CASE REPORT

Rare benign tumor of the larynx – laryngeal amyloidosis

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Abstract

We report a case of rare benign tumor of the larynx – amyloidosis of larynx – in a 52-year-old female who presented to the Department of Ear, Nose and Throat (ENT), Timişoara Municipal Hospital, Romania, with dysphonia. The patient’s first presentation was in June 2012, with a polypoid formation localized on left vocal cord. The patient underwent a suspended microlaryngoscopy (SM) with tumor removal followed by histopathological examination, which revealed a laryngeal amyloidosis. The tumor recurred in 2013, 2014, 2016, 2017 and 2018. The patient underwent tracheotomy, followed by SM with endoscopic CO2 laser procedures, in order to remove the tumor and to ensure the airway. The follow-up was for six years. In May 2018, amyloid was located in subglottis, glottis and left false vocal fold. The patient needed five revision surgical procedures. The patient did not developed systemic amyloidosis during the follow-up period.

Keywords: laryngeal amyloidosis, larynx tumor, recurrences, tracheostomy, CO2 laser.

Introduction

Amyloidosis is a disease characterized by the appearance of amyloid deposits in different organs. It can appear as systemic condition, when acellular deposits of amyloid can be noticed in any organs or a regional condition when only one organ is affected. At the cases affected by a systemic condition, in the head and neck area, macroglossia is the most common sign noticed, appearing at 15% to 20% of patients. The larynx amyloidosis is rarely encountered, consisting approximately 1.2% of all benign laryngeal tumors. Usually, laryngeal amyloidosis appears as local involvement, without other systemic symptoms [1, 2].

Using morphological stainings, laryngeal amyloid deposits appear as acellular, homogeneous, eosinophilic material in lamina propria of larynx [1, 2]. When stained with Congo Red, using polarized light, amyloid displays apple-green birefringence. Histochemical stainings as Crystal Violet and Methyl Violet highlight amyloid deposits as metachromatic substance in pink-violet [1, 2].

The goal of localized laryngeal amyloidosis treatment is to offer to the patient a long as possible disease-free interval with voice preservation and normal breathing. There are multiple therapeutic options [suspended microlaryngoscopy (SM) and tumor removal with CO2 laser or cold instruments] [3–8], even irradiation [9, 10]. The preferred method is complete excision.

Our study aim was to evaluate six years term effectiveness of the treatment applied in one case of localized laryngeal amyloidosis regarding disease progression, postoperative recurrence rate and voice characteristics.

Case presentation

We report a case of rare benign tumor of the larynx – amyloidosis of larynx – in a 52-year-old female (M.G.) who presented in June 2012 to Department of Ear, Nose and Throat (ENT), Timişoara Municipal Hospital, Romania, with dysphonia.

The patient was a non-smoker, non-diabetic, with a history of hoarseness for two previous years, a mild hearing loss on right ear and a pulsatile tinnitus in the same ear. At first admission, it was noted any difficulty in breath, nor pain when swallowing. An endoscopic laryngeal exam revealed a polypoid-like tumor localized on the left vocal cord, with normal vocal cord mobility. The larynx was evaluated by videolaryngoscopy with a 70° Karl Storz rigid telescope. The patient presented a left septal deviation, and tonal audiometry showed a mild sensorineural hearing loss.

Under general anesthesia with orotracheal intubation, the patient underwent a SM with endoscopic CO2 laser tumor removal followed by histopathological (HP) and immunohistochemical (IHC) studies.

The biological material harvested was fixed in 10%
neutral formalin solution and included in histological paraffin, according to the histopathology protocol. There were performed 4-μm serial sections stained with Hematoxylin–Eosin (HE), Goldner–Szekely (GS) green light trichrome and Periodic Acid–Schiff and Hematoxylin (PAS–H).

For the IHC study, we used the following antibodies:

▪ anti-cluster of differentiation (CD) 3 (monoclonal mouse anti-human CD3, clone F7.2.38, 1/50 dilution, Dako);
▪ anti-CD4 (monoclonal mouse anti-human CD4, clone MT310, 1/50 dilution, Dako);
▪ anti-CD8 (monoclonal mouse anti-human CD8, clone C8/144B, 1/100 dilution, Dako);
▪ anti-CD20 (monoclonal mouse anti-human CD20cy, clone L26, 1/50 dilution, Dako);
▪ anti-CD68 (monoclonal mouse anti-human CD68, clone KP1, 1/50 dilution, Dako);
▪ anti-CD79α (monoclonal mouse anti-human CD79α, clone JCB117, 1/50 dilution, Dako);
▪ anti-CD34 (monoclonal mouse anti-human CD34 Class II, clone QBEnd 10, 1/50 dilution, Dako);
▪ anti-tryptase (monoclonal mouse anti-human mast cell tryptase, clone AA1, 1/500 dilution, Dako);
▪ anti-CD34 (monoclonal mouse anti-human CD34 Class II, clone QBEnd 10, 1/50 dilution, Dako).

The HP study highlighted the presence of an amorphous, eosinophilic, relatively homogeneous, acellular material, with a nodular or lamellar arrangement in the conjunctive tissue of the laryngeal mucosa (Figure 1). Also, high quantities of amyloid were identified in the tunica media of the arterioles in the tumoral stroma (Figure 2). The amyloid deposits were intensely positive to the staining of the Periodic Acid–Schiff (Figure 3). The tumoral stroma was in a low quantity, in comparison to the deposits of amyloid. It was mainly formed of lax conjunctive tissue, with densifications of the collagen fibers at tumor periphery. In the stroma, there was identified the presence of a moderate diffuse chronic inflammatory infiltrate, with a heterogeneous distribution, mainly formed of lymphocytes and plasmocytes (Figure 4). The inflammatory infiltrate was present only in the conjunctive septa between the amyloid nodules and at the periphery of the tumor.

Figure 1 – Overall microscopic image of the laryngeal tumor where there is observed the presence of nodular or lamellar deposits of acellular, homogeneous and eosinophil, with a heterogeneous dissemination in the conjunctive stroma (HE staining, ×100).

Figure 2 – Arterioles of the tumoral stroma, with a thickened wall by the presence of a high quantity of amyloid in the media tunica (HE staining, ×400).

Figure 3 – Intensely positive reaction of the amyloid to the Periodic Acid–Schiff (PAS–H staining, ×200).

Figure 4 – Tumoral stroma at the tumor periphery with a high quantity of collagen fibers and a moderate inflammatory infiltrate (GS trichrome staining, ×200).
The IHC examination allowed us to identify the main types of cells present in the inflammatory infiltrate of the tumoral stroma. In our study, the inflammatory infiltrate contained high quantities of CD3+ T-lymphocytes, CD4+ T-lymphocytes, plasmocytes and macrophages (Figure 5, a–d) and low quantities of CD8+ T-lymphocytes, CD20+ B-lymphocytes and mastocytes (Figure 6, a–c). Immunomarking with anti-CD34 antibody highlighted a well-developed network of capillaries in the tumoral stroma, with the absence of blood vessels in the amyloid areas (Figure 7).

The HP and IHC exams confirmed the diagnosis of laryngeal amyloidosis.

Afterwards, we evaluated the patient in order to rule out the systemic amyloidosis performing a thorough clinical evaluation, following a standard protocol in order to assess heart, liver, kidney, bone marrow, and peripheral and autonomic nervous system, and performing biopsies of abdominal fat, rectum and bone marrow. The results of our investigations were negative, which made us believe that the patient is a case of primary laryngeal amyloidosis.

The tumor recurred in the following years, on 2013, 2014, 2016, 2017 and 2018. The sites where amyloid in the larynx was found on the first presentation and recurrences are displayed in Table 1, together with the surgical procedures applied.

Systemic amyloidosis was not found on repeated thorough investigations (normal immunoglobulin free light chain serum level (reference values: λ < 20 mg/L, κ < 32 mg/L) was found. Initial 24-hour urine specimen collected 4.2 g of protein, 63% of which was albumin by urine protein electrophoresis. Skeletal X-rays showed no lytic lesions.

Starting with 2014, the tumor involved the subglottis region, and the patient underwent a tracheotomy, followed by a general anesthesia with tracheal intubation (GA–TI) and SM with endoscopic CO2 laser tumor debulking.

The patient underwent six surgical procedures, four in GA– orotracheal intubation (OTI), and two in GA–TI, all SM, four endoscopic CO2 laser excisions and two tumors debulking. We performed a watchful waiting in two cases with residual disease (CO2 laser tumor debulking).

Postoperative voice of the patient was only mildly affected, even in debulking procedures. Our study aim was to evaluate six years term effectiveness of our approach in localized laryngeal amyloidosis regarding disease progression, postoperative recurrence rate and voice characteristics.

The patient evolution is with five recurrences and prognosis worsening in time due to tumor involving the subglottis and supraglottis region.

Figure 5 – Tumoral stroma infiltrated with numerous CD3+ T-lymphocytes (Anti-CD3 antibody immunomarking, ×200) (a) and CD4+ T-lymphocytes (Anti-CD4 antibody immunomarking, ×200) (b), plasma cells (Anti-CD79a antibody immunomarking, ×200) (c), and macrophages (Anti-CD68 antibody immunomarking, ×200) (d).
Discussions

Amyloidosis is a disease with an unknown etiopathogeny, characterized by deposits of abnormal cells, known as amyloid fibrils, in various tissues. There are multiple clinical forms of the disease: some are familial (genetic), while others are acquired; some are localized, while others are systemic [11–13]. Up to now, in humans, there were identified 36 distinct proteins forming amyloid fibrils. These are synthesized by various cells (plasmocytes, B-lymphocytes) in the bone marrow, liver, etc., and they are transported through blood to various deposit places, most often at distance from the synthesis location [12, 14].

We presented the case of an amyloidosis localized in the larynx, in a 52-year-old woman. The onset was insidious, with an essential symptom of macroglossia. Most studies state that localized amyloidosis is a rare condition, representing only 10–20% of the amyloidosis cases, most often occurring in the neck and head area [15–17]. Regarding laryngeal amyloidosis, this seems to
be the most frequent localization of head and neck amyloidosis [18]. Also, numerous authors showed that amyloidosis most often affects men aged between 50 and 70 years old, the men/women ratio being 3/1 [19, 20].

We studied the elements that conducted to a correct diagnosis and the effectiveness of our surgical approach (CO2 laser excision and debulking) in localized laryngeal amyloidosis [21–24]. There was described the presence of residual amyloid after more than 10 years [7, 8, 25–27]. Our patient presented a recurrence after six years. Macroglossia being the commonest ENT manifestation (between 15% and 20% of patients), in our patient, we did not encountered it [1].

The microscopic features of biopsied material noticing homogenous, acellular, eosinophilic nodules on HE staining can raise the suspicion of amyloidosis, but the “gold standard” for the diagnosis is represented by the highlighting of the pathognomonic green-yellow birefringence in polarized light with Congo Red staining and metachromatic pink-violet staining with Methyl Violet [28–30]. The performed microscopic study highlighted the presence of intensely acidophilic nodules of amyloid in the conjunctive tissue of the laryngeal mucosa. The staining with PAS-H highlighted the characteristic intense PAS positivity of the amyloid. Like other authors, we also observed that the conjunctive tissue of the laryngeal mucosa was disorganized by the presence of the amyloid [31]. It contained an inflammatory infiltrate mainly formed of CD3+ T-lymphocytes, CD4+ T-lymphocytes, plasma cells and macrophages.

As a rare benign tumor of the larynx, laryngeal amyloidosis should be differentiated on clinical and HP exam from other benign and malignant tumors of the larynx [32–35]. At the same time, we evaluated the possible etiology of sensorineural hearing loss and tinnitus performing a head magnetic resonance imaging (MRI) [36, 37]. Because of the anatomical particularity of the region and the paucity of the laryngeal amyloidosis, frequently the accurate diagnosis can be delayed for many months. Diagnosis is established on clinical exam, followed by HP examination. In the past, the “gold standard” for amyloidosis diagnosis consisted of a rectal biopsy that can demonstrate the presence of amyloid deposits in up to 80% of patients with systemic disease. In cases with systemic disease, nowadays, the more used method is core biopsy of otherwise clinically unaffected abdominal fat tissue of subcutis, which is positive in 95% of primary and in 66% of secondary cases with also 86% positivity of heredofamilial cases [2].

As in other pathologies, the goal of the localized laryngeal amyloidosis treatment is the preservation of voice and breathing. The state of art for the treatment of localized laryngeal amyloidosis impose the use of CO2 laser or cold instruments for microlaryngoscopy to realize removal of amyloid deposits with conservation of the surrounding tissue [38, 39]. The conservative methods of treatment are recommended by Lewis et al. (1992) even in the case of recurrences, that they consider rare [2]. Other studies, performed on small series of localized laryngeal amyloidosis patients with well-documented diagnosis and long-term follow-up showed that the patients were free of disease even after seven years from diagnosis without any re-intervention [6, 8, 39–42]. Radical removal of all amyloid is not indicated, due to the fact that localized laryngeal amyloidosis is a benign disease.

There are described other surgical ways of treatment by external approach [7], even a total laryngectomy [8, 25]. The size and localization of amyloid requires sometimes a tracheotomy [1, 26–28]. Amyloid may shift its location within the larynx.

### Conclusions

In our patient, laryngeal amyloidosis occurred in 2013, 2014, 2016, 2017 and 2018. The patient underwent two tracheotomies, followed by SM endoscopic CO2 laser procedures, in order to remove the tumor and to ensure the airway. The follow-up was for six years. The patient did not develop any systemic amyloidosis during the follow-up period. The postoperative voice was only mildly affected in most patients.

### Conflict of interests

The authors declare that they have no conflict of interests.

### Authors’ contribution

Ioana Delia Horhat and Nicolae Constantin Balica equally contributed to the manuscript.

### References


