test, *p<0.05, **p<0.01

Shibakita et al. reported that there is a correlation between strong Fas ligand release and a decrease in CD8 T-lymphocytes in esophageal squamous cell carcinomas (14). In the study of Kase S. et al., the frequency of Fas-ligand release was high in T3 squamous cell esophageal carcinoma, respectively, followed by T2 squamous cell esophageal carcinoma, and then dysplasia (14). Shibakita found no association between Fas-ligand release and survival (13). It has been reported that Fas-ligand release in esophageal squamous cell carcinomas may be associated with tumor progression and poor prognosis (15). Ewing sarcomas have more metastatic tumors than Fas-ligand primary tumors. It is secreted frequently and in larger volumes. Similarly, Fas-ligand is secreted less in non-aggressive non-Hodgkin lymphomas and more in aggressive non-Hodgkin lymphomas (16). Older patients, greater tumour size and lymph node positivity were found to be associated with high expression of FasL in tobacco-related intraoral squamous cell carcinoma (17).

It has been reported that papillary thyroid carcinomas with aggressive histology and extensive/locally invasive/recurrent poor prognostic features show stronger Fas-ligand immunoreactivity compared to thyroid carcinomas that are well-differentiated and do not have poor prognostic features (18).

Studies have been carried out using a Fas activation system in the treatment of several types of cancer including gastric, prostate, and glioblastoma. Positive results were obtained in these trials. These results recommend that FasL gene therapy may become a potent treatment for HNSCC. (19) In this study, a positive correlation was found between Fas and the inflammatory response (p=0.019). In cases with weak inflammatory response, staining with Fas is weak (1-10%) or not observed at all. As the inflammatory response increased, the rate of staining with Fas increased. In a recent study 20 years ago, no statistically significant relationship could be shown between Fas and survival in the cases.

Studies have shown that squamous cell carcinoma of the Head and Neck is sensitive to apoptotic inducers such as etoposide (VP-16), but resistance to agonistic antibodies such as CH11has been noticed. We mentioned above that this result was also found in cancerous cells in thyroid specimens. Such a result indicates that tumor cells are strongly Fas-resistant, protecting themselves from apoptosis via Fas-ligand on tumor cells or Fas-ligand in plasma/serum.

Head and neck squamous cell cancers contribute to escape from immune effector cells, such as Fas resistance, by secreting bcl-2 as an additional defense mechanism (3) Fas-related phosphatase-1, protein kinase C, and FLICE protein inhibitor FL1P, which inhibit apoptotic signals by acting on the COOH terminal of FAS, are examples of additional protection mechanisms. It is shown, that there are associations of FAS and FAS Ligand with elevated hematological toxicity, ototoxicity, and lessened survival of tobacco- and alcohol-related head and neck squamous cell patients homogeneously treated with chemoradiation (20).

CONCLUSION

In conclusion, we found that the Fas/Fas-L system was not associated with clinical parameters in laryngeal cancers in our short-term follow-up. However, when we repeated our follow-up 20 years later in the long term, we found that Fas system deficiency, although not in FAS Ligand, adversely affected survival in laryngeal cancer patients.

Ethics Committee Approval: This work was done under the principles of the Declaration of Helsinki. Because this was a retrospective study and there was no experimental intervention involved, ethical committee approval was not needed.

Informed Consent: Written informed consent was obtained.

Peer-Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- A.M.B., T.E.; Data Acquisition- A.M.B., T.E.; Data Analysis/Interpretation- A.M.B., T.E.; Drafting Manuscript- A.M.B., T.E.; Critical Revision of Manuscript- A.M.B., T.E.; Final Approval and Accountability- A.M.B., T.E.

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REFERENCES

1. Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer statistics, 2007. CA Cancer J Clin 2007;57(1):43-66.
2. Kerr JF, Winterford CM, Harmon BV. Apoptosis. Its significance in cancer and cancer therapy. Cancer 1994;73(8):2013-26.
3. Gastman BR, Atarshi Y, Reichert TE, Saito T, Balkir L, Rabinowich H, et al. Fas ligand is expressed on human squamous cell carcinomas of the head and neck and it promotes apoptosis of T lymphocytes. Cancer Res 1999;59(20):5356-64.
4. Chinnaiyan AM, Tepper CG, Seldin MF, O'Rourke K, Kischkel FC, Hellbardt S, et al. FADD/MORT1 is a common mediator of CD95 (Fas/APO-1) and tumor necrosis factor receptor-induced
5. Hug H. Fas-mediated apoptosis in tumor formation and defense. Biol Chem 1997;378(12):1405-12.
6. Sundelin K, Jadner M, Norberg-Spaak L, Davidsson A, Hellquist HB. Metallothionein and Fas (CD95) are expressed in squamous cell carcinoma of the tongue. Eur J Cancer 1997;33(11):1860-4.
7. Loro LL, Vintermyr OK, Johannessen AC, Liavaag PG, Jonsson R. Suppression of Fas receptor and negative correlation of Fas ligand with differentiation and apoptosis in oral squamous cell carcinoma. J Oral Pathol Med 1999;28(2):82-7.
8. Wang J, Gao J, Li Y, Zhao X, Gao W, Peng L, Yan D, et al. Functional polymorphisms in FAS and FASL contribute to risk of squamous cell carcinoma of the larynx and hypopharynx in a Chinese population. Gene 2013;524(2):193-6.
9. Muraki Y, Yoshioka C, Tateishi A, Fukuda J, Haneji T, Kobayashi N. Localization of Fas antigen in oral squamous cell carcinoma. Br J Oral Maxillofac Surg 1999;37(1):37-40.
10. Leithäuser F, Dhein J, Mechtersheimer G, Koretz K, Brüderlein S, Henne C, et al. Constitutive and induced expression of APO-1, a new member of the nerve growth factor/tumor necrosis factor receptor superfamily, in normal and neoplastic cells. Lab Invest 1993;69(4):415-29.

11. Gratas C, Tohma Y, Barnas C, Taniere P, Hainaut P, Ohgaki H. Up-regulation of Fas (APO-1/CD95) ligand and down-regulation of Fas expression in human esophageal cancer. Cancer Res 1998;58(10):2057-62.
12. Jäckel MC, Mitteldorf C, Schweyer S, Füzesi L. Clinical relevance of Fas (APO-1/CD95) expression in laryngeal squamous cell carcinoma. Head Neck 2001;23(8):646-52.
13. Lee JK, Sayers TJ, Brooks AD, Back TC, Young HA, Komschlies KL, et al. IFN-gamma-dependent delay of in vivo tumor progression by Fas overexpression on murine renal cancer cells. J Immunol 2000;164(1):231-9.
14. Shibakita M, Tachibana M, Dhar DK, Kotoh T, Kinugasa S, Kubota H, et al. Prognostic significance of Fas and Fas ligand expressions in human esophageal cancer. Clin Cancer Res 1999;5(9):2464-9.
15. Kase S, Osaki M, Adachi H, Kaibara N, Ito H. Expression of Fas and Fas ligand in esophageal tissue mucosa and carcinomas. Int J Oncol 2002;20(2):291-7.
16. Müllauer L, Mosberger I, Chott A. Fas ligand expression in nodal non-Hodgkin's lymphoma. Mod Pathol 1998;11(4):369-75.
17. Das SN, Khare P, Singh MK, Sharma SC. Fas receptor (CD95) & Fas ligand (CD178) expression in patients with tobacco-related intraoral squamous cell carcinoma. Indian J Med Res 2011;134(1):54-60.
18. Mitsiades N, Poulaki V, Mastorakos G, Tseleni-Balafouta ST, Kotoula V, Koutras DA, et al. Fas ligand expression in thyroid carcinomas: a potential mechanism of immune evasion. J Clin Endocrinol Metab 1999;84(8):2924-32.
19. Ji X, Jiang C, Liu Y, Bu D, Xiao S. Fas ligand gene transfer effectively induces apoptosis in head and neck cancer cells. Acta Otolaryngol 2011;131(8):876-81.
20. Costa EFD, Lima TRP, Lopes-Aguiar L, Nogueira GAS, Visacri MB, Quintanilha JCF, et al. FAS and FASL variations in outcomes of tobacco- and alcohol-related head and neck squamous cell carcinoma patients. Tumour Biol 2020;42(7):1010428320938494. doi: 10.1177/1010428320938494.

CASE REPORT
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Endoscopic Endonasal Approach to a Giant Dentigerous Cyst

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ABSTRACT

Dentigerous cysts constitute 20% of all odontogenic cysts and are often located in the mandible and maxilla. They are often seen at young ages and in men. Patients are usually asymptomatic, and the diagnosis is established by dental radiographs in routine scans. Surgery is recommended for dentigerous cysts because ameloblastoma, intraosseous mucoepidermoid carcinoma, or intraosseous squamous cell carcinoma may develop from them. Generally the transoral route is preferred over the endoscopic route for dentigerous cysts located in the maxilla. In addition to the question of the transnasal versus oral approach to dentigerous cysts, another controversy is the removal or marsupialization of the entire cyst. In our 10-year-old male patient, a cyst that completely filled the right maxillary sinus and eroded the lateral and anterior wall of the maxillary sinus was treated with transnasal endoscopic surgery. The cyst wall and 3 permanent teeth were removed endoscopically. No post-operative complications were observed. The patient's age and the location and size of the cyst play an important role in the choice of treatment. Furthermore, the most accurate way to choose the appropriate treatment is to make the treatment decision with a multidisciplinary approach.

Keywords: Endoscopic surgery, Dentigerous cyst, Odontogenic Cyst

INTRODUCTION

Dentigerous cysts constitute 20% of all odontogenic cysts. They are usually seen between the ages of 10 and 30 and are more common in men. They form around the crown of an unerupted tooth. The pressure of the erupting tooth on the follicle is the cause of the cyst. That pressure prevents normal blood flow, leading to fluid accumulation between the enamel membrane tissue and coronal of the tooth. This will cause inflammation and infection, and finally, a dentigerous cyst. Patients are usually asymptomatic, and the diagnosis is established by dental radiographs in routine scans. X-ray imaging shows a well-defined unilocular radiolucency surrounding the crown of the affected tooth, with a good sclerotic margin. While marsupialization may be preferred for large lesions in treatment, enucleation is generally preferred (1, 2).

CASE REPORT

Written informed consent was obtained from the patient's family for this case report.

A 10-year-old male patient applied to the dental clinic for a check-up examination. Mild swelling was observed on the right cheek. It was observed that the baby teeth numbered 1, 2, and 3 on the upper right were still in place. In the panoramic x-ray, radiopaque areas surrounded by the sclerotic border in the right maxillary sinus and thought to belong to 3 permanent teeth were observed (Figure 1). For detailed evaluation, a PNS CT was taken and a dentigerous cyst was observed, obliterating the right maxillary sinus almost completely and containing 3 unerupted permanent teeth, one on the floor of the maxillary sinus, the other on the medial wall, and the last one on the floor of the orbit (Figure 2). It was observed that the cyst had severely thinned the anterior and lateral walls of the

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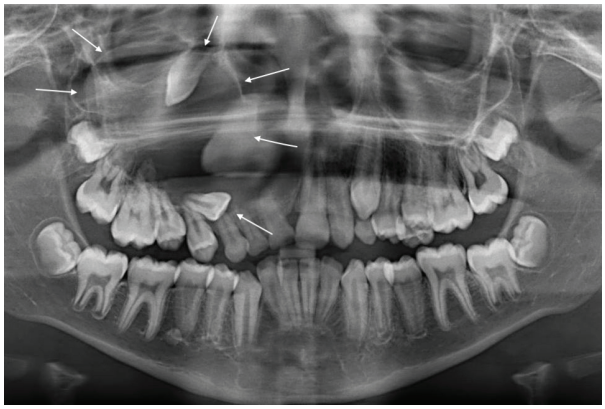

Figure 1: Panoramic X-Ray Imaging of Cyst

maxillary sinus. The patient was operated on endoscopically under general anesthesia and the cyst wall and 3 permanent teeth were removed endoscopically (Figure 3). Post-operative bleeding was controlled and a merocell tampon was placed in the nasal cavity. The patient's tampon was removed postoperative day 1 and the patient was discharged. The pathology result was confirmed as a dentigerous cyst and no postoperative complications were observed.

SURGICAL PROCEDURE

The patient was intubated orotracheal under general anesthesia. Appropriate field treatment was provided for endoscopic transnasal surgery. The operation was started

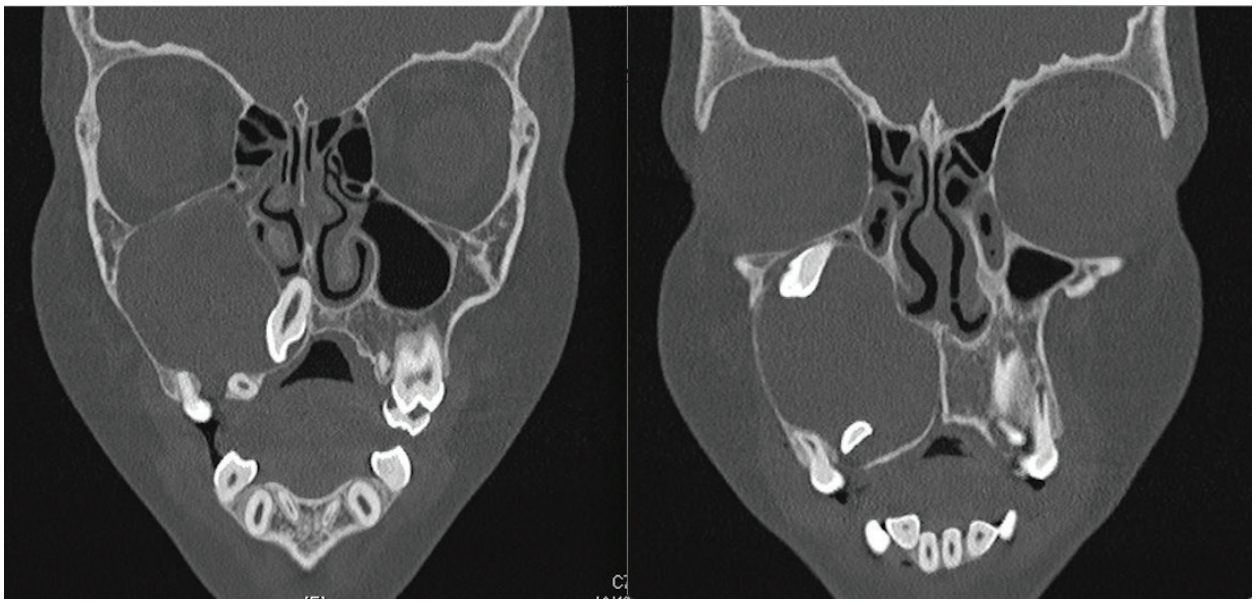

Figure 2: Computerized Tomography Imaging of Cyst

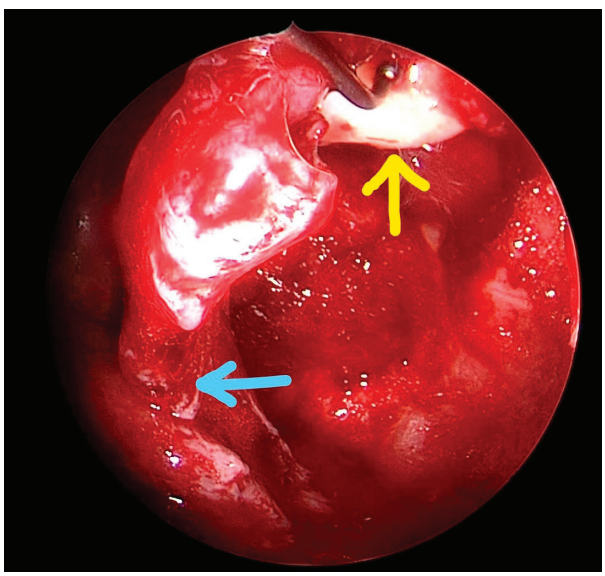

Figure 3: Endoscopic view of maxillary sinus (70 degree) Blue arrow: cyst wall, Yellow arrow: tooth on the orbital base

from the right nasal cavity with a 0 degree endoscope. The anterior 1/2 of the inferior turbinate and the anterior 1/3 of the middle turbinate were excised for visualization. Uncinectomy was performed and wide maxillary antrostomy was performed. The cyst wall was released from the maxillary sinus. The teeth at the base of the sinus and against the medial wall were removed with a 45-degree endoscope. The tooth at the base of the orbit was visible with a 70-degree endoscope and was removed. Then the entire cyst wall was removed (Figure 4).

DISCUSSION

Dentigerous cysts are most commonly seen in the mandible and secondarily in the maxilla. Generally the transoral route is preferred for dentigerous cysts located in the maxilla rather than the endoscopic route (2, 3). However, we did not prefer the transoral route for this cyst; it can cause serious weakening of the anterior and lateral wall of the maxillary sinus. Our main concern was to avoid creating bone fenestration inside the mouth as the result of an uncontrolled fracture or post-surgical

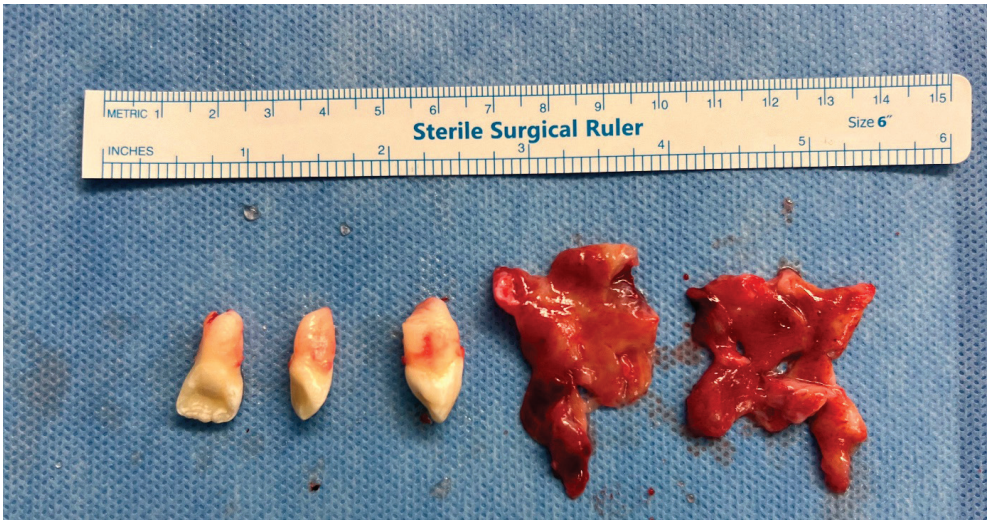

Figure 4: Postoperative Cyst wall and teeth

fistula formation. For all these reasons, we chose to remove the cyst by the endoscopic method.

In addition to the question of the transnasal versus oral approach to dentigerous cysts, another controversy is the removal or marsupialization of the entire cyst. With marsupialization, the cyst wall is sutured to the oral mucosa by mouthing. It is performed particularly in young patients to protect the permanent tooth germs in the cyst and significantly reduces postoperative morbidity. The most important point here is to leave the pathological cyst tissue behind because ameloblastoma, intraosseous mucoepidermoid carcinoma, or intraosseous squamous cell carcinoma may develop from the dentigerous cyst. Also, enucleation of especially enlarged cysts brings the risk of pathological fracture (4). However, many authors have stated that spontaneous bone regeneration is seen after enucleation, especially in young patients, and that grafting is not required in many patients (4, 5).

Patient's age and the location and size of the cyst play an important role in the choice of treatment. Furthermore, the most accurate way to choose the appropriate treatment is to make the treatment decision with a multidisciplinary approach.

Ethics Committee Approval: Not obtained because it was a case report.

Informed Consent: Written informed consent was obtained from the patient's family for this case report.

Peer-Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- E.Y., A.A.; Data Acquisition- E.Y., P.K., D.Y.; Data Analysis/Interpretation- A.A., D.Y., K.B.; Drafting Manuscript- E.Y., P.K.; Critical Revision of Manuscript- A.A., D.Y., K.B.; Final Approval and Accountability- E.Y., A.A., P.K., D.Y., K.B.

Conflict of Interest: Authors declared no conflict of interest.

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REFERENCES

1. Thompson LD. Dentigerous cyst. Ear Nose Throat J 2018;97(3):57.
2. McKinney SL, Lukes SM. Dentigerous cyst in a young child: a case report. Can J Dent Hyg 2021;55(3):177-81.
3. Thattarakkal VR, Saravanam PK, Rajan J. Endoscopic management of a giant dentigerous cyst. BMJ Case Rep 2021;14(2):e240070. doi:10.1136/bcr-2020-240070
4. Rajae EG, Karima EH. Dentigerous cyst: enucleation or marsupialization? (a case report). Pan Afr Med J 2021;40:149.
5. Arakeri G, Rai KK, Shivakumar HR, Khaji SI. A massive dentigerous cyst of the mandible in a young patient: a case report. Plast Aesthet Res 2015;2:294-8.

AIM AND SCOPE

The Turkish Journal of Ear Nose and Throat (Tr-ENT) aims to contribute to the literature by publishing high quality original articles, case clinical reports, surgical techniques and invited reviews focusing on key subjects and contemporary developments in the field. The scope of the journal includes otology, neurootology, rhinology, head and neck, general ORL, facial plastic surgery and laryngology. The journal welcomes articles from other disciplines as well, provided that these are related to the major subject area. The target audience of the journal consists of academicians, researchers, professionals, students, related professional and academic bodies and institutions.

The editorial and publication processes of the journal are shaped in accordance with the guidelines of the International Committee of Medical Journal Editors (ICMJE), World Association of Medical Editors (WAME), Council of Science Editors (CSE), Committee on Publication Ethics (COPE), European Association of Science Editors (EASE), and National Information Standards Organization (NISO). The journal is in conformity with the Principles of Transparency and Best Practice in Scholarly Publishing (doaj.org/bestpractice).

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Processing and publication are free of charge with the journal. No fees are requested from the authors at any point throughout the evaluation and publication process.

All expenses of the journal are covered by the İstanbul University.

INSTRUCTION TO AUTHORS

The Turkish Journal of Ear Nose and Throat (Tr-ENT) is an international, scientific, open acces and peer-reviewed publication of Istanbul University. It is published quarterly in March, June, September and December. The publication language of the journal is English. The journal started off in 1990 under the editorship of Professor Behbut Cevanşir. Since then it has been a significant source of knowledge in the field of otorhinolaryngology for researchers, residents and specialists. As of year 2018 Volume 28 Issue 1, the title of the journal was changed from “Kulak Burun Boğaz İhtisas Dergisi” (ISSN: 1300-7475, E-ISNN: 2147-67569) to “The Turkish Journal of Ear Nose and Throat” and the journal decided to publish articles only in English with a frequency of four issues per year. The Turkish Journal of Ear Nose and Throat aims to contribute to the literature by publishing high quality original articles, case reports, surgical techniques and invited reviews focusing on key subjects and contemporary developments in the field. The scope of the journal includes otology, neurootology, rhinology, head and neck, general ORL, facial plastic surgery and laryngology. The journal welcomes articles from other disciplines as well, provided that these are related to the major subject area. The target audience of the journal consists of academicians, researchers, professionals, students, related professional and academic bodies and institutions. Former title of the journal: Kulak Burun Boğaz İhtisas Dergisi (P-ISNN: 1300-7475 / E-ISNN: 2147-6756)

POLICIES

Publication Policy

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The subjects covered in the manuscripts submitted to the Journal for publication must be in accordance with the aim and scope of the Journal. Only those manuscripts approved by every individual author and that were not published before in or sent to another journal, are accepted for evaluation.

Changing the name of an author (omission, addition or order) in papers submitted to the Journal requires written permission of all declared authors.

Plagiarism, duplication, fraud authorship/denied authorship, research/data fabrication, salami slicing/salami publication, breaching of copyrights, prevailing conflict of interest are unethical behaviors. All manuscripts not in accordance with the accepted ethical standards will be removed from the publication. This also contains any possible malpractice discovered after the publication.

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Depending on the type of article and the percentage of similarity score taken from each article, the overall similarity score is generally expected to be less than 15 or 20%.

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After plagiarism check, the eligible ones are evaluated by the editors-in-chief for their originality, methodology, the importance of the subject covered and compliance with the journal scope. The editor provides a fair double-blind peer review of the submitted articles and hands over the papers matching the formal rules to at least two national/international referees for evaluation and gives green light for publication upon modification by the authors in accordance with the referees' claims.

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All parties involved in the publishing process (Editors, Reviewers, Authors and Publishers) are expected to agree on the following ethical principles.

All submissions must be original, unpublished (including as full text in conference proceedings), and not under the review of any other publication synchronously. Authors must ensure that submitted work is original. They must certify that the manuscript has not previously been published elsewhere or is not currently being considered for publication elsewhere, in any language. Applicable copyright laws and conventions must be followed. Copyright material (e.g. tables, figures or extensive quotations) must be reproduced only with appropriate permission and acknowledgement. Any work or words of other authors, contributors, or sources must be appropriately credited and referenced.

Each manuscript is reviewed by at least two referees under double-blind peer review process. Plagiarism, duplication, fraud authorship/denied authorship, research/data fabrication, salami slicing/salami publication, breaching of copyrights, prevailing conflict of interest are unethical behaviors.

All manuscripts not in accordance with the accepted ethical standards will be removed from the publication. This also contains any possible malpractice discovered after the publication.

Research Ethics
The journal adheres to the highest standards in research ethics and follows the principles of international research ethics as defined below. The authors are responsible for the compliance of the manuscripts with the ethical rules.

- Principles of integrity, quality and transparency should be sustained in designing the research, reviewing the design and conducting the research.

- The research team and participants should be fully informed about the aim, methods, possible uses and requirements of the research and risks of participation in research.
- The confidentiality of the information provided by the research participants and the confidentiality of the respondents should be ensured. The research should be designed to protect the autonomy and dignity of the participants.
- Research participants should participate in the research voluntarily, not under any coercion.
- Any possible harm to participants must be avoided. The research should be planned in such a way that the participants are not at risk.
- The independence of research must be clear; and any conflict of interest or must be disclosed.
- In experimental studies with human subjects, written informed consent of the participants who decide to participate in the research must be obtained. In the case of children and those under wardship or with confirmed insanity, legal custodian's assent must be obtained.
- If the study is to be carried out in any institution or organization, approval must be obtained from this institution or organization.
- In studies with human subject, it must be noted in the method's section of the manuscript that the informed consent of the participants and ethics committee approval from the institution where the study has been conducted have been obtained.

Ethics Committee Approval and Informed Consent

The Turkish Journal of Ear Nose and Throat (Tr-ENT) takes as principle to comply with the ethical standards of World Medical Association (WMA) Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects revised in 2003 and WMA Statement on Animal Use in Biomedical Research revised in 2016.

An approval of research protocols by the Ethics Committee in accordance with international standards mentioned above is required for experimental, clinical, and drug studies and for some case reports. If required, ethics committee reports or an equivalent official document will be requested from the authors. For manuscripts concerning experimental research on humans, a statement should be included that shows that written informed consent of patients and volunteers was obtained following a detailed explanation of the procedures that they may undergo. For studies carried out on animals, the measures taken to prevent pain and suffering of the animals should be stated clearly. Information on patient consent, the name of the ethics committee, and the ethics committee approval number should also be stated in the Materials and Methods section of the manuscript. It is the authors' responsibility to carefully protect the patients' anonymity. For photographs that may reveal the identity of the patients, signed releases of the patient or of their legal representative should be enclosed.

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All the authors of a submitted manuscript must have direct scientific and academic contribution to the manuscript. The author(s) of the original research articles is defined as a person who is significantly involved in "conceptualization and design of the study", "collecting the data", "analyzing the data", "writing the manuscript", "reviewing the manuscript with a critical perspective" and "planning/conducting the study of the manuscript and/or revising it". Fund raising, data collection or supervision of the research group are not sufficient roles to be accepted as an author. The author(s) must meet all these criteria described above. The order of names in the author list of an article must be a co-decision and it must be indicated in the Copyright Agreement Form. The individuals who do not meet the authorship criteria but contributed to the study must take place in the acknowledgement section. Individuals providing technical support, assisting writing, providing a general support, providing material or financial support are examples to be indicated in acknowledgement section.

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Editor-in-Chief evaluates manuscripts for their scientific content without regard to ethnic origin, gender, sexual orientation, citizenship, religious belief or political philosophy of the authors. He/She provides a fair double-blind peer review of the submitted articles for publication and ensures that all the information related to submitted manuscripts is kept as confidential before publishing.

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Reviewers must have no conflict of interest with respect to the research, the authors and/or the research funders. Their judgments must be objective.

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A reviewer who feels unqualified to review the topic of a manuscript or knows that its prompt review will be impossible should notify the editor and excuse himself from the review process.

The editor informs the reviewers that the manuscripts are confidential information and that this is a privileged interaction. The reviewers and editorial board cannot discuss the manuscripts with other persons. The anonymity of the referees must be ensured. In particular situations, the editor may share the review of one reviewer with other reviewers to clarify a particular point.

PEER REVIEW

Peer Review Policies
Only those manuscripts approved by its every individual author and that were not published before in or sent to another journal, are accepted for evaluation.

Submitted manuscripts that pass preliminary control are scanned for plagiarism using iThenticate software. After plagiarism check, the eligible ones are evaluated by editor-in-chief for their originality, methodology, the importance of the subject covered and compliance with the journal scope.

The editor hands over the papers matching the formal rules to at least two national/international referees for double-blind peer review evaluation and gives green light for publication upon modification by the authors in accordance with the referees' claims.

Responsibility for the Editor and Reviewers
Editor-in-Chief evaluates manuscripts for their scientific content without regard to ethnic origin, gender, citizenship, religious belief or political philosophy of the authors. Editor-in-Chief provides a fair double-blind peer review of the submitted articles for publication and ensures that all the information related to submitted manuscripts is kept as confidential before publishing.

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- Does the abstract clearly and accurately describe the content of the manuscript?
- Is the problem significant and concisely stated?
- Are the methods described comprehensively?
- Are the interpretations and consclusions justified by the results?
- Is adequate references made to other Works in the field?
- Is the language acceptable?

Reviewers must ensure that all the information related to submitted manuscripts is kept as confidential and must report to the editor if they are aware of copyright infringement and plagiarism on the author's side.

A reviewer who feels unqualified to review the topic of a manuscript or knows that its prompt review will be impossible should notify the editor and excuse himself from the review process.

The editor informs the reviewers that the manuscripts are confidential information and that this is a privileged interaction. The reviewers and editorial board cannot discuss the manuscripts with other persons. The anonymity of the referees is important.

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The manuscripts should be prepared in accordance with ICMJE-Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals (updated in December 2015 - http://www.icmje.org/icmje-recommendations.pdf). Author(s) are required to prepare manuscripts in accordance with the CONSORT guidelines for randomized research studies, STROBE guidelines for observational original research studies, STARD guidelines for studies on diagnostic accuracy, PRISMA guidelines for systematic reviews and meta-analysis, ARRIVE guidelines for experimental animal studies, and TREND guidelines for non-randomized public behavior.

Manuscripts can only be submitted through the journal's online manuscript submission and evaluation system, available at https://dergipark.org.tr/tr/journal/3565/submission/step/manuscript/new Manuscripts submitted via any other medium will not be evaluated.

Manuscripts submitted to the journal will first go through a technical evaluation process where the editorial office staff will ensure that the manuscript has been prepared and submitted in accordance with the journal's guidelines. Submissions that do not conform to the journal's guidelines will be returned to the submitting author with technical correction requests.

Author(s) are required to submit the following documents together with the manuscript and must ensure that the abstract and keywords are in line with the standards explained in below.

- **Copyright Agreement Form**
- **Author Form and ICMJE Potential Conflict of Interest Disclosure Form**
- **Ethics Committee Approval**
- **Cover Letter to the Editor**
- **Title Page**: A separate title page should be submitted with all submissions and this page should include:

- The full title of the manuscript as well as a short title (running head) of no more than 50 characters,
- Name(s), affiliations, academic degree(s) and ORCID ID(s) of the author(s),
- Grant information and detailed information on the other sources of support,
- Name, address, telephone (including the mobile phone number) and fax numbers, and email address of the corresponding author,
- Acknowledgment of the individuals who contributed to the preparation of the manuscript but who do not fulfil the authorship criteria.

Abstract: Abstract should be submitted with all submissions except for Letters to the Editor. The abstract of Original Articles should be structured with subheadings (Objective, Materials and Methods, Results, and Conclusion). Abstracts of Case Reports and Reviews should be unstructured. Abstracts should be 200-250 words.

Keywords: Each submission must be accompanied by a minimum of 3 to a maximum of 6 keywords for subject indexing at the end of the abstract. The keywords should be listed in full without abbreviations. The keywords should be selected from the National Library of Medicine, Medical Subject Headings database (http://www.nlm.nih.gov/mesh/MBrowser.html) .

Manuscript Types

Original Articles: This is the most important type of article since it provides new information based on original research. The main text of original articles should be structured with Introduction, Material and Method, Results, Discussion, and Conclusion subheadings..

Statistical analysis to support conclusions is usually necessary. Statistical analyses must be conducted in accordance with international statistical reporting standards (Altman DG, Gore SM, Gardner MJ, Pocock SJ. Statistical guidelines for contributors to medical journals. Br Med J 1983: 7; 1489-93). Information on statistical analyses should be provided with a separate subheading under the Materials and Methods section and the statistical software that was used during the process must be specified.

Units should be prepared in accordance with the International System of Units (SI).

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Tables

Tables should be included in the main document, presented after the reference list, and they should be numbered consecutively in the order they are referred to within the main text. A descriptive title must be placed above the tables. Abbreviations used in the tables should be defined below the tables by footnotes (even if they are defined within the main text). Tables should be created using the "insert table" command of the word processing software and they should be arranged clearly to provide easy reading. Data presented in the tables should not be a repetition of the data presented within the main text but should be supporting the main text.

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Figures, graphics, and photographs should be submitted as separate files (in TIFF or JPEG format) through the submission system. The files should not be embedded in a Word document or the main document. When there are figure subunits, the subunits should not be merged to form a single image. Each subunit should be submitted separately through the submission system. Images should not be labeled (a, b, c, etc.) to indicate figure subunits. Thick and thin arrows, arrowheads, stars, asterisks, and similar marks can be used on the images to support figure legends. Like the rest of the submission, the figures too should be blind. Any information within the images that may indicate an individual or institution should be blinded. The minimum resolution of each submitted figure should be 300 DPI. To prevent delays in the evaluation process, all submitted figures should be clear in resolution and large in size (minimum dimensions: 100 × 100 mm). Figure legends should be listed at the end of the main document.

All acronyms and abbreviations used in the manuscript should be defined at first use, both in the abstract and in the main text. The abbreviation should be provided in parentheses following the definition.

When a drug, product, hardware, or software program is mentioned within the main text, product information, including the name of the product, the producer of the product, and city and the country of the company (including the state if in USA), should be provided in parentheses in the following format: "Discovery St PET/CT scanner (General Electric, Milwaukee, WI, USA)"

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Limitations, drawbacks, and the shortcomings of original articles should be mentioned in the Discussion section before the conclusion paragraph.

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When submitting a revised version of a paper, the author must submit a detailed "Response to the reviewers" that states point by point how each issue raised by the reviewers has been covered and where it can be found (each reviewer's comment, followed by the author's reply and line numbers where the changes have been made) as well as an annotated copy of the main document. Revised manuscripts must be submitted within 30 days from the date of the decision letter. If the revised version of the manuscript is not submitted within the allocated time, the revision option may be canceled. If the submitting author(s) believe that additional time is required, they should request this extension before the initial 30-day period is over. Accepted manuscripts are copy-edited for grammar, punctuation, and format. Once the publication process of a manuscript is completed, it is published online on the journal's webpage as an ahead-of-print publication before it is included in its scheduled issue. A PDF proof of the accepted manuscript is sent to the corresponding author and their publication approval is requested within two days of their receipt of the proof. The latest status of the submitted manuscripts and other information about the journal can be accessed at http://tr-ent.com. The editorial and publication processes of the journal are conducted in accordance with the guidelines of the International Council of Medical Journal Editors (ICMJE), the World Association of Medical Editors (WAME), the Council of Science Editors (CSE), the Committee on Publication Ethics (COPE), the European Association of Science Editors (EASE), and National Information Standards Organization (NISO). The journal conforms to the Principles of Transparency and Best Practice in Scholarly Publishing (doaj.org/bestpractice). An ORCID ID is required for all authors during the submission of the manuscript. The ID is available at http://orcid.org with free of charge.

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Periodicals: Author(s) Last Name initial(s) name of author(s) (if there are six or fewer authors, all authors should be written; if the number of authors are seven or more, only the first six of the authors should be written and the rest as "et al"). The title of the article, the abbreviated name of the journal according to the Index Medicus, Year; Volume (Issue): The first and last page numbers.

Example: Robson A, Greene J, Ansari N, Kim B. Eccrine porocarcinoma (malignant eccrine poroma): a clinicopathologic study of 69 cases. The American Journal of Surgical Pathology 2001;25:710-20. Books: Surname of the author(s) initial name(s) of author(s). The name of the book. The edition number. Place of publication: Publisher, Publication year.

Book chapters: The author (s) surname of the chapter initial (s) letter of the name. Section title. In: Surname of editor (s) initial (s) letter of first name (s) ed / eds. The name of the book. Edition number. Place of publication: Publisher, year of publication: The first and last page numbers of the chapter. Web address: If a "web" address is used as the reference address, the web address date should be given in brackets with the address. The DOI (Digital Object Identifier) number must be provided, when a web access article used in the text as a reference.

Example: AB Author, CD Author. Title of document. Retrieved from http://Web address (Accession date: aa/bb/2016).

Congress papers:

Thesis: Maden KL. Experimental investigation of the ..... Master Thesis, Health Science Institute of Ankara University, Ankara, 2005.

SUBMISSION CHECKLIST

- Cover letter to the editor
  - The category of the manuscript
  - Confirming that "the paper is not under consideration for publication in another journal".
  - Including disclosure of any commercial or financial involvement.
  - Confirming that the statistical design of the research article is reviewed.
  - Confirming that last control for fluent English was done.
  - Confirming that journal policies detailed in Information for Authors have been reviewed.
  - Confirming that the references cited in the text and listed in the references section are in line with NLM.
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- Permission of previous published material if used in the present manuscript
- Acknowledgement of the study "in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration.
- Statement that informed consent was obtained after the procedure(s) had been fully explained. Indicating whether the institutional and national guide for the care and use of laboratory animals was followed as in "Guide for the Care and Use of Laboratory Animals".
- Title page
  - The category of the manuscript
  - The title of the manuscript
  - Short title (running head)
  - All authors' names and affiliations (institution, faculty/department, city, country), e-mail addresses
  - Corresponding author's email address, full postal address, telephone and fax number
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  - The title of the manuscript
  - Abstract 200-250 words
  - Key words: 3 - 6 words
  - Main article sections
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  - All tables, illustrations (figures) (including title, description, footnotes)

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