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Original article

Prevalence and effect of *PIK3CA* H1047R somatic mutation among Indian head and neck cancer patients

Arjita Ghosh [1], Anbalagan Moorthy [1,*]

Department of Integrative Biology, School of Bioscience and Technology (SBST), Vellore Institute of Technology (VIT), Vellore, Tamil Nadu, India

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ABSTRACT

PIK3CA is one among the several mutated genes in cancer, including head and neck squamous cell carcinoma (HNSCC). H1047R is a hotspot somatic mutation in *PIK3CA* that occurs most frequently in several forms of cancers. Distribution of *PIK3CA* H1047R mutation in Indian HNSCC patients was screened and its effect on disease progression and response to treatment was analysed in this study. Genomic DNA was extracted from tumour biopsies of HNSCC patients (n = 48) and polymerase chain reaction coupled restriction fragment length polymorphism (PCR-RFLP) technique was used to screen for the mutation. Overall survival (OS) and Progression-free survival (PFS) of the patients were calculated in order to study effect of this mutation on survival and response to treatment respectively. Results showed that irrespective of patients' criteria, twenty-five patients (52 %) carried a heterozygous form of mutation (His/Arg) and the rest (48 %) were wild type (His/His). The mean OS of the cohort with the mutation was 20.451 months (SE ± 1.710 months) while 26.31 months (SE ± 2.431) was in wild type population. PFS of the patients with the mutation was 18.612 months (SE ± 2.072), and for the wild type population, it was 26.31 months (SE ± 2.431). These observations suggest that Indian HNSCC patients with *PIK3CA* H1047R mutation have poor prognosis.

1. Introduction

Cancer is a major disease burden all over the world which has been increasing alarmingly in recent times; attributed to changes in lifestyles. Every year, millions of patients are reported to be diagnosed with cancer and more than half of them die. Global Cancer Observatory (GLOBOCAN) reported an estimate of 19.3 million cancer incidents worldwide in the year 2020 (Sung et al., 2021). In mouth, squamous cells are present in inner surface of the mouth, pharynx, larynx, etc. in head and neck region, these cells maintain the surface moist. Cancer that affect squamous cells present in the mucosal are collectively called as Head and Neck Squamous Cell Carcinoma (HNSCC). Worldwide, HNSCC stands in top sixth position in the list of most frequently occurring cancer (Zhou et al., 2016). In India, it stands second of all kinds of cancers reported. It is estimated that every year 6 lakh HNSCC cases are reported annually out of which 3.5 lakh die (Higginson et al., 1993; Sathishkumar et al., 2022).

The cause of HNSCC varies depending upon regions and countries. Approximately 70–80 % of HNSCC cases can be correlated directly to tobacco and alcohol abuse (Jethwa & Khariwala, 2017). Besides, alcohol and tobacco usage, human papillomavirus (HPV) infection also causes HNSCC, especially in population where disoriented sexual habit is practiced (Johnson et al., 2020). Indian HNSCC patients are mostly of HPV negative cluster caused mainly due to tobacco usage (Murthy et al., 2017). The Cancer Genome Atlas (TCGA) reports that several genes are mutated in HNSCC; both in tumour suppressor genes as well as in oncogenes (G. Zhou et al., 2016).

PI3K is a heterodimeric protein comprising a catalytic p110 subunit complexed with a regulatory p85 subunit. This is a kinase, bound to inner surface of plasma membrane which mediates signalling associated with physiological events such as proliferation, differentiation, cell mobility and survival (Lui et al., 2013). Both the catalytic subunit and regulatory subunits with gain of function are reported in cancer. Hyper activation of signalling pathway involving PI3K is related to neoplastic transformation and tumour progression (Lui et al., 2013; Qiu et al., 2006). It is reported that the PI3K pathway is deregulated in several cancers including HNSCC (Samuels & Waldman, 2010).

PIK3CA gene which encodes p110 subunit of PI3K is one of the

* Corresponding author at: School of Bioscience and Technology (SBST), Vellore Institute of Technology, Vellore 632014, India.
E-mail addresses: arjita.ghosh209@vitstudent.ac.in (A. Ghosh), anbalagan.m@vit.ac.in (A. Moorthy).
[1] Address- School of Bioscience and Technology (SBST), Vellore Institute of Technology, Vellore- 632014, India.

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oncogenes that is found to be increasingly mutated in HNSCC (Qiu et al., 2006). The most common activation mutations of *PIK3CA* are E542K, E545K and H1047R. These mutant forms of the *PIK3CA* gene are reported in various cancers like colorectal (Li et al., 2016a), breast (Reinhardt et al., 2022), ovarian (Campbell et al., 2004), and lungs (Scheffler et al., 2014). H1047R is a strong driver in the development of tumours when compared to E542K, and E545K mutants (Janku et al., 2013). H1047R somatic mutation is caused due to single nucleotide change of 3140 A > G in exon 20 of the *PIK3CA* gene. H1047R results in increased phosphorylation of AKT and ERK 1/2 resulting in growth factor-independent cell survival (Guo et al., 2020). It is also reported that H1047R mutation is found mostly in HPV-negative HNSCC patients (Cochicho et al., 2022).

Considering the important role of the H1047R variant of the *PIK3CA* gene in cancer in general and particularly in HNSCC, the current study was performed to screen tumour cells of HNSCC patients from India for the mutation and study effect of this mutation on their survival rate and response to treatment.

2. Materials and Method

2.1. Patients and samples

Forty-eight tumour samples were collected (n = 48) from 42 male and 6 female patients with HNSCC, who were surgically treated at Chennai Apollo Hospital, India. The tumour samples were snap-frozen as soon as they were surgically removed and delivered to the laboratory. All information related to the patients' treatment protocol, recurrence of tumor and number of survival days were recorded (Table 1). Patients were staged following the standard TNM staging system. Specimens were majorly from the oral cavity and oropharynx with major sub-sites, tongue and buccal mucosa and histopathologically of mostly squamous cell carcinoma type followed by adenocarcinoma. Patients with HNSCC had records with/without tobacco usage for duration < 5 to > 10 years. All patients were of Indian origin and attended hospital from different states of India.

2.2. Ethical committee

This current study was approved by the Institute's human ethical committee (IEC/IRB No: IECH/2013/Dec18-006). All procedures performed in studies involving human participants were as per the ethical standards of the Institutional and/or National Research Committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

2.3. Inclusion and exclusion criteria

The criteria for inclusion observed for this study were as follows: i) patients with HNC of non-nasopharyngeal origin such as cancers of the laryngeal, oropharyngeal and oral cavity; ii) patient's biopsy with squamous cell cancer; iii) patients who are undertaking surgery or radical radiation therapy (accompanying chemoradiation therapy); iv) patients from stages I-IV A with potentially curable disease; and v) Patient who consented to this study and also its follow-up.

The conditions for exclusion for this study were observed as follows: i) patients diagnosed with nasopharyngeal or thyroid cancer; ii) patients carrying metastatic diseases in any visceral organs like liver, lungs or bones; iii) patients having a prior history of disease or have previously undergone radiation or chemotherapy; iv) Patients with expected survival less than six months and having poor performance status (KPS < 70); and iv) Patients carrying Li Fraumeni syndrome. Also, there was no age cut-off.

Table 1
Patient Data Table.

All Patients Criteria	Total n = 48	H1047R mutation n = 25	Wild Type n = 23
<45 years	21 (43.75 %)	10 (40 %)	11 (47.82 %)
>45 years	27 (56.25 %)	15 (60 %)	12 (52.18 %)
Gender			
Male	42 (87.5 %)	22 (88 %)	20 (87 %)
Female	6 (12.5 %)	3 (12 %)	3 (13 %)
Diagnosis			
Oral cavity	33 (68.75 %)	15 (60 %)	18 (78.27 %)
Oropharynx	5 (10.42 %)	3 (12 %)	2 (8.69 %)
Hypopharynx	4 (8.34 %)	2 (8 %)	2 (8.69 %)
Larynx	2 (4.16 %)	1 (4 %)	1 (4.35 %)
Maxilla	1 (2.08 %)	1 (4 %)	0
Others	3 (6.25 %)	3 (12 %)	0
Sub-site			
Tongue	12 (25 %)	8 (32 %)	4 (17.38 %)
Buccal mucosa	15 (31.25 %)	9 (36 %)	6 (26.09 %)
Gingivo-buccal sulcus	6 (12.5 %)	3 (12 %)	3 (13.05 %)
Hard palate	2 (4.16 %)	1 (4 %)	1 (4.35 %)
Base of tongue	3 (6.25 %)	1 (4 %)	2 (8.69 %)
Pyriform fossa	3 (6.25 %)	1 (4 %)	2(8.69 %)
Tonsil	1 (2.09 %)	1 (4 %)	0
Vocal cord	1 (2.09 %)	0 (0 %)	1 (4.35 %)
Maxilla	2 (4.16 %)	1 (4 %)	1 (4.35 %)
Others	3 (6.25 %)	0 (0 %)	3 (13.05 %)
Stage			
Stage I	12 (25 %)	5 (20 %)	7 (30.44 %)
Stage II	4 (8.34 %)	1 (4 %)	3 (13.05 %)
Stage III	10 (20.84 %)	5 (20 %)	5 (21.73 %)
Stage Iva	15 (31.25 %)	8 (32 %)	7 (30.43 %)
Stage IVb	5 (10.41 %)	4 (16 %)	1 (4.35 %)
Stage IVc	2 (4.16 %)	2 (8 %)	0
Histopathology Report			
Squamous Cell Carcinoma	44 (91.66 %)	23 (92 %)	21 (91.31 %)
Adeno Carcinoma	4 (8.34 %)	2 (8 %)	2 (8.69 %)
Others	0	0	0
Grade			
Grade I	15 (31.25 %)	10 (40 %)	5 (21.72 %)
Grade II	21 (43.75 %)	12 (48 %)	9 (39.14 %)
Grade III	12 (25 %)	3 (12 %)	9 (39.14 %)
Symptoms			
Ulcer	36 (75 %)	17 (68 %)	19 (82.61 %)
Bleeding	3 (6.25 %)	2 (8 %)	1 (4.35 %)
Cheek swelling	3 (6.25 %)	2 (8 %)	1 (4.35 %)
Swallowing difficulty	3 (6.25 %)	3 (12 %)	0
Voice change	2 (4.17 %)	0	2 (8.69 %)
Foreign body sensation	1 (2.08 %)	1 (4 %)	0
Symptom duration			
<3 months	15 (31.25 %)	10 (40 %)	5 (21.74 %)
3–6 months	22 (45.83 %)	9 (36 %)	13 (56.52 %)
>6 months	11 (22.92 %)	6 (24 %)	5 (21.74 %)
Tobacco Usage			
Yes	33 (68.75 %)	17 (68 %)	16 (69.57 %)
No	15 (31.25 %)	8 (32 %)	7 (30.43 %)
Tobacco usage duration			
No tobacco usage	14 (29.16 %)	8 (32 %)	6 (26.09 %)
<5 yrs	10 (20.84 %)	5 (20 %)	5 (21.74 %)
5–10 yrs	19 (39.58 %)	12 (48 %)	7 (30.43 %)
>10 yrs	5 (10.42 %)	0	5 (21.74 %)
Treatment			
Radical Surgery only	10 (20.83 %)	9 (36 %)	1 (4.35 %)
Surgery + Post-OP RT	37 (77.09 %)	15 (60 %)	22 (95.65 %)
Radical RT	1 (2.08 %)	1 (4 %)	0

2.4. DNA extraction

DNA was extracted from the 48 tumour samples by the High Salt Method (Gauthaman & Moorthy, 2020). In brief, the tumour tissue sample were digested in a microfuge tube containing 1 ml of TNES buffer with proteinase-k, for overnight at 45° C. The genomic DNA was ethanol precipitated in presence of NaCl. The DNA was pelleted by centrifugation and washed with 70 % ethanol. After ethanol wash, the DNA pellet was air dried and suspended in sterile distilled water. The quality and

quantity of the DNA was tested using spectrophotometer and Agarose gel electrophoresis.

2.5. PCR-RFLP

The 126 bp region of *PIK3CA* gene including the H1047R mutation site was PCR amplified using specific primers (Li et al., 2016b). The PCR was performed in a 50 µl reaction mixture consisting of 10x buffer (5 µl), 2 µl of each primer, 2 µl of dNTPs mixture (2.5 mM), 0.35 µl of Taq polymerase (1U), template DNA (100 ng) – 1 µl, 0.5 µl of DMSO, and 37.15 µl of H_2O. The PCR conditions used in the Eppendorf thermal cycler (Eppendorf) were as follows: Initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 45 s, annealing at 60 °C for 45 s, extension at 72 °C for 45 s and a single time final extension for 5 min at 72 °C.

The primer sequences used are as follows (Li et al., 2016b): Exon 20 forward primer 5′- GGAGTATTTCATGAAACAAATGAATGATGCG-3′, and reverse primer: 5′- GAGCTTTCATTTTCTCAGTTATCTT-3′. In the forward primer, a mismatched nucleotide was introduced (indicated by underline, G). This mismatch nucleotide introduces a new recognition site for the restriction enzyme FspI (TGCGCA). The PCR products were separated on 10 % polyacrylamide gel (PAGE) and viewed under UV light and documented in gel documentation system (Axygen).

To screen for *PIK3CA* H1047R mutation, the PCR products restriction digested with FspI enzyme (Thermo Fisher Scientific – 400 units). Due to the presence of the mismatched nucleotide (G) in the forward primer, PCR product (126 bp) on digestion produced 2 (96 bp and 30 bp – wild type) and/ or 3 (126 bp, 96 bp and 30 bp – heterozygous allele) products. The bands were resolved in 12 % polyacrylamide gel, observed under UV light and documented.

2.6. Sequencing

To collaborate the PCR-RFLP data sanger sequencing was performed on respective heterozygous and wild type samples. The anti- sense stand (i.e, the reverse primer) was used for sequencing. Sequencing data was outsourced.

2.7. Statistical analysis

Patients' data was collected and analyzed using SPSS 20 statistical software. Kaplan-Meier survival analysis was used to calculate the overall survival (OS) and progression-free survival (PFS) rate of the patients.

3. Results

Forty-eight HNSCC patients were distributed between 42 males and 6 females (87.5 % *versus* 12.5 %) with primary tumours located in the oral cavity (n = 33, 68.75 %), followed by oropharynx (n = 5, 10.42 %) and hypopharynx (n = 4, 8.34 %) respectively. Among major sub-sites, tongue (n = 12, 25 %), buccal mucosa (n = 15, 31.25 %) and gingiva-buccal sulcus (n = 6, 12.5 %) were there followed by base of the tongue, maxilla and other sub-sites. Collected samples represented all stages of HNSCC with the highest occurrence of stage IVa (31 %), followed by stage III (20.84 %), grade II (43.75 %) and grade I (31.25 %). Patients reported symptoms with the highest incidence of ulcer (n = 36, 75 %), followed by bleeding, cheek swelling (6.25 %), swallowing difficulties, voice change and foreign body sensation. The recorded duration of symptoms was less than 3 months to more than six months. Among them, 33 patients were tobacco users (68.75 %) and 15 did not (Table 1).

3.1. Screening of H1047R mutation

PIK3CA- 126 bp gene fragment flanking the mutation site was PCR amplified from genomic DNA isolated from 48 patient samples. The H1047R mutation of *PIK3CA* in exon 20 converts CAT to CGT, which converts the amino acid histidine to arginine. The forward primer used for PCR contains one mismatched nucleotide, which creates a new recognition site for the restriction enzyme FspI (TGCGCA) in the PCR product. If the product is obtained from a wild type allele, on digestion with the enzyme produces 96 bp and 30 bp products. As the H1047R mutation abolishes the restriction site, the 126 bp PCR product is not digested by the FspI enzyme. Therefore, all three bands of 126 bp, 96 bp and 30 bp were observed in heterozygous mutation. Using this strategy, we screened 48 HNSCC patients for the mutation and found that 25 patients in the cohort carried a heterozygous form of mutation (i.e. His/Arg) remaining 23 were wild type (i.e. His/His) and none were with homozygous mutation (Fig. 1).

Twenty-five HNSCC patients (52.1 %) with H1047R mutation observed in different diagnosed sites and sub-sites, stages, grades and symptoms showed no significant differences from 23 patients (47.9 %) without *PIK3CA* mutation (Table 1). The observed results from RFLP analysis could be considered as a good and affordable tool to differentiate between mutated and wild type populations of clinico-pathologically diagnosed HNSCC patients.

3.2. Sequencing Analysis

The PCR-RFLP data was verified by randomly selecting samples and sequencing the PCR products. Sample data of sequencing results of both wild type and heterozygous mutation are given below. In sense strand CAT (WT) is changed to CGT (Mutation), similarly in anti-sense strand, (ATG to ACG). Sanger's sequencing results for WT (Fig. 2a) and heterozygous mutation (ATG to AT/CG) (Fig. 2b) are shown below as predicted by PCR-RFLP.

3.3. Statistical Analysis

The overall survival and progression free survival were calculated by using Kaplan-Meier survival curve analysis. Our analysis shows that the mean overall survival of patients with heterozygous mutation H1047R was 20.451 months (SE ± 1.710 months), whereas wild type patients had an overall survival rate of 26.31 months (SE ± 2.431) (Fig. 3). Similarly, the overall median survival for the wild type was −30.6 months; whereas for heterozygous patients it was – 19 months. The mean progression-free survival for the population with the heterozygous mutation was 18.612 months (SE ± 2.072) whereas for the wild type patients, the mean progression-free survival was 26.31 months (SE ± 2.431) (Fig. 4). The median progression-free survival for the wild type was – 30 months, and for the heterozygous type was – 17.8 months. The P value was not statistically significant due to less number of events (OS: p-value- 0.727; PFS: p-value- 0.150).

4. Discussion

Cancer occurs either due to activation mutations in proto-oncogenes that turn them into oncogenes or inactivating mutations in tumour suppressor genes (G. Zhou et al., 2016). Cancer cells are also known to possess somatic oncogenic mutations that give them an added advantage for an increased rate of proliferation. H1047R mutation in catalytic subunit p110a, substitutes histidine with arginine, leading to conformational changes in the protein complex (Lui et al., 2013). It is well established in several cancers that H1047R is an oncogenic mutation.

In breast cancer, the H1047R mutation of *PIK3CA* was reported to induce multi-lineage and multi-potency in the mammary tumour, also compared to other hotspot mutations of *PIK3CA*, H1047R occurs mostly in breast cancer (Reinhardt et al., 2022; J. Zhou et al., 2022). In colon cancer, this mutation is reported to cause the development of mucinous adenocarcinomas and hyperplasia (Yueh et al., 2016). Based on these lines of evidence, we have screened for *PIK3CA* H1047R mutations in

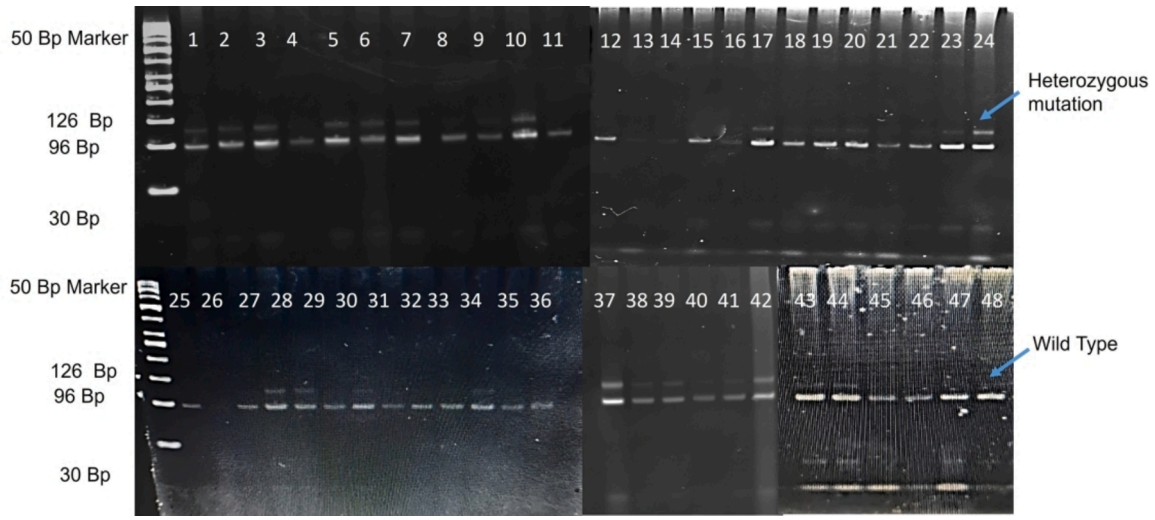

Fig. 1. RFLP analysis of PCR products (126 bp) obtained from 48 HNSCC patients. The FspI digestion of PCR product from patients with wild-type *PIK3CA* yielded two bands of 96 bp and 30 bp, while from heterozygous mutation of *PIK3CA* yielded 3 bands (intact 126 bp, 96 bp and 30 bp). Lanes 1–48 represent RFLP of 48 samples where Lane (4, 8, 11, 12, 13, 14, 15, 16, 21, 22, 25, 26, 27, 30, 31, 32, 33, 35, 36, 45, 46, 47, 48) represents wild type and Lanes (1, 2, 3, 5, 6, 9, 10, 17, 19, 20, 23, 22, 24, 28, 29, 30, 34, 37, 38, 39, 40, 41, 42, 43, 44) represents the heterozygous mutation. A 50 bp marker was used in 12 % PAGE.

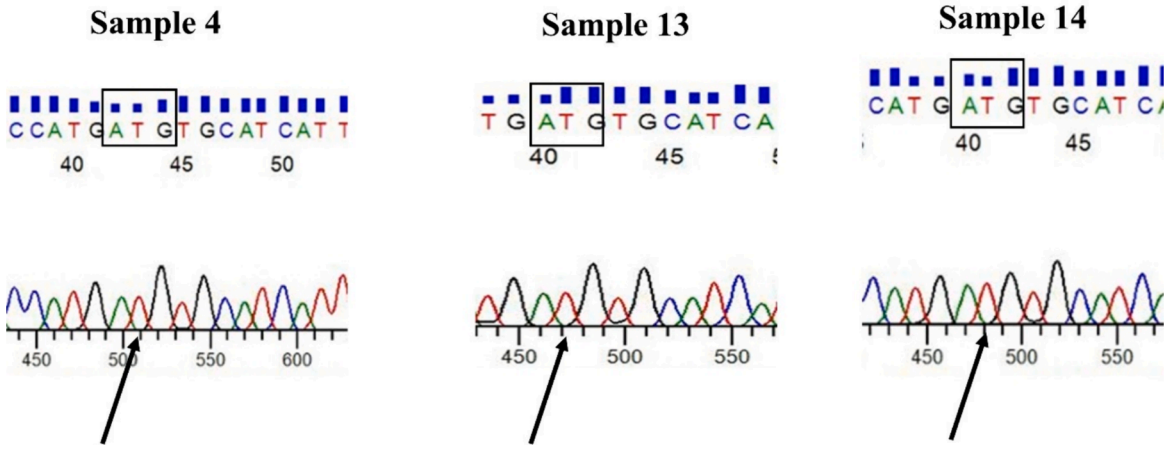

Fig. 2a. Wild type samples seen in patient sample 4, 13,14. Blue is C, red is T, black is G, and green is A.

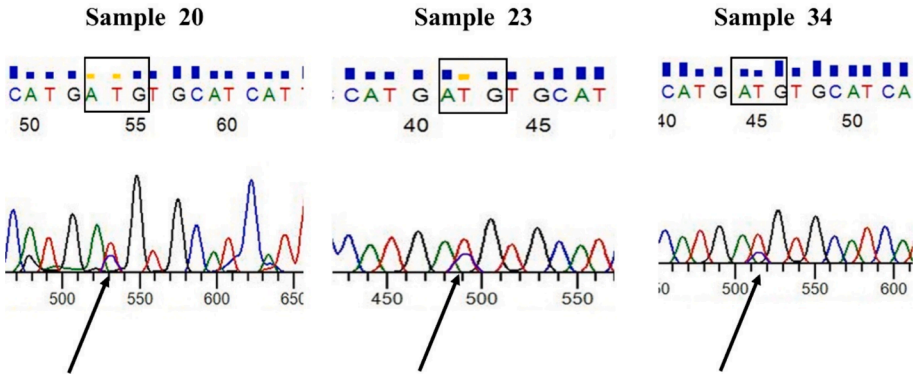

Fig. 2b. Heterozygous mutation observed in the patient samples 20,23,34. The C allele (represented by blue) is interposed with the T allele (represented by red) as seen in the figures showing the mutation.

our cohort.

Studies carried out in other cohorts of HNSCC patients have reported a low frequency of homozygous H1047R mutation in the *PIK3CA* gene. In Poland, 8 % of the patients carried the mutation (Borkowska et al., 2021), 3 % in Portugal (Cochicho et al., 2022), 2 % in the USA (Feldman et al., 2016) and 0.8 % in Japan (Suda et al., 2012). In this study, we used the PCR-RFLP technique, an affordable, advantageous and reliable tool with easy operation and suitability in regular laboratory. To our

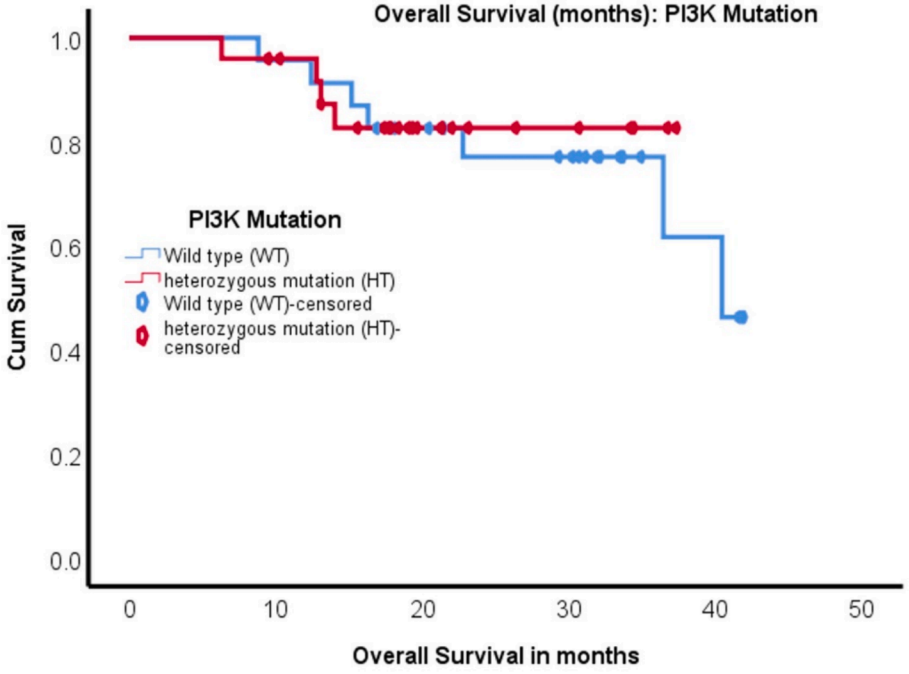

Fig. 3. Kaplan-Meier curve for overall survival analysis of 48 HNSCC patients.

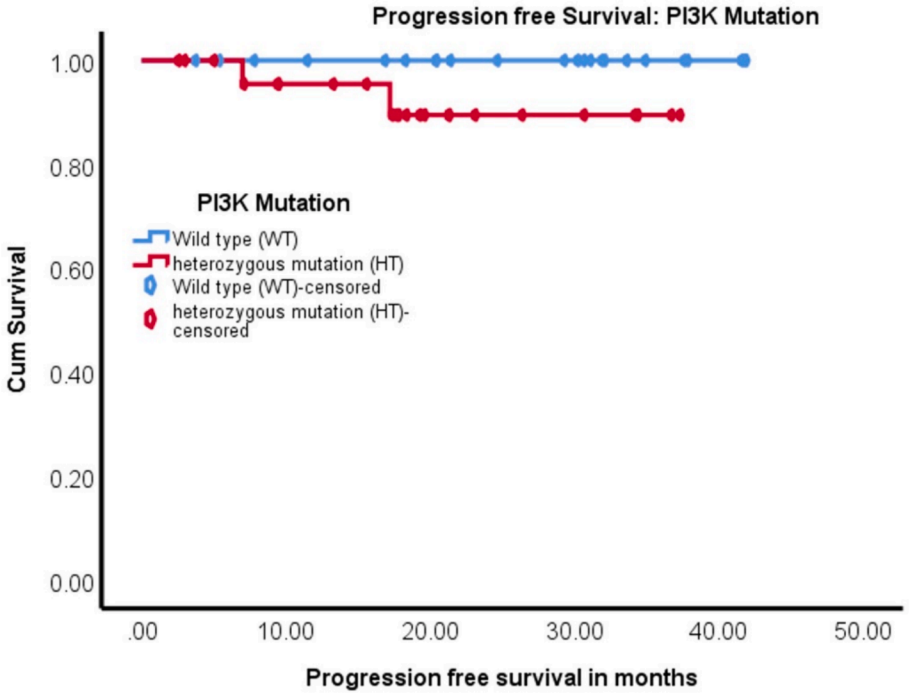

Fig. 4. Kaplan-Meier curve for progression-free survival analysis of 48 HNSCC patients.

understanding, this is the only study in India on H1047R mutation in HNSCC patients. In our cohort, 52 % of the patients possessed the H1047R heterozygous mutation. The huge difference in the percentage of mutations in the cohort could be attributed to the type of cancer, the stage at which the patients were enrolled in the study and the lifestyle of the patients.

Though we did not find any homozygous mutation in the cohort, being an oncogene, a heterozygous mutation is sufficient to cause a cancerous effect on the cell which is reflected in the survival curve analysis. Overall survival (OS) which reflects the number of survival days was less in the patients with H1047R mutation compared to the patients with wild type allele; 20.45 months vs 26.31 months. Progression-free survival, which measures the patient's response to treatment was also poor in patients with H1047R mutation compared to wild type allele; 18.61 months vs 26.3 months. These observations suggest that HNSCC patients with H1047R have a poor prognosis.

Identifying and correlating specific mutations in patients to the prognosis of cancer helps to understand their role in cancer progression, as well as this type of study promotes in personalizing treatment protocols to treat individual patients. In this connection, it is important to

note that in clinical trials, those patients having tumours with H1047R mutation responded to PI3K inhibitors (Janku et al., 2013; Hector & Guddati, 2020). Studies have also shown that patients with H1047R mutation responded more to PI3K/AKT/mTOR inhibitors compared to other PI3K mutations (Janku et al., 2013; Shi et al., 2023).

Prior studies from our laboratory have indicated that Indian HNSCC patients have a high incidence of oncogenic mutations such as Ras G12A (Gauthaman & Moorthy, 2021) and B-Raf V600E (Gauthaman & Moorthy, 2018). More advanced techniques such as whole genome sequencing of each patient in the cohort would give the global mutational status of HNSCC patients and may help to redefine the treatment strategy for better prognosis of the patients.

5. Conclusion

PIK3CA H1047R is a missense mutation found across various cancers, but its highest prevalence occurs in breast cancer. In head and neck cancer it also plays relatively a major role. The present study focused on finding hotspot mutation H1047R in the HNSCC population of Indian origin. As observed, 52 % mutation of heterozygous nature was observed in our study. No homozygous mutation was observed. By comparing the OS and PFS values we can conclude that patients who possess even heterozygous mutation of H1047R in *PIK3CA* have decreased survival rates.

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CRediT authorship contribution statement

Arjita Ghosh: Writing – review & editing, Writing – original draft, Methodology, Formal analysis. **Anbalagan Moorthy:** Resources, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Borkowska, E.M., Barańska, M., Kowalczyk, M., Pietruszewska, W., 2021. Detection of pik3ca gene mutation in head and neck squamous cell carcinoma using droplet digital pcr and rt-qpcr. Biomolecules 11 (6). https://doi.org/10.3390/biom11060818.

Campbell, I.G., Russell, S.E., Choong, D.Y.H., 2004. Mutation of the PIK3CA gene in ovarian and breast cancer. Cancer Res. 64 (21), 7678–7681. https://doi.org/10.1158/0008-5472.CAN-04-2933.

Cochicho, D., Esteves, S., Rito, M., Silva, F., Martins, L., Montalvão, P., Cunha, M., Magalhães, M., Gil da Costa, R.M., Felix, A., 2022. PIK3CA gene mutations in HNSCC: systematic review and correlations with HPV status and patient survival. Cancers 14 (5). https://doi.org/10.3390/cancers14051286.

Feldman, R., Gatalica, Z., Knezetic, J., Reddy, S., Nathan, C.A., Javadi, N., Teknos, T., 2016. Molecular profiling of head and neck squamous cell carcinoma. Head Neck 38, E1625–E1638. https://doi.org/10.1002/hed.24290.

Gauthaman, A., Moorthy, A., 2018. BRAF V600 mutation in Indian Head and Neck cancer patients. Front. Biosci. Elite 10 (3), 520–527.

Gauthaman, A., Moorthy, A., 2020. R248W mutations in p53 gene are rare among Indian patients with head-and-neck cancer. Indian J. Med. Paediatr. Oncol. 41 (4), 519–522. https://doi.org/10.4103/ijmpo.ijmpo_248_19.

Gauthaman, A., Moorthy, A., 2021. Prevalence of K-ras Codon 12 Mutations in Indian Patients with Head and Neck Cancer. Indian J. Clin. Biochem. 36 (3), 370–374. https://doi.org/10.1007/s12291-020-00882-w.

Guo, S., Loibl, S., Minckwitz, G.V., Darb-Esfahani, S., Lederer, B., Denkert, C., 2020. PIK3CA H1047R Mutation Associated with a Lower Pathological Complete Response Rate in Triple-Negative Breast Cancer Patients Treated with Anthracycline-Taxane-Based Neoadjuvant Chemotherapy. Cancer Res. Treat. 52 (3), 689–696. https://doi.org/10.4143/crt.2019.497.

Hector, P., Guddati, A.K., 2020. HNSCC resistant to chemotherapy 20.8.23. Am J Cancer Res 10 (9), 2742–2751.

Higginson, J., Muir, C.S., Munoz, N., 1993. Human Cancer. Eur. J. Cancer Prev. 2 (3), 285. https://doi.org/10.1097/00008469-199305000-00017.

Janku, F., Wheler, J.J., Naing, A., Falchook, G.S., Hong, D.S., Stepanek, V.M., Fu, S., Piha-Paul, S.A., Lee, J.J., Luthra, R., Tsimberidou, A.M., Kurzrock, R., 2013. PIK3CA mutation H1047R is associated with response to PI3K/AKT/mTOR signaling pathway inhibitors in early-phase clinical trials. Cancer Res. 73 (1), 276–284. https://doi.org/10.1158/0008-5472.CAN-12-1726.

Jethwa, A.R., Khariwala, S.S., 2017. Tobacco-related carcinogenesis in head and neck cancer. Cancer Metastasis Rev. 36 (3), 411–423. https://doi.org/10.1007/s10555-017-9689-6.

Johnson, D. E., Burtness, B., Leemans, C. R., Lui, V. W. Y., Bauman, J. E., & Grandis, J. R. (2020). Head and neck squamous cell carcinoma. In *Nature Reviews Disease Primers* (Vol. 6, Issue 1). Nature Research. DOI: 10.1038/s41572-020-00224-3.

Li, W.M., Hu, T.T., Zhou, L.L., Feng, Y.M., Wang, Y.Y., Fang, J., 2016. Highly sensitive detection of the PIK3CA H1047R mutation in colorectal cancer using a novel PCR-RFLP method. BMC Cancer 16 (1). https://doi.org/10.1186/s12885-016-2493-9.

Lui, V.W.Y., Hedberg, M.L., Li, H., Vangara, B.S., Pendleton, K., Zeng, Y., Lu, Y., Zhang, Q., Du, Y., Gilbert, B.R., Freilino, M., Sauerwein, S., Peyser, N.D., Xiao, D., Diergaarde, B., Wang, L., Chiosea, S., Seethala, R., Johnson, J.T., Grandis, J.R., 2013. Frequent mutation of the PI3K pathway in head and neck cancer defines predictive biomarkers. Cancer Discov. 3 (7), 761–769. https://doi.org/10.1158/2159-8290.CD-13-0103.

Murthy, V., Calcuttawala, A., Chadha, K., D'Cruz, A., Krishnamurthy, A., Mallick, I., Nair, S., Teni, T., Pawar, S., Talapatra, K., Patil, A., Bhatt, A., Chatterjee, S., Swain, M., Narayanan, P., Ghadyalpatil, N., Singhal, M., Kuriakose, M., Prabhash, K., … Parikh, P. (2017). Human papillomavirus in head and neck cancer in India: Current status and consensus recommendations. In *South Asian Journal of Cancer* (Vol. 6, Issue 3, pp. 93–98). Wolters Kluwer Medknow Publications. DOI: 10.4103/sajc.sajc_96_17.

Qiu, W., Schönleben, F., Li, X., Ho, D.J., Close, L.G., Manolidis, S., Bennett, B.P., Su, G.H., 2006. PIK3CA Mutations in Head and Neck Squamous Cell Carcinoma. Clin. Cancer Res. 12 (5), 1441–1446.

Reinhardt, K., Stückrath, K., Hartung, C., Kaufhold, S., Uleer, C., Hanf, V., Lantzsch, T., Peschel, S., John, J., Pöhler, M., Bauer, M., Bürrig, F.K., Weigert, E., Buchmann, J., Kantelhardt, E.J., Thomssen, C., Vetter, M., 2022. PIK3CA-mutations in breast cancer. Breast Cancer Res. Treat. 196 (3), 483–493. https://doi.org/10.1007/s10549-022-06637-w.

Samuels, Y., Waldman, T., 2010. Oncogenic mutations of PIK3CA in human cancers. Phosphoinositide 3-Kinase in Health and Disease 2, 21–41. https://doi.org/10.1007/82_2010_68.

Sathishkumar, K., Chaturvedi, M., Das, P., Stephen, S., Mathur, P., 2022. Cancer incidence estimates for 2022 & projection for 2025: result from national cancer registry programme India. Indian J. Med. Res. 156 (4), 598–607. https://doi.org/10.4103/ijmr.ijmr_1821_22.

Scheffler, M., Bos, M., Gardizi, M., König, K., Michels, S., Fassunke, J., Heydt, C., Künstlinger, H., Ihle, M., Ueckeroth, F., Albus, K., Serke, M., Gerigk, U., Schulte, W., Töpelt, K., Nogova, L., Zander, T., Engel-Riedel, W., Stoelben, E., Wolf, J., 2014. PIK3CA mutations in non-small cell lung cancer (NSCLC): Genetic heterogeneity, prognostic impact and incidence of prior malignancies. Oncotarget 6 (2).

Shi, Q., Xuhong, J., Luo, T., Ge, J., Liu, F., Lan, Y., Chen, Q., Tang, P., Fan, L., Chen, L., Liang, Y., Wang, M., Hu, Y., Zhang, Y., Bian, X., Qi, X., Jiang, J., 2023. PIK3CA mutations are associated with pathologic complete response rate to neoadjuvant pyrotinib and trastuzumab plus chemotherapy for HER2-positive breast cancer. Br. J. Cancer 128 (1), 121–129. https://doi.org/10.1038/s41416-022-02021-z.

Suda, T., Hama, T., Kondo, S., Yuza, Y., Yoshikawa, M., Urashima, M., Kato, T., Moriyama, H., 2012. Copy number amplification of the PIK3CA gene is associated with poor prognosis in non-lymph node metastatic head and neck squamous cell carcinoma. BMC Cancer 12. https://doi.org/10.1186/1471-2407-12-416.

Sung, H., Ferlay, J., Siegel, R.L., Laversanne, M., Soerjomataram, I., Jemal, A., Bray, F., 2021. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J. Clin. 71 (3), 209–249. https://doi.org/10.3322/caac.21660.

Yueh, A.E., Payne, S.N., Leystra, A.A., Van De Hey, D.R., Foley, T.M., Pasch, C.A., Clipson, L., Matkowskyj, K.A., Deming, D.A., 2016. Colon cancer tumorigenesis initiated by the H1047R mutant PI3K. PLoS One 11 (2). https://doi.org/10.1371/journal.pone.0148730.

Zhou, J., Imani, S., Shasaltaneh, M.D., Liu, S., Lu, T., Fu, J., 2022. PIK3CA hotspot mutations p. H1047R and p. H1047L sensitize breast cancer cells to thymoquinone treatment by regulating the PI3K/Akt1 pathway. Mol. Biol. Rep. 49 (3), 1799–1816. https://doi.org/10.1007/S11033-021-06990-X/METRICS.

Zhou, G., Liu, Z., Myers, J.N., 2016. TP53 Mutations in Head and Neck Squamous Cell Carcinoma and Their Impact on Disease Progression and Treatment Response. J. Cell. Biochem. 11 (May), 2682–2692. https://doi.org/10.1002/jcb.25592.