# Loss of heterozygosity in laryngeal cancer

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## Abstract

**Purpose:** Head and neck cancers account for about 6% of all human cancers. Molecular changes leading to the disease development and progression still remain not fully explained. Examination of loss of heterozygosity (allelic loss, LOH) using the specific microsatellite markers is a method of choice in assessing tumour suppressor genes (TSGs) localisation in human genome.

**Material and methods:** The study was performed in a group of 46 male patients, aged 42-77 years. Forty three patients underwent total laryngectomy with lymph nodectomy, two patients – chordectomy and one patient – partial laryngectomy.

Tumour tissue specimens and reference peripheral blood samples were obtained during surgical resections. Standard methods were used for DNA isolation. Fluorescent multiplex PCR was used to amplify microsatellite loci included in commercially available human identification kits.

**Results:** LOH was found at the following loci: BAT26, D3S1358, FGA, CSF1PO, D5S818, D8S1179, VWA, D13S317, D18S51. The highest LOH frequency was found in the tumor samples where the neighbouring cervical lymph nodes were affected but the incidence of LOH at BAT26 was statistically insignificant ($p = 0.07$).

**Conclusions:** High incidence of LOH is considered an unfavourable prognostic factor accompanying an aggressive nature of the tumour and indicating an involvement of certain genome regions in cancerogenesis. In head and neck cancers LOH was found on the following chromosomes: 3p, 5q, 8p, 9p, 9q, 11q, 17p, 17q, 18p, 18q.

**Key words:** laryngeal cancer, loss of heterozygosity.

## Introduction

Head and neck cancers account for about 6% of all human cancers. They are more common in men than in women (the ratio about 3 to 1). Cytogenetic changes, including MYC or RAS proto-oncogene inactivation and inactivation of tumour suppressor genes (TSGs) of cancerous transformation are discussed as etiological factors. On the other hand, suppressor gene products may inhibit cancerous phenotype. Molecular changes leading to the disease development and progression still remain not fully explained. Examination of loss of heterozygosity (allelic loss, LOH) using the specific microsatellite markers is a method of choice in assessing TSGs localisation in human genome. More detailed understanding of these disturbances will help to establish possible prognostic factors in the disease treatment. LOH, or loss of normal allele in heterozygotic locus, may result from different mechanisms, including chromosomal deletion, mitotic recombination (MR), gene conversion, point mutation, or intragenic allele inactivation [1]. Consequently, the locus may become homozygous due to mitotic recombination, gene conversion or chromosome loss with reduplication, hemizygous due to deletion or chromosome loss, complex heterozygote due to introduction of another point mutation at the locus, or it may remain heterozygous (with relation to nucleotide sequence), if one allele is inactivated intragenically [2].

High incidence of LOH is considered an unfavourable prognostic factor accompanying aggressive nature of the tumour and indicating involvement of certain genome regions in cancerogenesis. In head and neck cancers LOH was found

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*Table 1.* **Chromosomal distribution of LOH frequency in laryngeal cancer**

| Marker | Chromosomal localisation | LOH frequency (%) |
|---|---|---|
| BAT26 | 2p16.3-q21 | 19/45 (42%) |
| D3S1358 | 3p | 6/45 (13%) |
| FGA | 4q28 | 2/45 (4%) |
| CSF1PO | 5q33.3-34 | 8/45 (18%) |
| D5S818 | 5q21-q31 | 5/45 (11%) |
| D8S1179 | 8q | 1/45 (2%) |
| VWA | 12p12 | 3/45 (7%) |
| D13S317 | 13q22-q31 | 4/45 (9%) |
| D18S51 | 18q21 | 7/45 (16%) |

*Figure 2.* **LOH incidence in laryngeal cancer**

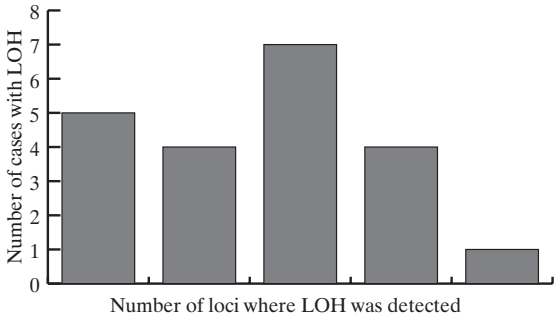
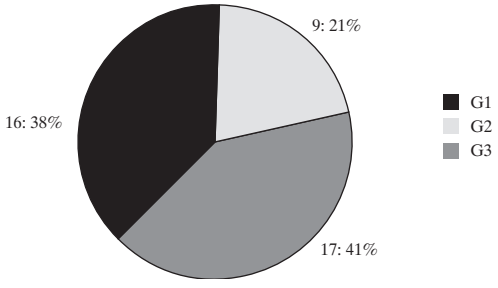



*Figure 1.* **LOH incidence and histopathological grade (G1, G2, G3): number of cases; % of cases**




on the following chromosomes: 3p, 5q, 8p, 9p, 9q, 11q, 17p, 17q, 18p, 18q. Changes in other DNA regions are also possible [3].

## Material and methods

The study was performed in a group of 46 male patients, aged 42-77 years. Forty three patients underwent total laryngectomy with lymph nodectomy, two patients – chordectomy and one patient – partial laryngectomy. Tumour tissue specimens and reference peripheral blood samples were obtained during surgical resections. Standard methods were used for DNA isolation. Additional microcolumn purification was performed when necessary. Fluorescent multiplex PCR was used to amplify microsatellite loci included in commercially available human identification kits: AmpFlSTR Profiler and AmpFlSTR SGM Plus (Applied Biosystems, USA) according to the manufacturer's recommendations. Genotyping was performed in 310 ABI Prism Genetic Analyzer (Applied Biosystems, USA) using the GeneScan Analysis v3.1 and Genotyper v2.5 software. The examined microsatellite markers and targeting genes are presented in *Tab. 1*. Amplification of the BAT26 locus was performed in a separate reaction using PCR Core Kit (Qiagen, USA) and commercially synthesized primers of the following sequences: forward primer 5'-tga cta ctt ttg act tca gcc, reverse primer 5'- aac cat tca aca ttt tta acc. PCR conditions were as follows: initial denaturation at 94°C - 1 min, 35 cycles of 94°C - 30 s, 49°C - 40 s and 72°C - 40 s with the final elongation at 72°C for 10 min. PCR products were separated using horizontal non-denaturing PAGE in a discontinuous buffer system and silver stained. The analyses were performed twice, so the results were highly reproducible. LOH was defined as a decrease (at least 50%) in peak height of an allele compared with that of the other determined after comparison of normal and pathologic DNA. Amplicons were sized according to 100 bp ladder (Gibco, BRL). Frequency of LOH was calculated for respective samples. Results were analyzed using a statistical analysis program commonly applied in biomedical assays (t-Student test for pairs of variables).

## Results

On histopathologic specimens, G1 grade cancers were diagnosed in 9 patients (21%), G2 grade cancers in 17 patients (41%) and G3 grade cancers in 16 patients (38%) (*Fig. 1*). Lymph node metastases were found in 16/45 cases, with G1 in 2 patients, G2 in 5 patients and G3 in 9 patients. LOH was detected in 31% cases: one patient (2%) displayed LOH at 5 loci, 4 patients (9%) at 4 loci, 7 patients (16%) at 3 loci, 4 (9%) patients at 2 loci, 5 patients (11%) at 1 locus (*Fig. 2*). LOH frequency was statistically significant in high G grade cancers ($P=0.0034$).

Overall, 39 (87%) of 45 patients displayed LOH at the following loci: BAT26 – 19/45 (42%), D3S1358 – 6/45 (13%), FGA – 2/45 (4%), CSF1PO – 8/45 (18%), D5S818 – 5/45 (11%), D8S1179 – 1/45 (2%), VWA – 3/45 (7%), D13S317 – 4/45 (9%), D18S51 – 7/45 (16%). No LOH was found at the other microsatellite markers. No evidence of LOH was found at the other markers.

The highest LOH frequency was found in the tumor samples where the neighbouring cervical lymph nodes were affected. In 8/16 histopathologically verified cases with lymph node metastases LOH was found at BAT26 and at other studied STR loci, in other two cases – only at BAT26. The incidence of LOH at BAT26 was statistically insignificant ($p=0.07$). In 5/29 patients with non-involved cervical lymph nodes LOH was present at BAT26 and the particular chromosomal localisation (*Tab. 1*).

## Discussion

In the present study, LOH was found in 39 out of 45 cases (87%). Comparable data was obtained by El-Naggar et al., whereas Jin et al. found no statistically significant association between major karyotypic features and histological differentiation or TNM stage in the laryngeal cancer [4,5]. Microsatellite markers are commonly used in the analysis of allelic imbalance. Presence of LOH at numerous loci in tumor specimens may contribute to the development of a model of cancerogenous progression [6]. Application of microsatellite loci as genetic markers is common due to their abundance in the genome, extreme polymorphism and amplification by the PCR reaction. In 1996 Califano et al. proposed a model of genetic changes in head and neck squamous cell epitheliomas, which assumes positive correlation of LOH and histological grade, spanning from mild hyperplasia to invasive cancer [7] .Veltman et al. reported LOH at 18q21 in pre-malignant laryngeal lesions, which suggests involvement of this chromosomal region in the laryngeal carcinogenesis [8].

El-Naggar et al. analysed LOH in 20 patients suffering from head and neck squamous cell epithelioma [3]. According to these authors, in non-invasive cancers LOH was observed on the following chromosomes: 9p (28%), 9q and 18q (10%), 11q and 17p (7%), 3p and 18p (5%). High incidence of LOH in invasive cancers was observed on chromosomes: 9p (72%), 8p (53%), 3p (47%), 9q (35%) and 11q (33%). LOH was also correlated with DNA aneuploidy, high grading and low histologic differentiation [3]. In the present study the authors failed to obtain statistically significant correlation between LOH frequency and metastases to the lymph nodes.

## Conclusions

1. Our findings suggest positive correlation between LOH incidence and G grade in laryngeal cancers.
2. No statistically significant correlation was found between LOH frequency and metastases to the cervical lymph nodes.

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