

# Comparison of Loss of Heterozygosity as a Marker of Various Histological Grades of Squamous Cell Cancer

Dr Nitu Sharma<sup>1</sup>, Dr Rajat K Sharma<sup>2</sup>

<sup>1</sup>Asst Professor Pathology, Command Hospital, Udhampur, India

<sup>2</sup>Senior Consultant Radiology St Stephens Hospital, Delhi, India

**Abstract:** Cancer is the second leading cause of death globally and Head and neck squamous cell carcinoma (HNSCC) is the sixth most common malignancy. Tumors have always been graded to assess the biological behaviour and predict the prognosis. However with development of Molecular science Loss of heterozygosity (LOH) has come out as an important marker for predicting disease progression. We need to correlate specific markers in various tumor grades to predict the overall prognosis. **Methods:** An observational study aimed at studying the LOH at 3p & 9p by PCR in HNSCC. **Results:** 30 cases were studied and LOH either at 3p or 9p was seen progressively more in MDSCC & PDSCC. **Interpretation:** More studies for risk stratification and LOH collectively need to be done before incorporating LOH as a biomarker for predicting prognosis.

**Keywords:** Abbreviations Loss of heterozygosity LOH, , Head and neck squamous cell carcinoma HNSCC)

## 1. Introduction

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common malignancy, with an annual incidence of 300,000 new cases diagnosed worldwide. (1)

As per Indian data Cancers of oral cavity and lungs account for 25% cancer deaths in males and cancer of breast and oral cavity account for 25% cancers in females. (2) India has one third of oral cancers in the world (3). Head and neck tumorogenesis is a multistep process that involves the accumulation of multiple genetic and epigenetic alterations. (4)

Genetic alterations known to occur during carcinogenesis including point mutations, amplifications, rearrangements, and deletions. Concept of Loss of heterozygosity (LOH) developed with the discovery of tumor suppressor gene. LOH occurs in a somatic cell as a result of loss of genomic material specifically affecting the single retained copy of a fundamental allele.(1)

Allelic imbalances have been reported in HNSCC in chromosomes 3,4, 5, 6, 8, 9,10, 12, 13, 16, 18, 21 and 22. In the series of sequential accumulation, the earliest to be lost are 9p and 3p followed closely by 17p and others in the more advanced lesions.(4) Studies had demonstrated that loss of heterozygosity (LOH) at different loci were likely markers for prognosis in HNSCC. Loss of heterozygosity (LOH) was reported at 9p21–p22 in 72% of tumors (5) Allelic loss of 3p and 9p and other regions containing tumor suppressor genes has also been reported in precursor lesions of oral cancer showing varying degrees of dysplasia. Patrick et al quoted Allelic imbalance at one or more loci within 3p24–26, 3p21, 3p13, and 9p21 was associated with reduced survival, with a 25-fold increase in mortality rate compared with patients retaining heterozygosity at these loci (6) Various studies across the world have emphasized the role of LOH

especially at 3p and 9p as biomarkers in oral premalignant lesions however there are hardly any studies which have tried to correlate the LOH in various grades of cancer.

### 1.1 Aims and Objectives

To compare loss of heterozygosity, as a marker of various histological grades of cancer.

### 1.2 Principle of the study technique

We used Polymerase chain reaction (PCR) as the baseline technique for studying LOH. The technique employed was to extract the DNA from the affected tissue, run a PCR for the required locus and compare a loss of intensity of bands along with a control and a cut off of > 50 % loss of intensity was taken as LOH.

### 1.3 Source of case collection

A total of 30 consecutive cases of histologically defined invasive squamous cell carcinoma of the head and neck regions diagnosed at a tertiary care hospital were studied over a period of 2 years. SCC of the lip, tongue, buccal mucosa and retromolar trigone were included in the study. Only those cases where hemi-mandibulectomy with RND was performed were taken for the study. A part of the tumor tissue was collected in sterile eppendorf tubes on ice, frozen immediately after careful removal from the surrounding normal tissues and stored at -70°C until extraction of DNA was done.

The remaining resected sample was fixed in 10% buffered neutral formalin and routinely processed for histopathology. Peripheral blood was also collected in EDTA vacutainers from all of the above patients for DNA extraction. This was meant to serve as control DNA for comparison for

constitutive homozygosity while looking for loss of heterozygosity.

#### 1.4 DNA Extraction

DNA from fresh frozen sample was extracted by the standard Phenol chloroform extraction Method (Sambrook et al 1989). DNA from peripheral blood collected using EDTA as anticoagulant was extracted by a kit-based method (QIAamp DNA Blood Mini Kit, Qiagen, USA), following the manufacturers protocol. DNA extracted was quantified spectrophotometrically and 250 ng used as template for the polymerase chain reaction (PCR) amplification procedure. The extracted DNA was stored at  $-20^{\circ}\text{C}$ .

#### 1.5 LOH-PCR

PCR was performed on all the DNA samples (i.e. fresh frozen tumor, and peripheral blood) for each case. Six sets of primers were used as microsatellite markers (Table 1). Primers were obtained from Qiagen or Sigma. PCR amplification was performed in a total reaction volume of 50 $\mu\text{l}$ , as described in literature. Amplicons were studied by gel electrophoresis using both 4% agarose gel and 12% polyacrylamide gel electrophoresis and visualized by ethidium bromide staining. The intensities of the signals in tumor DNA were compared with those of the corresponding normal (blood derived) DNA. **Loss of heterozygosity, if any, was defined as a reduction in signal intensity of at least greater than 50%.**

## 2. Data Analysis

Data on LOH was correlated with grade of tumor. The data was analyzed statistically by the Fischer test.

**Table 1:** Primers used for LOH-PCR at various loci in this study

SN	Chromosome Marker	Forward/Reverse	Sequence
1	3p21-D3S1284	F	5' GCC TTG GGG GTA AAT ACT CT 3'
		R	3' GGA ATT ACA GGC CAC TGC TC 5'
2	9p21-D9S171	F	5' AGC TAA GTG AAC CTC ATC TCT GTC T 3'
		R	3' ACC CTA GCA CTG ATG GTA TAG TCT 5'

#### 2.1 LOH and Histological Grades

Grading of cancers is determined by cytological appearance, based on the idea that poorly differentiated tumors have a more aggressive behavior. Though most of the times the prognosis is related to the stage the correlation between histologic appearance and biologic behavior is less than perfect. We classify squamous cell carcinomas into three histological grades as Well differentiated, moderately differentiated and poorly differentiated. Study by Fonseca-Silva *et al.* assessed the histological parameters used to grade dysplasia with the LOH profile (7). The different grades of dysplasia did not show differences in the frequencies of LOH, but their study highlighted that histological features of dysplasia were associated with specific LOH. In fact Dual LOH on 3p and 9p helps distinguish premalignant lesions likely to progress to

invasive carcinoma from those lesions not likely to progress (1,8)

Prognostically various studies have tried to find out the role of LOH in overall survival and prognosis. Study by Kiran B Jadhav et al quotes LOH at 3p region in early stage of OSCC was significantly correlated with reduced disease free and overall survival (LOH positive patient survival was 42 months) (10) William WN Jr et al states the 3-year cancer-free survival rate was significantly lower for LOH-positive compared with LOH-negative groups (74% vs. 87%, hazard ratio: 2.19; 95% CI: 1.25–3.83;  $P = 0.01$ ). The authors have stressed that LOH testing in the management of oral premalignant lesions could be incorporated as a prognostic indicator in clinical practice. (9)

The aim of this study was to find a correlation of genetic alteration at 3p&9p chromosome in HNSCC with the histological grades of tumor. Out of the 30 cases studied 08(26.6%) were well differentiated squamous cell carcinoma, 21 (79%) of MDSCC and only 1(3.3%) PDSCC. Amongst these in WDSCC 3/8(37.5%) showed LOH at 3p and 50% at 9p. 03/8(37.5%) showed LOH for both 3p and 9p.(Table2) Similarly in MDSCC 61% (13/21) showed LOH at 9p, 19%( 4/21) for both 3p and 9p. Single case of poorly differentiated carcinoma included showed LOH for both 3p and 9p but cannot be compared with. The p value for 3p vs grade was 0.24 and 9p vs grade 0.81. Though incidence wise there is an increase in LOH at 9p for MDSCC 61% (13/21) but due to a limitation of small sample size it cannot be given a statistical correlation. Not many studies correlate the grades with LOH loss. Bockmuhl and colleagues reported deletions of chromosome 3p, 5q, and 9p with 3q gain in well differentiated tumors, whereas in poorly differentiated tumors deletions of 4q, 8p, 11q, 13q, 18q, and 21q and gains in 1p, 11q, 13, 19, and 22q were identified, thus suggesting an association with tumor progression (11). Most of the studies have tried to restrict themselves with tumor staging and progression with LOH.

## 3. Conclusions

LOH is being considered as a biomarker for predicting tumor progression and assess the prognosis, hence it becomes all the more necessary to substantiated its role in various tumor grades

**Table 2:** Tumor grade and LOH

	Total cases	3p loss	9p loss	both	none
<b>Grade WD</b>	08	03 (37%)	04 (50%)	03 (37%)	04
<b>MD</b>	21	05 (23%)	13 (61%)	04 (19%)	07
<b>PD</b>	01	01	01	01	none
<b>Total</b>	<b>30</b>	<b>09</b>	<b>18</b>	<b>08</b>	<b>11</b>

More definite studies need to be undertaken to establish the correlation with the grade before this can be taken up as a bio marker to predict tumor progression.

## 4. Conflict of Interest

No conflict of interest is declared.

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