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ORIGINAL ARTICLE

Laryngeal dysplasia: A long-term follow-up study

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Summary

Purpose: *To assess the progression of precancerous laryngeal lesions to squamous cell carcinoma (SCC), defined by specific histopathological criteria, in patients with long-term follow-up.*

Methods: *Patients with laryngeal dysplasia, followed/treated between 1985 and 2008, were retrospectively evaluated and classified according to the World Health Organization classification system (WHO). The investigated outcome parameters were progression of dysplasia to SCC, time interval to malignant transformation and continuation of smoking as potential risk factors.*

Results: *Fifty-nine patients were studied. Progression of dysplasia to SCC between the first and the final histological examination was statistically significant (p<0.0001). Malignant transformation appeared in 29 patients (49.2%). Serious dysplasia was more likely to progress to SCC (64.8%) compared to mild (41.7%) or moderate (44.4%) (p<0.0001). However, the time interval needed in these 29 cases to progress to cancer was not statistically related to the initial histological diagnosis. Continuation of smoking did not affect the progression of disease. However, the mean time from dysplasia to laryngeal cancer was much longer in patients who quitted smoking (33.5 months) vs those who continued smoking (19.5 months), with a marginal statistical difference (p=0.057).*

Conclusion: *All patients with laryngeal dysplasia should be followed up at regular intervals. The progression of dysplasia to SCC did not seem to be directly related to the continuation of smoking or not. However, large long-term follow-up studies taking into account the degree of exposure (e.g. time of exposure, number of cigarettes) are needed in order to clarify risk factors and proper management. Consensus guidelines in diagnosis, follow-up, and treatment would improve substantially the current clinical practice.*

Key words: *laryngeal cancer, laryngeal dysplasia, premalignant lesions, smoking*

Introduction

Although laryngeal cancer management is the focus of many research papers, the literature regarding the process leading to invasive carcinoma is rather limited. However, it is well known that prior to becoming laryngeal cancer, the epithelium of the larynx undergoes a sequence of precancerous changes. The term precancerous covers both the various stages of dysplasia as well as carcinoma *in situ* (CIS). Such dysplastic lesions range between 2 and 10 cases per 100,000 population [1]. Reports have shown that malignant transformation rates range greatly, between 2% and 74% of the cases [2,3].

The management of precancerous lesions depends on early diagnosis and timely application of the appropriate treatment, in order to avoid further progression to SCC. However, due to the absence of widely accepted follow-up guidelines and the lack of any robust prognostic factors, the management and follow-up strategies for these lesions vary considerably worldwide. Among the serious weaknesses of the related studies are the various criteria used by different pathologists and the short follow-up time.

The aim of the present study was to assess the progression of precancerous lesions to SCC,

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defined by specific histopathological criteria in a significant number of patients with long-term follow-up time.

Methods

The present study included 70 adult patients who underwent a microlaryngoscopy under general anaesthesia at the Athens University Otorhinolaryngology Department and whose the initial histology report revealed laryngeal dysplasia. These patients had a second microlaryngoscopy during the follow-up period. Follow-up was set to a minimum of 5 years in order to have a valid and reasonable time frame to assess any progression from dysplasia to laryngeal cancer. Cases that had progressed to cancer earlier with a minimum time interval of more than 6 months from the initial microlaryngoscopy were also included. All patients underwent excision of the entire lesion, either with cold instruments or using a CO_2 laser. In order to have an homogeneous sample, all histological reports were reevaluated by the same pathologist with special experience in laryngeal pathology and were classified according to the WHO classification system [4-6].

Histological characterizations

The histological diagnosis of dysplasia was based on both cytomorphologic and maturation abnormalities criteria. These alterations include loss of maturation, nuclear pleomorphism (variations in the size and shape of the nuclei), loss of cellular organization or polarity, increase in the nuclear chromatin (hyperchromasia) with irregular distribution, increased nucleus/cytoplasm ratio, and increased mitoses, including atypical forms in all epithelial layers. Key diagnostic parameters in the assessment of laryngeal dysplasia are the locations of the architectural and cytomorphologic changes within the epithelium [7,8]. There is no definite association between dysplastic changes and keratosis or dyskeratosis.

Taking all the above into consideration, the pathological evaluation of the cases was carried out as follows: a) in cases with no evidence of keratosis, the degree of dysplasia was mainly defined in accordance to the thickness of the epithelial lining occupied by atypical cells (1/3- mild dysplasia, 2/3- moderate dysplasia, almost 3/3 severe dysplasia or CIS); and b) in cases with keratosis, the grade was mainly based on the architecture of the epithelium, the severity of atypia and the mitotic activity/presence of atypical mitoses.

Patients

Of the 70 cases initially included in the present study, 11 cases were excluded due to insufficient material, invasive carcinoma, or non dysplasia specimen. Of the remaining 59 patients, 57 (96.6%) were male and 2 (3.38%) female. Their mean age was 63.5 years ±11.57 SD. The follow-up period ranged between 6 and 276 months (mean 61.36±48.4.

Statistics

Statistical analysis was performed with PASW Statistics 18 (SPSS-IBM, U.S.A.). The significance level was set at $p \leq 0.05$. Pearson's x^2 test was used to compare initial histological results with smoking. Related samples Kendall's coefficient of concordance were used to evaluate the progression of disease. The investigated outcome parameters were progression of dysplasia to SCC, time interval to malignant transformation, and the continuation of smoking as potential risk factors.

Results

Progression to cancer

From the 59 patients included in the present study, the initial histopathological evaluation revealed mild dysplasia in 24 (40.67%) patients, moderate dysplasia in 18 (30.50%), and severe dysplasia/CIS in 17 (28.81%) patients. With regard to the 24 patients with mild dysplasia in the initial diagnosis, the final histopathological assessment remained the same in 4 (16.7%) patients, progressed to moderate in 5 (20.8%), progressed to severe/CIS in another 5 (20.8%) patients, and to SCC in 10 (41.7%) patients. With regard to the 18 patients with moderate dysplasia in the initial diagnosis, 1 (5.6%) did not progress, 9 (50%) progressed to severe dysplasia/CIS, and 8 (44.4%) cases progressed to SCC. Finally, 6 (35.3%) patients of the severe dysplasia group did not progress, while 11 (64.7%) progressed to SCC (Table 1). Statistical analysis revealed that there was statistically significant difference between the initial and the final histopathological evaluation (Related sam-

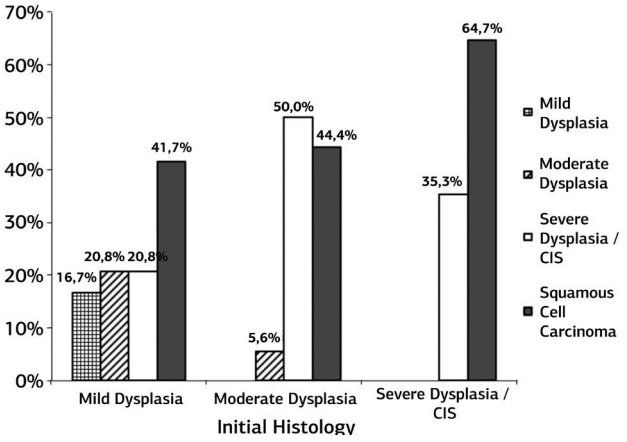

Figure 1. Disease progression from the initial to final pathology report according to the grade of dysplasia ($p<0.0001$).

Table 1. Progression of the 1ST to the last histopathology report

First histopathology report	*Last histopathology report*				*Total N (%)*
	Mild dysplasia N (%)	*Moderate dysplasia N (%)*	*Severe dysplasia / CIS N (%)*	*SCC N (%)*	
Mild dysplasia	4 (16.7)	5 (20.8)	5 (20.8)	10 (41.7)	24 (100)
Moderate dysplasia	0 (0)	1 (5.6)	9 (50.0)	8 (44.4)	18 (100)
Severe dysplasia/CIS	0 (0)	0 (0)	6 (35.3)	11 (64.7)	17 (100)
Total	4 (6.8)	6 (10.2)	20 (33.9)	29 (49.2)	59 (100)

SCC: squamous cell carcinoma, CIS: carcinoma *in situ*

ples Kendall's coefficient of concordance control; p<0.0001).

Overall, malignant progression appeared in 29 of the 59 patients (49.2%). The statistical analysis revealed that serious dysplasia was more likely to progress to SCC (64.8%) compared to a mild (41.7%) or a moderate one (44.4%; p<0.0001; Figure 1). However, the time interval needed in these 29 cases to progress to cancer was not related statistically to the initial histopathological diagnosis (p= 0.770). In patients with mild dysplasia the mean time for progression to SCC was 25 months (median 14 months), in patients with moderate dysplasia 31.6 months (median 30 months), and in patients with severe dysplasia/CIS 28.8 months (median 20 months).

Continuation of smoking

At the time of the initial histology report, 4 (6.8%) patients were non-smokers and 55 (93.2%) smokers. Two of the non-smokers had mild dysplasia and the remaining 2 had moderate dysplasia. On the other hand, the initial histology among the 55 smokers showed 22 (40%) patients with mild dysplasia, 16 (20.09%) with moderate and 17 (30.90%) with severe dysplasia / CIS. Although there was a strong recommendation to all patients to quit smoking, 35 did quit and 20 continued to smoke.

For the 35 ex-smokers, the last histology report revealed that 3 (8.6%) of them presented with mild dysplasia, 4 (11.4%) with moderate, 11 (31.4%) with severe dysplasia/CIS, and 17 (48.6%) with laryngeal cancer. The respective percentages in the 20 patients who continued smoking were: 5% (1 patient) with mild dysplasia, 10% (2 patients) with moderate, 35% (7 patients) with severe dysplasia/CIS, and 50% (10 patients) with laryngeal cancer (Table 2). There was no statistical difference in the two groups (p=0.9). Moreover, continuation of smoking was not found to be correlated with progression to cancer (p=0.9). However, the mean time to progression from dysplasia to laryngeal cancer was 19.5 months (median 13) for those who continued smoking and 33.5 months (median 30) for those who did quit smoking. Statistical analysis revealed that this difference had only a marginal statistical significance (p=0.057).

Discussion

It is very interesting to note that although it is well known for more than 100 years that precancerous lesions of the vocal cords often precede laryngeal cancer, the exact process that these lesions follow in order to progress to invasive carcinoma is still unknown [9].

Durant was the first author to describe a case of laryngeal leucoplakia (white cicatrices) in 1880. However, the theory of the precancerous diseases of the larynx was first developed in the early 20th century by Chevalier Jackson [10], who gave emphasis on the timely diagnosis and management in order to avoid the progression of these lesions to cancer. Almost at the same time (1920) Pierce [11] described leucoplakia laryngitis. However, Chevalier Jackson was the one who

Table 2. Smoking and last histopathology report

Smoking	*Last histopathology report*				*Total N (%)*
	Mild dysplasia N (%)	*Moderate dysplasia N (%)*	*Severe dysplasia / CIS N (%)*	*SCC N (%)*	
Ex-smokers*	3 (8.6)	4 (11.4)	11 (31.4)	17 (48.6)	35 (100.0)
Smokers	1 (5.0)	2 (10.0)	7 (35.0)	10 (50.0)	20 (100.0)
Total	4 (7.3)	6 (10.9)	18 (32.7)	27 (49.1)	55 (100.0)

*Smoking before the first histolopathology report. SCC: squamous cell carcinoma, CIS: carcinoma *in situ*

introduced in 1930 the relation between leucoplakia and cancer, and eloquently recognized atypia's malignant potential by defining it as the "mobilization of an army preparatory to invasion". In 1942, Graham reported that precancerous conditions of the larynx were well recognized during his time [1,12,13] . A decade later, in 1953, Putney and O´Keefe reported that invasive carcinoma had been developed in 27 out of 68 patients (40%) with keratinization of the larynx [14]. This study was the first that had a specific follow-up interval, suggesting that treating precancerous lesions may arrest their progression to cancer.

The difficulty in the diagnosis of laryngeal dysplasia lies in the distinction between normal and dysplastic tissue. Therefore, it is generally accepted that there is an element of subjectivity. In other words, a mild dysplasia according to one pathologist might be a moderate one according to another pathologist, especially when the diagnosis is made on small biopsy fragments. Therefore, all pathology reports in the present study were prepared by the same pathologist who had special experience in laryngeal diseases, and this is one of the strengths of this study. In addition, it is well known that specific and strict diagnostic criteria should be used to limit the related variability in diagnosis [7,8]. Consequently, in order to exclude, as much as possible, any false pathologic diagnoses in cases with no evidence of keratosis in the present study, the degree of dysplasia was defined according to the thickness of the epithelial lining occupied by atypical cells. On the other hand, in cases with keratosis, the above criterion was secondarily taken into account. In these cases, the location of the architectural and cytomorphologic changes within the epithelium was recorded but the grading was based on the severity and not the location of the architectural and nuclear abnormalities.

It is well acknowledged that the transition from a normal epithelium to SCC of the larynx is a lengthy, dynamic, and rather unpredictable process. Some cases of laryngeal precancerous lesions are self-limiting and reversible. Others respond to proper treatment, while others persist or progress to SCC, and this may occur despite careful follow-up and management. Table 3 illustrates the reported rate of malignant transformation of laryngeal dysplasia in various studies published in the literature. It is obvious that there is a considerable variation in each individual grade of dysplasia. This may very well be attributed to the inconsistent use of morphological criteria, the inclusion and exclusion criteria, the type of treatment, and the length of follow-up [15]. In the present study the minimum follow-up period for the patients who did not progress to cancer was 60 months, a quite long time in comparison with the majority of the other studies. This gives an additional strength to the results of the present study that are not in disagreement with the above mentioned studies as a positive connection between the degree of dysplasia and the risk of developing cancer was found. However, it is very interesting to note that in the patients of the present study, besides severe dysplasias that were found to have a high risk for malignant transformation, mild and moderate dysplasias seemed to have a considerable risk as well (41.7 and 44.4%, respectively). The corresponding percentages in the literature range widely; 0-20% for mild dysplasias and 0-44.5% for the moderate dysplasias. Of course this may be explained by the differences in the design of the

Table 3. Reported rates of malignant transformation of laryngeal dysplasia in various studies published in the literature

Authors [Ref no.]	*Patients N*	*Mean follow-up (months)*	*Min follow-up (months)*	*Mild N (%)*	*Moderate N (%)*	*Severe/CIS N (%)*
Hellquist et al.(1982) [24]	147	NR	<1 year	2/98 (2)	3/24 (12)	9/25 (38)
Stenersen et al. (1988) [25]	41	109	12			19/41 (46)
Sllamniku et al. (1989) [26]	317	NR	60	15/204 (7)	4/23 (17)	25/90 (28)
Hojslet et al. (1989) [27]	147	64	NR	6/128 (4.7)	4/9 (44.5)	4/10 (40)
Blackwell et al.(1995) [28]	59	73	12	3/26 (12)	5/15 (33)	5/18 (28)
Uno et al. (1997) [29]	26	59	13	2/10 (20)	0/9 (0)	2/7 (28.6)
Pich et al. (1998) [17]	99	146	60	0/63 (0)	0/25 (0)	6/11 (54.5)
Gallo et al. (2001) [30]	116	101	15	4/56 (7)	6/28 (21)	3/32 (9)
Ricci et al. (2003) [15]	101	NR	84	2/44 (5)	5/36 (14)	3/21 (14)
Jeannon et al. (2004) [31]	113	72	15	1/23 (4.5)	13/64 (20)	13/26 (50)

NR: not reported, min: minimum, CIS: carcinoma *in situ*

studies, the inclusion of cases that have never had a second biopsy, and the methods of treatment.

The results of the present study also revealed that the time of progression to laryngeal cancer was independent of the grade of dysplasia. This is in agreement with a recent review of Weller et al [3]. The considerable risk of malignant transformation of mild and moderate dysplasias, as well as their similar time interval to malignancy in comparison with the time interval to malignancy of severe dysplasias support the recommendation that all patients with laryngeal dysplasia should be closely monitored and properly managed irrespective of the grade of dysplasia. However, in a recent review by Spielmann et al., the authors suggested that if, after a follow-up of 3 years, the mild and moderate dysplasias do not progress, patients could be discharged [16]. In an older study by Plch et al. the authors did not notice any malignancy in patients with mild and moderate dysplasia in a time span of 20 years, suggesting that the role of follow-up in those patients is questionable [17]. This is in contrast with the results of the present study where 4 cases with mild/moderate dysplasias progressed to SCC in a period longer than 5 years.

Smoking, is universally accepted as a major risk factor for the development of laryngeal cancer, especially when combined with alcohol abuse [18]. However, Weller et al. concluded in a meta-analysis that there is insufficient data, as derived from the majority of the related studies, regarding the effect of risk factors, such as smoking and alcohol consumption on laryngeal dysplasia progressing to cancer [3]. In the patients of the present study, continuation of smoking did not seem to influence progression to malignant transformation. However, the mean progression time from dysplasia to laryngeal cancer was much longer in patients who quitted smoking (33.5 months) in comparison to those who continued smoking (19.5 months), with a marginal statistical significance (p=0.057). However, these results are weakened by the small numbers of patients in the related groups and the fact that important factors such as the number of cigarettes per day and the total duration of smoking have not been investigated [12,19,20]. Nevertheless, other studies in the literature also suggest that smoking cessation does not alter the prognosis of the disease [21,22] and it remains uncertain whether laryngeal epithelium ever returns to its normal histological structure after smoking cessation [4,19,20,23].

Studies with long-term follow-up (as the present one) with large numbers of patients (probably multicentric) of high quality with strict criteria, including all patients with dysplasia with regular follow-ups, are needed to clarify the importance of factors predisposing to laryngeal cancer from an initial dysplasia.

Conclusion

The results of the present study suggest that certain diagnostic criteria should be applied universally in the histopathological diagnosis of laryngeal dysplasia. All patients with dysplasia should be followed up at regular intervals and even patients with mild dysplasias should not be discharged early. Progression to SCC does not seem to be directly related with the continuation of smoking or not, although large studies with long-term follow-up taking into account the degree of exposure (e.g. time of exposure and number of cigarettes) are needed in order to clarify risk factors and proper management. Consensus guidelines in diagnosis, follow-up, and treatment would improve substantially the current clinical practice.

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