Review Article

# TP53 Mutation as Molecular Marker in the Assessment of Surgical Margin Status in Head and Neck Squamous Cell Carcinoma

**Rayamajhi A[1], Acharya B[1], Khanal B[2]**

[1]Department of Clinical Oncology, [2]Department of Dental Surgery, NAMS, Bir hospital, Kathmandu

**ABSTRACT**

Genetic alteration of tumor suppressor and oncogene plays important role in development and progression of cancer. Tumor suppressor gene, Tp53 also knows as 'guardian of genome' has very important role in head and neck squamous cell carcinoma (HNSCC). Mutation of Tp53 has been commonly observed in other carcinomas including HNSCC, comprising of 75-85% of head and neck cancer, with highest in larynx and hypopharynx followed by oral cavity. Tp53 mutation is more commonly seen in Human Papilloma Virus (HPV)-ve and wild type Tp53 in HPV+ve carcinomas. Role of Tp53 mutation help us to know about the prognostic state, molecular stage and chance of local recurrence. Different studies have highlighted the importance of assessing Tp53 mutation in the negative surgical margin, which showed mutation of Tp53 in the negative surgical margin resulted increase in chance of local recurrence. These studies provide significance of moving conventional method of histopathological assessment to molecular assessment, that gives advantage of proper management decision as well as prognosis of tumor. Regarding techniques of these assessments, molecular technique has been always superior to immunohistochemistry (IHC) but is somewhat tedious, so more clinical analysis on the alternatives of IHC combined with Next generation sequencing (NGS) gives the platform to perform more Tp53 mutation test in the surgical margin. This is the need of time to incorporate molecular staging in the conventional staging for proper treatment and outcome in the management of head and neck cancers.

**Key words:** Head and Neck squamous cell carcinoma P53 mutation Molecular techniques Molecular

## Highlights

- Tp53 also known as guardian of genome is the most important gene that plays role in carcinogenesis as tumor suppressor.

- Mutation of Tp53 has been observed in 75-85% of head and neck cancer, with the most common being larynx and hypopharynx followed by oral cavity.

- Histopathologically negative surgical margins when molecular analysis done showed Tp53 mutation.

- Local recurrence was common in margin showing Tp53 mutation, showing the importance of molecular analysis in histopathological asessement.

## Introduction

Head and neck cancer constitute cancer of oral cavity, oropharynx, hypopharynx, nasopharynx, paranasal sinuses and salivary glands. According to GLOBOCAN 2018, head and neck cancer constitute about 3.9% of total cases worldwide, being the 8 th most common cancer in the world. In Nepal, head and neck cancer comprises 10.3 % cases cumulatively which comes out to be third most common after lung and cervix. Oral cavity being the most common among the different subsites. [1] More than 90% of cancer in head and neck are squamous cell carcinoma and chewing betel, areca nuts, smoking bidis being some of the common reason in Indian subcontinents. The management of head and neck cancer includes

*Address of correspondence: Asmita Rayamajhi, MD, Consultant Radiation oncologist*
*Department of Clinical Oncology,*
*asmita.7714@gmail.com, 9849563298 NAMS, Bir hospital, Kathmandu*

different modalities like surgery, radiation therapy, chemotherapy and their combinations depending on the sites, subsites and intention of treatment. [2] Majority of tumors present with the lymph node metastasis at the time of diagnosis and about 40-60% of patients experience recurrence. [3][4] Despite of improvement in treatment modalities 5-year overall survival of head and neck squamous cell carcinoma (HNSCC) have not changed much. [5][6] HNSCC classification and clinical management depends mainly on anatomic location, phenotype, clinical stage which are explained by AJCC (American Joint Committee on Cancer) TNM (Tumor Node and Metastasis) staging. After surgical resection of the primary cancer and cervical nodes, pathological stage of the neoplasm depends on histopathological examination and the management is based on TNM staging as well as histopathological description as marginal status, extracapsular extension, depth of invasion.[7] Despite of complete surgical removal, local recurrence occur in up to half of the patients with negative surgical margins that leads to treatment failure. [8] There are two theories for the recurrence of tumor. One is residual cancer cells that have been undetected by pathologist in surgical margins and other one is tumor related mucosal precursor lesion and genetically altered field that goes unnoticed and give rise to invasive cancer later. [9] Carcinogenesis is commonly explained by genetic alteration in the oncogene and tumor suppressor genes. Among the various tumor suppressor genes, p53 has been the most common tumor suppressor gene to be mutated in head and neck cancer. [10] After surgical resection, minimum of 1 cm margin has been taken for margin clearance to prevent the recurrence but true frequency of margin positivity is not just explained by margin clearance but also molecular studies. Various studies have been conducted to see the chance of recurrence in the negative surgical margin with presence of p53 mutation in the tissues, that gives real picture of margin status and re staging of tumor. This assessment and restaging of tumor help in providing proper treatment and answering of recurrence of tumor despite of treatment. [11][12] In this review we will focus about the role of p53 in head and neck cancer in assessing the surgical marginal status, recurrence of disease, different techniques to assay p53 level, its role in molecular staging and its application.

## Molecular Progression and Implication of P53

The basis of molecular staging is explained by the concept of clonality. With the several debates whether monoclonal or multiclonal concept of tumor cells, Dr. Weinberg concluded majority of tumor cells are monoclonal i.e., developed from single progenitor cells. Though monoclonality is fundamental concept, tumor heterogeneity exists with several genetic mutation and tumorigenesis occur due to the result of series of these genetic mutations.[13] As previously mentioned about the importance of p53 among different molecular markers. Tp53 gene consists of 11 exons and p53 protein consists of 393 amino acids and four regions with different functions. Wild type p53(WT p53), also known as "guardian of genome" exerts its tumor suppressive function by regulating different downstream target genes involved in cell cycle arrest, apoptosis, senescence, DNA repair and metabolism.[14] Normally when there is no physiological stress, p53 has very short life, maintained at low level and is degraded by E2 ubiquitin ligase, MDM2,pir h2 and COP1.[15] When there is physiological stress then there is rise in p53 protein, where by post translation modification and stabilization occurs by phosphorylation, acetylation and increase in protein levels to combat the stress by cell cycle arrest, senescence, apoptosis, metabolism and differentiation.[16] Tp53 mutation can coexist with wild type Tp53 but has dominant negative effect of mutated Tp53 and at varying periods there is loss of wild type p53 due to its oligomerization and sequestration caused by mutated P53 owing to loss of heterozygosity and development and progression of tumor. In case of HNSCC wild type p53 is more common in Human Papilloma Virus (HPV)+ve cases and mutated type in HPV-ve cases.[17] Tp53 mutation comprises of 75-85% in head and neck cancer when assessed by next gene sequencing method (NGS). Among the different subsites, tumor of larynx and hypopharynx have highest mutation rate (83.5%) followed by oral cavity (75.6%) whereas tumors of oropharynx including tonsils and base of tongue have lowest mutation rate (28.6%). Lowest TP53 mutation in oropharyngeal cancer compared to other subsites is given the status that most of the oropharyngeal

carcinomas are HPV+.[18] [19] [20] [21] Tp53 mutation are usually missense mutation and the codons that are most common hotspots mutations are R248, R273, G245, R175, R282 and H19. Different studies like Boyle et al suggested that alteration in Tp53 occurs early in progression, similarly another Study by el-Naggar et al demonstrated normal margin of 3 cm from carcinoma did not show p53 mutation but the dysplastic lesion showed mutation which indicates occurrence of Tp53 mutation in the early phase of disease. As p53 mutation occurs early in the disease progression and results in overexpression of protein so surgical margin can be immuno stained to identify dysplastic precursor lesion or residual cancer cells. For a molecular marker to imply as cancer staging it most fulfill certain criteria and p53 has been successful to fulfill all these criteria and has been the scope of study for molecular staging. These criteria are marker or genetic mutation should be associated with development of cancer, genetic mutation should precede or occur at the time of invasion and markers should provide growth of tumor and to be present in all neoplastic cells. [22] [23]

## Technique to Assay P53 Mutation

Despite the surgical margin assessment, sampling of lymph node also provides the hinderance for the actual assessment of micro metastasis. Small foci of metastatic cancer have higher chance of being missed because of sampling problem. A single 5 micrometer section through 1 cm lymph node samples only 1/2000 of the node. In this scenario polymerase chain reaction (PCR) has been established as the efficient measure and has capacity to detect 1 mutant cancer cells among 10,000 normal cells. So, this molecular technique has been used by various studies previously to assess mutation in the tumor cells, to study presence of mutation in the surgical margins that provides one of the reasons for recurrence of tumor and focusses on the need of molecular staging. Earlier molecular technique for the assessment of p53 in stool sample in colorectal cancer, urine in bladder cancer have been frequently used Similarly, molecular techniques to assess p53 mutation in head and neck cancer have been done in various studies. There are sequence of events starting form deoxyribonucleic acid (DNA) extraction from the sample. These sample for DNA extraction might

be surgical margins, lymph node, sputum, and oral rinses. This is followed by amplification pf p53 encompassing exons 5-9 where the PCR products were then cloned to bacteriophage vector and amplified further in Escherichia coli. After the amplification there occurs the process of molecular probing whereby the clones were transferred to nylon membrane and hybridized with oligonucleotide probe. After hybridization membrane is washed and exposed to the x ray film. This hybridization leads to identify the mutant p53 gene. These oligonucleotide probes are unique and specific for mutant p53 gene. This technique is tedious, robust but with high sensitivity and has been used in different studies previously by Brenner et al and Partridge et al and Houten et al.[7,9,11] Other technique is immunohistochemistry analysis of p53 protein. This technique is quite popular due to easy performance and less expensive but its range of sensitivity is variable compared to molecular technique. During IHC analysis monoclonal antip53 antibody is used to detect residual or precursor lesions. IHC analysis has been inferior to molecular analysis due to increase in false positive and false negative rates. 28% of p53 mutations produces truncated protein which is difficult to assess by immunohistochemistry. [7, 24] In a study by Houten et al where histopathologically negative surgical margin was assessed for p53 mutation by molecular analysis as well as IHC, immunohistochemistry missed some of the mutated Tp53 which has been shown positive by molecular analysis and resulted in local recurrence in those which were missed by immunohistochemistry. So, this study also concluded that molecular analysis was superior to immunohistochemistry in the analysis.[9] Some studies in gynecological malignancy has provided insights that IHC along with Next generation sequencing (NGS) can improve the accuracy of test but more of the clinical analysis is required for this. So more of the clinical studies is required to assess p53 mutation by both of these techniques in head and neck cancer as well.[25]

## Discussions

Importance of molecular staging has highlighted after the local recurrence of tumor despite of surgery, radiation and chemotherapy. Local recurrence is defined as occurrence of another

tumor within 3 years and distance of less than 2 cm from the primary tumor.[26] Several studies have been carried to assess whether the histopathological negative margin means true negative or study of molecular analysis in the margin provide the idea about restaging of tumor. Light microscopy detects cancer cells when population is exceeded by 5 % of total cancer cells, whereas molecular study helps to assess tumor cells when they comprise less than 0.01% of total cell population. [7,27,28,29] In a study by Brennen et al. molecular analysis was done among the histopathological negative margins with molecular techniques.[8] High incidence of residual tumor cells was seen in the histopathological negative margins which was suggested by Tp53 mutation in the margins and later there was local recurrence in these molecular positive margins. In the other study by Partridge et al molecular assessment was done in 18 patients of post-operative oral cavity cancer patients with conventional clear margins.[11] Margins were assessed by molecular technique. Out of which 6 of the cases had Tp53 mutation and among these 5 cases had locoregional recurrence. Similar study by Houten et al, where out of 76 molecular positive margins, 50 had local recurrences.[9] All these studies have been together summarized in a table. The summarization of above studies clearly projects that there might be molecularlypositive margins despite of histopathologically negative margins and whatever local recurrenceshave occurred all has occurred in molecular positive margin. Whenever there is molecular negative margin almost all of the above studies showed that there is no recurrence except one study, where recurrence was seen in two cases despite negative margins.

But there is discordant of the fact that, in this study margin was assessed by immunohistochemistry analysis unlike other studies where molecular analysis has been done for the assessment. As mentioned earlier immunohistochemical analysis though simple to conduct than molecular analysis, but is inferior to molecular analysis and chances of false negative margin status is also higher than molecular analysis. So, in this one study where recurrence occurred in negative p53 mutation margin, scenario might have been different if mutation status was conducted or re analysed through molecular technique to assess true negative status of that margin.

Another study by Tabor et al, which was retrospective study conducted in 13 recurrent cases where different factors were studied like the concept of 'minimal residual cells (MRC) for the recurrence of tumor, next is second field tumor (SFT), Tp53 mutation in the margin status. Concept of minimal residual cells for the recurrence itself includes the mutation status of Tp53 or Microsatellite instability (MSI). In 5 of 13 cases of recurrence, genetically altered field related to primary tumor was absent. Absence of genetically field gave clear indication that recurrence was due to presence of minimal residual cells. All five pair shared common Tp53 mutation and two shared common MSI pattern. In other 8 of 13 cases, genetically altered field was present. Among these genetically altered field as well there were total 3 cases which showed Tp53 mutation in primary tumor and one margin, 3 cases common MSI was observed, 2 patients common loss of heterozygosity (LOH) was present.

**Table I:** Studies showing molecular positive margin correlation with local recurrence

| Studies | Total number of patients | No. of cases with conventional negative margins | No. of molecularly assessed positive margins | No. of locoregional recurrence in Molecularly positive margins | Number of recurrences in Molecularly negative margins |
|---|---|---|---|---|---|
| Brenner et al. 1995 | 30 | 25 | 13 | 5 | 0 |
| Ball et al 1997 | 24 | 24 | 14 | 8(By IHC technique not molecular analysis) | 2 |
| Partridge et al 2000 | 18 | 18 | 6 | 5 | 0 |
| Houton et al2004 | 176 | 76 | 50 | 9(locoregional recurrence) 3(regional recurrence) | 0 (local recurrence) 1(regional recurrence) |

## Application of Molecular Staging

Molecular staging helps to decide during the decision of treatment making, whether to opt for aggressive treatment or less aggressive treatment and no requirement of adjuvant treatment in early stage diseases post-surgery. More aggressive treatment in higher stages helps to increase 5-year survival. If the histopathological margin is negative because of conventional sampling method, margin status could be properly assessed through molecular staging that would finally upstage the tumor and need of chemotherapy along with radiation in the concurrent basis and planning field and volume is also properly assessed during radiotherapy (RT). Similarly, chance of occult nodal metastases is also common in head and neck cancer. This technique of molecular analysis will surely help to assess the occult micromets and helps in upstaging of tumor and proper regional treatment by radiation in case of positive nodes.[7] Molecular staging not only helps in decision of treatment making but also helps to provide early salvage therapy post treatment for the cases where Tp53 mutation is seen. As discussed, earlier Tp53 mutation is seen very early in the precursor state, so assessment in the post treatment state in case of any dysplastic lesion also guide towards the timely therapy. Incorporation of molecular staging in head and neck cancer also help us to know about the real scenario of disease in the individual basis and guide the physicians for proper management as well as council the patient about prognosis and possibility of recurrence of disease. It also provides logical answer to the physician level about the recurrence of tumor despite of therapy. Its application can also be importance in post treatment counselling where P53 mutation is more common in smokers, so post treatment proper counselling can be done to patient about the carcinogenesis effect of smoking that increases the mutation and results in local recurrence. [24]

## Limitations of Molecular Assessment

Limitations of Molecular Assessment As discussed earlier, sensitive molecular analysis process is laborious and immunohistochemistry is not that sensitive as molecular analysis marker. So, despite of importance of p53 status that has been highlighted in different studies for the upstaging of tumor not many studies have been carried out.

The technique of assessment has itself provide limitation for the study, which can be solved by researches and alternatives. Alternatives like IHC combined with NGS can be used to increase the sensitivity of IHC technique alone and clinical researches can be done in this field to use of these techniques for proper surgical margin assessment.

## Conclusion

Tumor suppressor gene Tp53 that encodes p53 protein has important role in head and neck carcinomas. Assessment of Tp53 mutation provides the knowledge about the aggressive nature of tumor, prognostic status, upstaging of head and neck cancer postoperatively by truly assessing the marginal status. Proper surgical assessment gives us advantage of proper treatment strategy. Since the oncology has moved from generalized to personalized medicine with the help of molecular markers as well as targeting the molecular markers, assessing mutation of this important protein, p53 in head and neck carcinomas helps to lead to proper management and answers to our question of recurrence despite of treatment.

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