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EXPRESSION OF P53 (WILD) IN RESECTED MUCOSAL MARGINS OF ORAL CANCERS

Moitrayee Sharma,[1*] Mridul Kumar Sarma,[2] Ranadeep Gogoi,[3] Devajit Choudhury,[4]

Maihur Basumatary,[5] Mrinmoy Mayur Choudhury,[6] Asim Debnath[7]

[1]BDS, MDS, Junior Resident, Department of Head & Neck Oncology, State Cancer Institute, Government Medical College, Guwahati, Assam*
[2]MS [ENT], Associate Professor, Department of Head & Neck Oncology, State Cancer Institute, Government Medical College, Guwahati, Assam
[3] MSc, PhD [PGDHRM], Assistant Professor, Department of Molecular Oncology, State Cancer Institute, Government Medical College, Guwahati, Assam
[4]Associate Professor, Department of General Surgery, State Cancer Institute, Government Medical College, Guwahati, Assam
[5]MSC [Molecular Biology], Junior Resident, Department of Surgical Oncology, State Cancer Institute, Government Medical College, Guwahati, Assam
[6]MS [ENT], Assistant Professor, Department of Head & Neck Oncology, State Cancer Institute, Government Medical College, Guwahati, Assam
[7]Senior Resident, Department of Head & Neck Oncology, State Cancer Institute, Government Medical College, Guwahati, Assam

***Corresponding Author:**
Dr. Moitrayee Sharma,
Email: Drmoitrayee@gmail.com

ABSTRACT

Background: Oral squamous cell carcinoma (OSCC) is the sixth most common cancer globally and the third most common in developing countries, caused by genetic changes and tumor suppressor gene mutations.

Aim: To study the expression of *Wild- type* p53 in resected mucosal margins of oral cancers.

Methods: Resected mucosal margins samples were collected with informed consent and stored at -80^{O}C. DNA was extracted using a QiAMP Tissue DNA Extraction Kit and quantified the same at A260/A280 in the ratio of 1.7-2.0. Then the DNA were subjected for the gradient Polymerase Chain Reaction following standard PCR cycling protocol followed by 1.5% agarose gel run for 90 minutes at 100V. The electrophoretogram was subjected for imaging and documentation for targeted P53 Exons 5, 6 and 7.

Results: Expression of Exons 5, 6 and 7 indicates the marked presence of *Wild- type* p53. Six cases (19%) of the study cohort showed the expression of *Wild-Type* p53 resected from the free margin tissues.

Conclusion: p53 expression in non-malignant mucosa, suggesting alterations in early carcinogenesis. Further study may be needed to compare p53 expression in free margin tissues and tumor margin tissues.

Keywords: P53 (Wild), Mucosal margins, Oral cancers

INTRODUCTION

Oral cancer refers to malignant epithelial tumors forming in the buccal mucosa, maxillary gingiva, mandibular gingiva, hard palate, tongue, and oral floor.[1] Oral squamous cell carcinoma (OSCC) is the sixth most common cancer globally and the third most common cancer in developing countries.[2] Squamous cell carcinoma is caused by genetic changes, resulting in abnormal or increased protein production, and tumor suppressor gene mutations, leading to uncontrolled cell growth and invasion.[3] Oral cancer affects 370,000 patients annually, with over 170,000 dying. OSCC is highly invasive, with lymph node metastasis common in advanced cases. [4,5]

The p53 gene, a key tumor suppressor, regulates cell cycle and DNA damage apoptosis. Mutations disrupt its transactivating activity, causing uncontrolled cell growth.[3] Kerdpon et al.'s 1997 study revealed that all cases of normal oral mucosa examined by IHC were p53-negative.[6] IHC detection of p53 in normal epithelium is due to physiological stabilization of wild type p53 due to genotoxic stress, UV radiation, hypoxia, and viral proteins. P53 mutations are linked to oral squamous cell carcinomas, a prevalent global malignancy, accounting for up to 40% of all malignancies in India and South East Asia.[7-9] The p53 gene, a nuclear phosphoprotein regulating cell growth and proliferation, is crucial in multistep carcinogenesis, as it can be altered or inactivated, promoting tumor development when inactivated.[10]

Lam et al. found 78% positivity for p53 in OSCC samples from buccal mucosa, mouth loor, and tongue.[11] Kerdpon et al. found that in Southern Thailand, 70% of cases of OSCC positive for p53,[12] while Thongusakai et al. found p53 positivity in 38.5% of cases.[13] Schoelch et al. found that 50% of OSCC expresses p53 expression, which increases as lesions progress from keratosis to dysplasia to carcinoma.[14] Cruz et al discovered supra basal p53 expression in non-malignant mucosa adjacent to p53 positive carcinomas, indicating p53 alterations can occur in early carcinogenesis.[15]

MATERIALS AND METHODS

The study included resected mucosal margins from thirty two treated oral cancer patient samples using sterile techniques with informed consent form after getting institutional ethical clearance from State Cancer Institute, Gauhati Medical College, Guahati, between June 2023 to May 2024 and stored them at -80^{O}C in the Molecular Oncology Laboratory. DNA was extracted from an approximately 25mg specimen using a QiAMP Tissue DNA Extraction Kit (Qiagen, Hilden, Germany),[16] and its concentration and purity were measured using a Multiscan Spectrophotometer (ThermoScientific, USA). The OD260/OD280 ratio indicates nucleic acid purity, with good-quality DNA having an A260/A280 ratio of 1.7-2.0. Specific primers were designed targeting the P53 gene using available bioinformatics blast tools shown in the table I.

Veriti Thermal Cycler (Applied Biosystems, Singapore) was applied for the Polymerase Chain Reaction of the targets. The PCR reaction mix were prepared on ice following the instructions (PCR Super Mix)**:** For each reaction, the following components were combined in a sterile micro centrifuge tube: 2µl of template DNA (usually 50-100ng), 1µl of forward primer (10µM), 1µl of reverse primer (10µM), 45µl of PCR Super Mix and sterile distilled water to make up the final volume to 50µl.

Prepared control reactions using nuclear free water instead of the template DNA. The reaction tubes were mixed gently and briefly centrifuged to collect the contents at the bottom.

- PCR cycling conditions applied in the protocol are given below:

- Step 1. Initial denaturation: 3 minutes at 95°C for 1 cycle,
- Step 2. Denaturation: 60 seconds at 95°C,
- Step 3. Annealing: 30 seconds at 55°C
- Step 4. Extension: 60 seconds at 72°C.

Repeated the steps 2-4 for 35 numbers of cycles. Final extension: 10 minutes at 72°C and holding stage: 4°C until further analysis. 1.5% Agarose gel (HiMedia) was prepared with 100 bp DNA ladder as reference at 100 V for 90 minutes. Visualization of DNA bands was made using Gel Documentation using iBright 1500 (Thermo Fisher Scientific, USA). Examined the gel image and compared the banding patterns of the patient samples with DNA Ladder and negative controls. The presence of specific DNA bands were observed that indicate the presence of P53 wild type with specific exons (Exon 5, 6, 7). Noted the size of the amplified DNA fragments and compared them to the expected amplicon size based on the primer design.

Finally, the data generated from gradient PCR and gel documentation analysis were subjected for the clinical interpretation of the subjects targeted.

RESULTS

The total number of Extracted DNA were quantified and down streamed for PCR. The PCR run was done in batch of 3, each consisting 10 samples. All the Gel runs were in 3 batches performed under the same conditions. The PCR product of different samples gave sharp band indicating the presence of each from *Wild-type* p53 of Exon 5, 6 and 7 (Figure 1-4). The gels were mixed with 5 µl Ethidium Bromide and placed on the gel casting tray. The gels were then loaded with 2 µl 50bp DNA Ladder in both the segments of gel electrophoresis run. The gels were then loaded with 5µl of PCR product along with loading dye with a ratio of 1:1. After the gel run the visualization was done in iBright Gel Documentation system. In figure 1, Sample 10 shows clean Band for Exon 5, 6 and 7 indicating the clear presence of *Wild- type* p53. In figure 2, 3 and 4, *Wild-type* p53 band was observed. Out of 31 cases, 6 cases (19%) showed the *Wild-Type* p53 which were resected from the free margin tissues.

DISCUSSION

p53 has an important role in the Head and Neck Cancer. Patients with tumor growth through histological studies show the expression of p53 Gene which is a tumor Suppressor Gene. Expression of p53 in non-tumor tissue may indicate the spreading of Cancer, or recurrence of Cancer post-surgery.

The present study supported the findings of Yang *et. al.,*[17] who stated that p53 overexpression is a risk factor for tumor recurrence and reduced survival in early OSCC patients. In the present study, out of the six samples showed wild type p53 gene expression, one sample showed the recurrence bearing T4 grade even after application of radiotherapy (Table:1). p53 in resected tissues may indicate DNA Repair or Apoptosis or inhibiting suppression of overexpression. In the present study, out of total of 11 exons of p53 gene, only Exon 5, 6 and 7 were observed in the free margin tissues of the sample cohort supporting the findings of Cruz et.al. 2000[15], who explained the expression of p53 gene in non-malignant mucosa of the resection margins of OSCCs might be a valuable predictor for local recurrences. However, absence of *Wild-type* p53 in rest of cases cannot be ruled out until all the exons are observed.

CONCLUSION

In the present study, samples showed *Wild-Type* p53 expressions were under gone recurrence bearing T4 grade category even after application of radiotherapy. Further, it may require to study with more samples for p53 in free margin tissues in comparison with p53 in tumor margin tissues of same cohort.

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Exon code	**Primer Sequence**	**Melting temperatures (Tm)**	**Product Length**
5	Forward primer: AGATAGCGATGGTGAGCAGC Reverse Primer: CTGGAGGGCCACTGACAAC	59.97 60.30	Product length 268
6	Forward primer: CTTGCCACAGGTCTCCCCAA Reverse Primer: GGGGATGTGATGAGAGGTGG	62.07 59.53	Product length 297
7	Forward primer: TCTCTGGCTTTGGGACCTCT Reverse primer: AGTGCTAGGAAAGAGGCAAGG	60.18 59.72	Product length 409

Table I: Primer Sequences of P53 of Exon 5, Exon 6 and Exon 7 with product length and Tm.

Patient's Age	Gender	Status	TNM	RT	Site
67	M	No Recurrence	T4aN1M0	No	Free margin
49	M	No Recurrence	T3N1M0	No	Free margin
40	M	Recurrence	T4aN0M1	Yes	Free margin
56	F	No Recurrence	T4a N3bM0	No	Free margin
55	F	No Recurrence	T2 N_O M_O	No	Free margin
50	M	No Recurrence	T3 N_O	No	Free margin

Table 1: Data of six patients showed *Wild-Type* p53

TNM, tumor-node-metastasis; RT, treated with postoperative radiotherapy

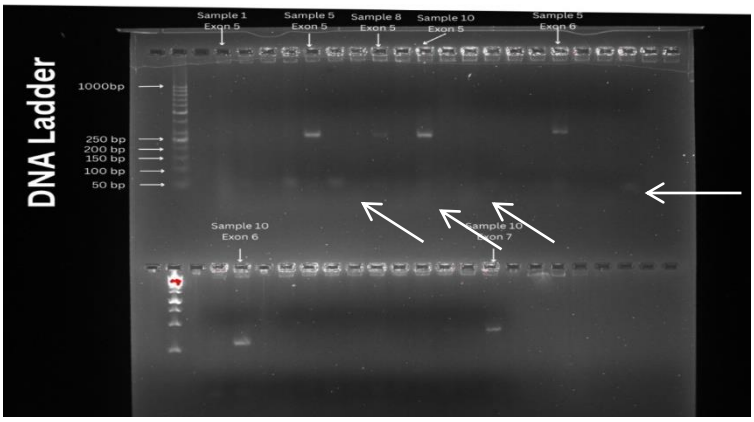

Fig 1: Migration of *Wild*-type p53 PCR amplicons of free margin tissue samples of Cancer Patients. Lane 4 to Lane 13 is for detection of p53 Exon 5, Lane 15 to Lane 29 is for detection of p53 Exon 6. Lane 31 to Lane 40 is for detection of p53 Exon 7.Lane 2& 26 DNA Ladder 50bp.

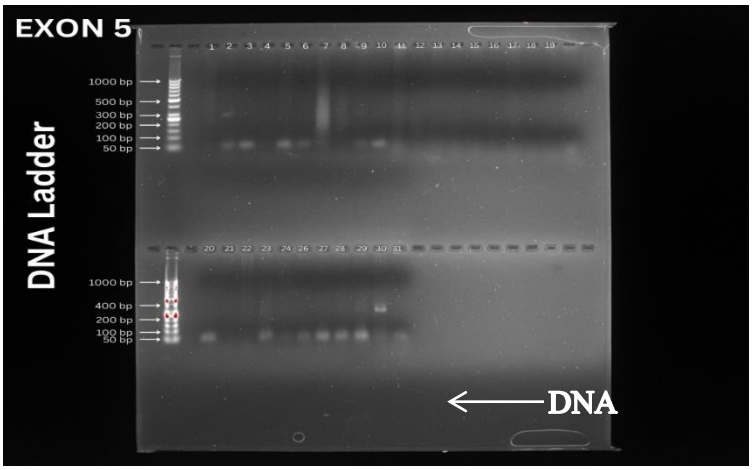

Fig 2. Migration of *Wild*-type p53 PCR amplicons of free margin tissue samples of Cancer Patients. Lane 2 & Lane 26 DNA Ladder 50bp. Gel-Run was observed for only Exon 5 of p53 with length of 268bp.

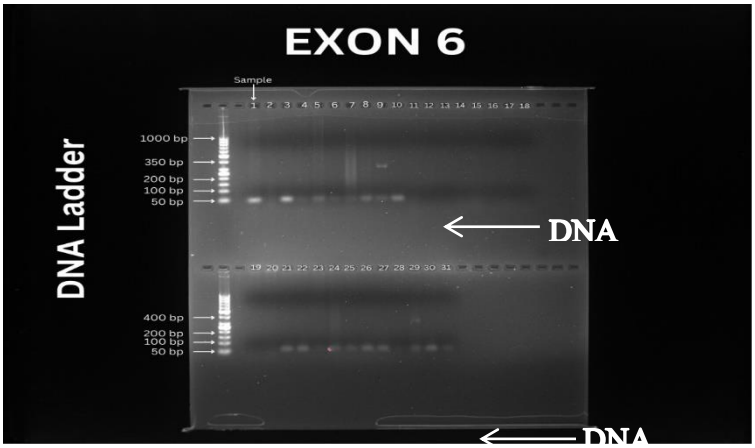

Fig 3. Migration of *Wild*-type p53 PCR amplicons of free margin tissue samples of Cancer Patients. Lane 2 & Lane 26 DNA Ladder 50bp. Gel-Run was observed for only Exon 6 of p53 *Wild-type* with length of 297bp.

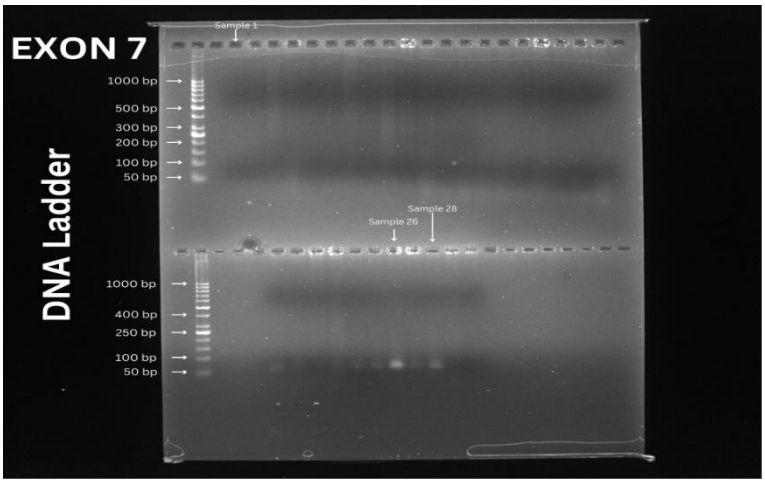

Fig 4. Migration of *Wild*-type p53 PCR amplicons of free margin tissue samples of Cancer Patients. Lane 2 & Lane 26 DNA Ladder 50bp. Gel-Run was observed for only Exon 7 of p53 *Wild-type* with length of 409bp.