RESEARCH ARTICLE

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High Prevalence of *EGFR R479K* (rs2227983) Polymorphism in Indian Head and Neck Cancer Patients: Association with Unfavourable Clinical Outcome

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Abstract

Objective: The Epidermal Growth Factor Receptor (*EGFR*) gene, is host to several single nucleotide polymorphisms (SNPs), R497K (rs2227983) is one such example. The current study was conducted to screen a cohort of Indian Head and Neck Squamous Cell Carcinoma patients (HNSCC), for R497K variant of *EGFR* gene and to correlate effect of the SNP on survival parameters of the cohort. **Method:** Tumour samples were collected from 50 HNSCC patients from Apollo Hospital, Chennai. Genomic DNA from the samples was then extracted by using the high-salt method. The extracted DNA was then screened for *EGFR R497K* SNP by polymerase chain reaction coupled with restriction fragment length polymorphism (PCR-RFLP) technique. The PCR-RFLP results were then re-confirmed by Sanger sequencing. Then Kaplan-Meier statistical analysis was used to corelate between the SNP data to the survival rates (overall survival (OS) and progression free survival (PFS) of our patients. **Results:** Our study found, That among 50 patients 84% (42/50) in the cohort carried this SNP (78% were heterozygous, and 6% were homozygous), and only 16% (8/50) were wild type. Median OS for homozygous, heterozygous and wild type variant were 30.8 months (SE ±3.302; 95% CI: 24.39–37.36), 34.7 months (SE ±2.152; 95% CI: 30.51–38.94), and 41.4 months (SE ±0.511; 95% CI: 39.96–42.37), respectively with p-value of 0.409. Similarly, median PFS for homozygous, heterozygous and wild type variant were 30.0 months (SE ±3.952; 95% CI: 13.08–47.09), 33.5 months (SE ±2.404; 95% CI: 28.66–38.39), and 40.4 months (SE ±1.162; 95% CI: 39.49–41.44) respectively, with 0.553 p-value. Though it is not statistically significant, our study reports a declining trend in the OS and PFS of patients carrying the R497K polymorphism compared to the wild type patients. **Conclusion:** The SNP R497K of *EGFR* gene has high prevalence in our Indian study population, which corelates with poor prognosis for HNSCC.

Keywords: *EGFR* gene, Polymerase Chain Reaction coupled with Restriction Fragment Length Polymorphism (PCR-RFLP),

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Introduction

Head and Neck Squamous cell carcinoma is one of the most prevalent cancers in the World. It stands sixth in the list of most commonly occurring cancers. Each year about 600,000 cases get reported, out of which, about 355,000 are reported to die mostly due to the development of resistance to therapeutic agents [1]. The sites for HNSCC occurrence are the larynx, pharynx, oral cavity, paranasal sinuses, head and neck, etc. In India, HNSCC accounts for one-third of all reported cancer cases [2]. Mostly men are affected by this cancer compared to females due to tobacco usage both by chewing and smoking. Alcohol consumption, betel nut chewing, Epstein Barr virus, etc are the other minor causes of HNSCC [3].

The epidermal growth factor receptor (*EGFR*) belongs to the family of receptor tyrosine kinases which is activated by its ligand, epidermal-like growth factor (EGF). Ligand–receptor interaction leads to the activation of several signalling pathways associated with cell proliferation and survival such as PI3K/Akt/mTOR, JAK/STAT, and MAPK [4] Deregulation of *EGFR* pathway utilizing overexpression, mutation, and receptor variants all three are implicated in various types of cancer; including HNSCC [5]. The role of *EGFR* in cancer is evident by the fact that expression levels and mutations in the receptor are considered major prognostic marker in cancer patients [6] In HNSCC patients, 80-90% of cases show either overexpression of the receptor or some kind of mutation in their cohort, which is reported to impact the overall survival and progression-free survival of the patients usually towards poor prognosis [7].

Across populations, polymorphisms occur in both coding and non-coding sequences of the genome which influences the cause and response to treatment in cancer patients. Some of the major forms of polymorphisms

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reported in *EGFR* are -191C/A, -216G/T, and *R497K*. The non-coding polymorphisms -191 C/A and -216 G/T are located in the promoter region of the *EGFR* gene and contribute to the *EGFR* protein levels by regulating the transcription of the gene. *R497K* (rs2227983) is a variant form of the gene where arginine is substituted with lysine amino acid at codon 497 of the gene [8, 9]. This variant form of *EGFR* is reported to affect the cause, progression, and response to treatment of various cancers [10]. Cancer patients with *EGFR R497K* variant are reported to have poor prognostic factors. Studies have shown *R497K* functions in growth stimulation, ligand binding; and also, activation of tyrosine kinase leading to activation of proto-oncogenes like C-Fos, C-Myc, and C-Jun [11].

While globally, many studies have been conducted on the prevalence and prognostic effects of *EGFR R497K*, it is interesting to note that there is a significant lack of data concerning this SNP in Indian HNSCC patients. Till now, there has been no systematic evaluation of the clinical significance and distribution of *EGFR R497K* in Indian HNSCC patients, despite India carrying one of the highest global burdens for HNSCC. This study was done to fill the absence of data from population-specific studies and to understand the ethnic and geographic variation of this SNP and its association with outcomes of clinical treatment. This study contributes novel insights to cancer prognostics in the Indian context.

This work was carried out to study the distribution of the gene variant in the Indian HNSCC cohort and compare their survival functions by calculating overall survival and progression-free survival.

Materials and Methods

Patients and Specimens

Tumour samples were obtained from 50 HNSCC patients for this study. Patient recruitment was conducted at Apollo Hospital, Chennai where due to logistical challenges, and time constraints, only 50 patient samples were allowed to be collected. The tumour samples after resection were snap-frozen and transported. Informed consent forms were obtained from all the patients and the institute's human ethical committee approved this study (IEC/IRB No: IECH/2013/Dec18-006). 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. Patients with HNSCC had records with/without tobacco usage for duration <5 to >10 years, as mentioned extensively in Table 1. The samples were obtained from Indian patients from different parts of the country like West Bengal, Manipur, Andhra Pradesh, Tamil Nadu, and Pondicherry. This study was done in accordance with the ethical standards laid down by the 1964 Declaration of Helsinki and its later amendments. A smaller sample size can cause statistical limitations to this study, however rigorous inclusion, exclusion criteria, and data analysis were employed to ensure the validity of our study.

Exclusion and Inclusion Criteria

The criteria for inclusion observed for this study were as follows: 1) patients with HNC of non-nasopharyngeal origin such as cancers of the laryngeal, oropharyngeal and oral cavity; 2) patient's biopsy with squamous cell cancer; 3) patients who are undertaking surgery or radical radiation therapy (accompanying chemoradiation therapy); 4) patients from stages I-IV A with potentially curable disease; and 5) Patient who consented to this study and also its follow-up.

The conditions for exclusion for this study were observed as follows: 1) patients diagnosed with nasopharyngeal or thyroid cancer; 2) patients carrying metastatic diseases in any visceral organs like liver, lungs or bones; 3) patients having a prior history of disease or have previously undergone radiation or chemotherapy; 4) patients with expected survival less than six months and having poor performance status (KPS<70), also, there was no age cut-off.

DNA Extraction

High salt method was applied to extract DNA from tumour samples. The tumour samples were ground with liquid nitrogen in a mortar and pestle. The tissues were then digested in 1ml buffer (0.6% SDS, 100mM EDTA, 6M NaCl, 10mM Tris, and proteinase K 20mg/ml) overnight at 45ºC and incubated overnight. After the overnight treatment 227uL of 6M NaCl was mixed, and the solution was centrifuged for 10 minutes at 12,000 rpm. The supernatant was transferred and to it, equal amounts of 100% ethanol were added. This solution is again centrifuged for 10 minutes at 12,000 rpm. The supernatant was then removed and the DNA pellet remained. This pellet was washed and then air-dried. Then in 20-100ul of sterile water, the DNA was suspended. The DNA was quantified using a Nano-drop instrument (Thermo-Fisher Scientific). To check the quality of DNA it was also run through agarose gel electrophoresis. The DNA was then visualized and documented in Axygen documentation system.

PCR-RFLP

The genomic DNA of 50 patient samples was used as a template to PCR amplify a 155bp DNA region spanning the variant sequence using forward primer of sequence -TGCTGTGACCCACTCTGTCT and reverse primer of sequence -CCAGAAGGTTGCACTTGTCC. The primers were designed specifically to amplify the region encompassing *EGFR R497K* (rs2227983). This specific sequence of *EGFR* was retrieved from the NCBI GenBank database (AH006650.2). The tool NCBI Primer BLAST was used to design primers for this study. The 50µl PCR mixture consisted of 5µl of 10X buffer, 2µl of dNTP (2.5mM each dNTP), 2µl each of primers (100µM concentration each), 0.35µl of Taq polymerase (3 units/µl) (Genei, India), 0.5µl of DMSO, 1µl of gDNA (50ng/µl), and 37.15µl of sterile water. The PCR conditions were 94ºC of initial denaturation for 5 minutes, 40 cycles of denaturation - 94ºC for 40 seconds, primer annealing for 60.1ºC for 1 minute, primer extension of 72ºC for 1 minute, and then followed by final extension for 72ºC for 5 minutes. An aliquot of PCR products was separated on a 10% polyacrylamide gel (PAGE) to observe 155 bp DNA

Table 1. Patient Demographic Data for *EGFR R497K* (n=50)

All Patients Criteria	Total n=50	R497K Heterozygous Variant (Arg/Lys) n= 39	R497K Homozygous Variant (Lys/Lys) n=3	Wild Type n = 8
Age				
<45 years	22 (44%)	16 (41%)	0	6 (75%)
>45 years	28 (56%)	23 (59%)	3 (100%)	2 (25%)
Gender				
Male	40 (80%)	33 (84.7%)	3 (100%)	4 (50%)
Female	10 (20%)	6 (15.3%)	0	4 (50%)
Diagnosis				
Oral cavity	35 (70%)	29 (74.3%)	2 (66.7%)	4 (50%)
Oropharynx	5 (10%)	3 (7.7%)	0	2 (25%)
Hypopharynx	4 (8%)	4 (10.25%)	0	0
Larynx	2 (4%)	0	1 (33.3%)	1 (12.5%)
Maxilla	1 (2%)	1 (2.57%)	0	0
Others	3 (6%)	2 (5.18%)	0	1 (12.5%)
Sub-site				
Tongue	12 (24%)	10 (25.6%)	2 (66.7%)	0
Buccal mucosa	16 (32%)	13 (33%)	0	3 (37.5%)
Gingivo-buccal sulcus	6 (12%)	5 (12.8%)	1 (33.3%)	0
Hard palate	2 (4%)	2 (5.1%)	0	0
Base of tongue	3 (6%)	2 (5.1%)	0	1 (12.5%)
Pyriform fossa	3 (6%)	3 (8.3%)	0	0
Tonsil	1 (2%)	0	0	1 (12.5%)
Vocal cord	1 (2%)	1 (2.5%)	0	0
Maxilla	2 (4%)	1 (2.5%)	0	1 (12.5%)
Others	4 (8%)	2 (5.1%)	0	2 (25%)
Stage				
Stage I	13 (26%)	10 (25.6%)	0	3 (37.5%)
Stage II	4 (8%)	2 (5.1%)	0	2 (25%)
Stage III	10 (20%)	10 (25.6%)	0	0
Stage IV a	16 (32%)	12 (30.3%)	1 (33.3%)	3 (37.5%)
Stage IV b	5 (10%)	3(8.3%)	2 (66.7%)	0
Stage IV c	2 (4%)	2 (5.1%)	0	0
Grade				
Grade I	16 (32%)	10 (25.6%)	0	6 (75%)
Grade II	22 (44%)	19 (48.8%)	1 (33.3%)	2 (25%)
Grade III	12 (24%)	10 (25.6%)	2 (66.7%)	0
Symptoms				
Ulcer	37 (74%)	31 (79.1%)	3 (100%)	3 (37.5%)
Bleeding	4 (8%)	2 (5.1%)	0	2 (25%)
Cheek swelling	3 (6%)	1 (2.5%)	0	2 (25%)
Swallowing difficulty	3 (6%)	3 (8.3%)	0	0
Voice change	2 (4%)	1 (2.5%)	0	1 (12.5%)
Foreign body sensation	1 (2%)	1 (2.5%)	0	0
Symptom duration				
<3 months	16 (32%)	12 (30.7%)	0	4 (50%)
3-6 months	23 (46%)	20 (51.3%)	0	3 (37.5%)
>6 months	11 (22%)	7 (18%)	3 (100%)	1 (12.5%)
Tobacco Usage				
Yes	33 (66%)	28 (71.8%)	2 (66.7%)	3 (37.5%)
No	17 (34%)	11 (28.2%)	1 (33.3%)	5 (62.5%)

Table 1. Continued

All Patients Criteria	Total n=50	R497K Heterozygous Variant (Arg/Lys) n= 39	R497K Homozygous Variant (Lys/Lys) n=3	Wild Type n = 8
Tobacco usage duration				
No tobacco usage	17 (34%)	11 (28.2%)	1 (33.3%)	5 (62.5%)
<5 yrs	10 (20%)	8 (20%)	0	2 (25%)
5-10 yrs	17 (34%)	15 (39%)	2 (66.7%)	0
>10 yrs	6 (12%)	5 (12.8%)	0	1 (12.5%)
Treatment				
Radical Surgery only	10 (20%)	8 (20%)	0	2 (25%)
Surgery+ Post-OP RT	38 (76%)	30 (77.5%)	3 (100%)	5 (62.5%)
Radical RT	2 (4%)	1 (2.5%)	0	1 (12.5%)

bands. The PCR products were then digested by restriction enzyme BstNI (ThermoFisher 10U/ µl) at 37ºC for two hours. The restriction-digested PCR products were then separated in 12% PAGE gel. The banding pattern of the samples was then recorded and documented in the gel documentation system (Axygen).

Sanger Sequence Analysis

To collaborate the PCR-RFLP data Sanger sequencing was performed on random samples. The PCR product of homozygous, heterozygous, and wild-type samples was gel purified via a gel purification kit (Qiagen) and then sent for analysis.

Statistical Analysis

Analysis of data was done using SPSS 20 statistical software. Overall survival and progression-free survival rates of patients were checked by using Kaplan- Meier survival analysis.

Results

Screening of EGFR R497K polymorphism by PCR-RFLP

In the codon 497 of exon 13 of the *EGFR* gene, due to a polymorphic variation of a single nucleotide, G is turned to A, which causes the amino acid arginine (Arg) to be substituted to lysine (Lys) [4]. The 155bp PCR product of a specific sequence carrying the site of polymorphism, when digested with BstNI restriction enzyme, cuts the PCR product in two sites, creating three fragments of length- 67, 50, and 38 base pairs. If there is *R497K* substitution, one of the restriction site gets abolished, and the PCR product produces only two fragments of length- 117, and 38 base pairs. This is a homozygous variant (Lys/Lys). If the variant is of a heterozygous nature (Arg/Lys), 4 DNA fragments are obtained of the length- 117, 67, 50, and 38 base pairs as shown in Figure 1.

Of the 50 patient samples that were screened, 42 patients showed the presence of *R497K* substitution; of which 39 were heterozygous (Arg/Lys) and 3 were homozygous (Lys/Lys). The remaining 8 patients were

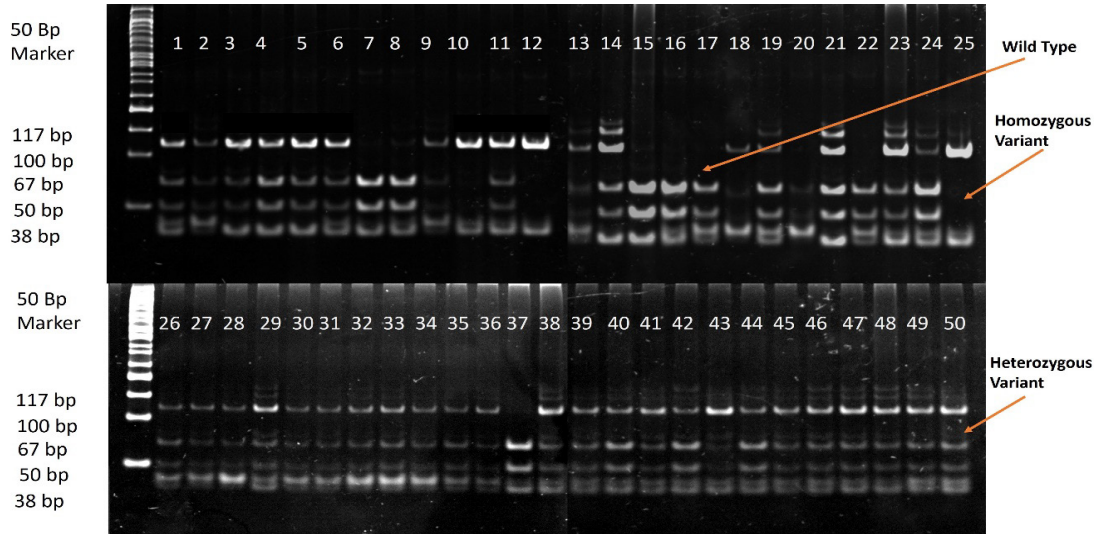

Figure 1. Screening of *EGFR R497K* Polymorphism in HNSCC Patients (n=50). Analysis of 50 patient samples using PCR-RFLP method. A 50 bp marker was used as a reference, and the bands were separated in 12% PAGE gel. The Homozygous variant is represented by two bands (117 and 38 bp), as seen in samples -10, 12, and 25. The heterozygous variant is represented by 4 bands (117, 67, 50, 38 bp) as seen in samples – 1, 2, 3, 4, 5, 6, 9, 11, 13, 14, 18, 19, 21, 23, 24, 26, 27, 28, 29,30, 31, 32, 33, 34, 35, 36, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50. The wild type is represented by 3 bands (67, 50, 38 bp), as seen in samples 7, 8, 15, 16, 17, 20, 22, 37.

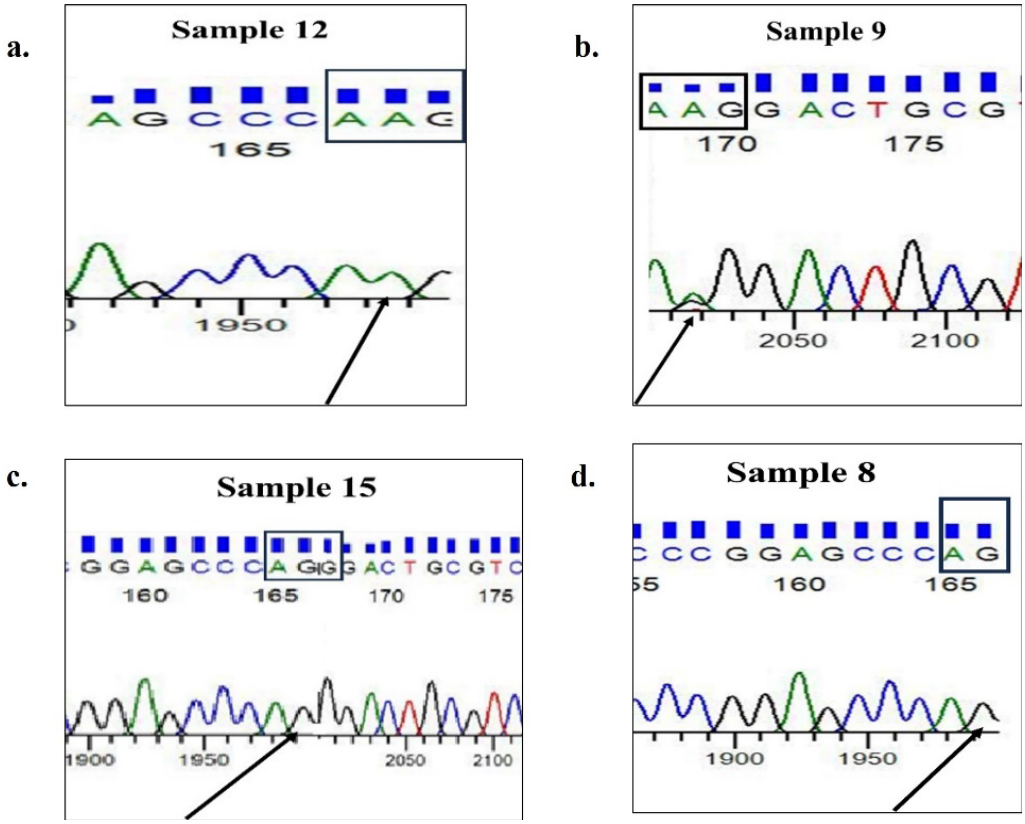

Figure 2. Sanger Sequencing of *RGFR R497K* Variants in Patient Samples. (a) The homozygous variant represented by sequence AAG (A is seen as green peak). The nucleotides are represented by the following colours C- blue, T- red, A- green, and G- black. (b) Heterozygous variant. The overlapping peak of A/G is seen (overlapping of green and black peaks). (c and, d) Wild type variant (AGG).

wild type (Arg/Arg).

Sequencing Analysis of Samples

Sanger sequencing results collaborated with our PCR-RFLP data. Forward primer was used to analyse the PCR products. The wild-type sequence was AGG, whereas the whereas the SNP was represented by AAG. The samples given represent homozygous (Figure 2a), heterozygous (Figure 2b), and wild-type samples (Figure 2c and 2d).

Statistical Analysis

When analysing the Overall survival (OS) by using

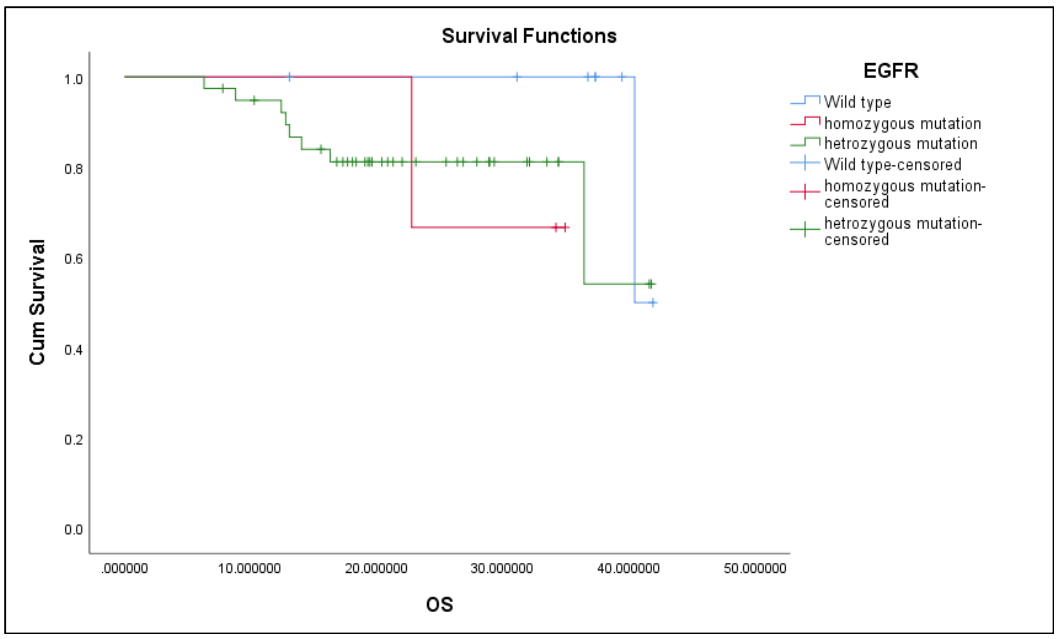

Figure 3. Kaplan-Meier Curve for Overall Survival Analysis of 50 HNSCC Patients. (X- axis represents the overall survival value in months, and Y-axis represents cum survival percentage)

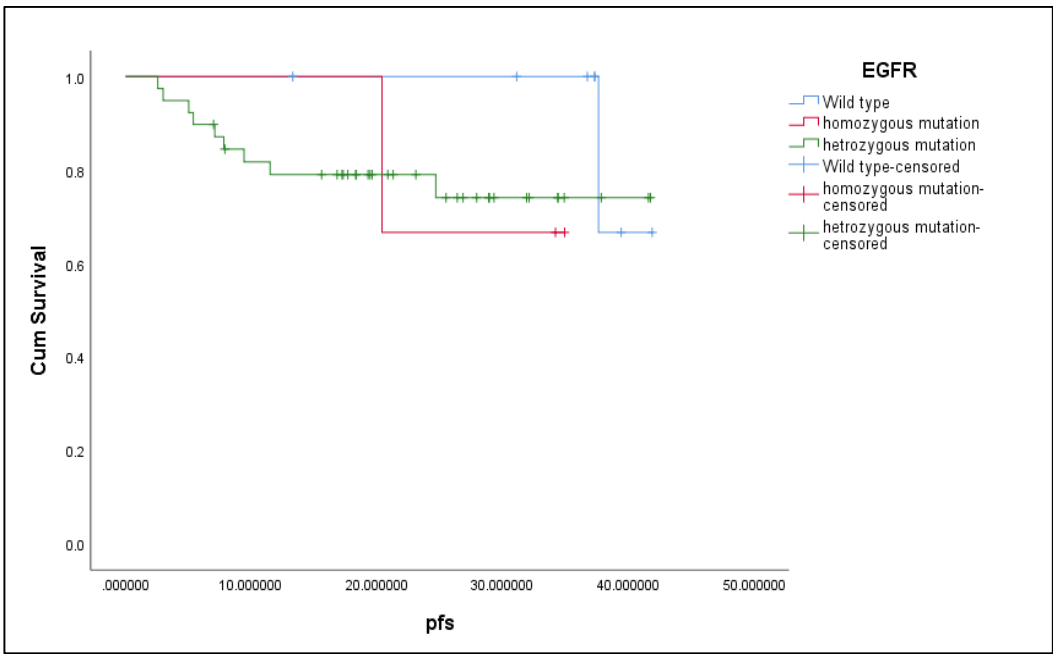

Figure 4. Kaplan-Meier Curve for Progression-Free Survival Analysis of 50 HNSCC Patients. (X- axis represents the progression free survival value in months, and Y-axis represents cum survival percentage)

the Kaplan Meir curve, the following observations were made- the patients who carried a homozygous variant of this gene survived for 30.8 months (SE ±3.302) with a 95% Confidence Interval (CI) - (24.39- 37.36), the heterozygous variant survived for 34.7 months (SE ± 2.152) (95% CI -30.51- 38.94). Patients with wild-type genotypes survived for 41.4 months (SE ± 0.511) (95% CI -39.958 - 42.374) (p-value = 0.409). The Kaplan-Meier curve for OS is depicted in Figure 3.

The progression-free survival (PFS) analysis also showed a similar trend to the overall survival. Where patients with the homozygous gene variant survived for 30 months (SE ± 3.952) (95% CI -13.075 - 47.093), patients with the heterozygous variant survived for 33.5 months (SE ± 2.404) (95% CI =28.660 - 38.390). In contrast, patients without the variant survived for 40.4 months (SE ± 1.162) (95% CI 39.49 - 41.435) (p-value = 0.553). The p-value is not statistically significant as number of incidences is lower. The Kaplan-Meier curve for PFS is depicted in Figure 4.

Although the OS and PFS values did not reach statistical significance (OS: p-value = 0.409; PFS: p-value = 0.553), due to small sample size and limited number of events. However, a consistent decreasing trend in survival rates from wild types to heterozygous and homozygous variants was observed. This decreasing gradient in survival rates suggests a potential correlation between the presence of the SNP *R497K* to poorer clinical outcomes. These results warrant further investigation of this SNP in a larger cohort to validate its prognostic significance.

Discussion

The *EGFR R497K* polymorphism causes amino acid arginine to be substituted to lysine at codon 497 located within the extracellular domain of the *EGFR* protein. Studies have shown that the lysine variant affects the ligand binding activation of the protein, which affects the *EGFR*-mediated signalling. Reports also suggest that the *R497K* variant can modulates downstream pathways such as *PI3K/AKT, MAPK*, and *JAK/STAT*, essential for regulating cellular proliferation, survival, and apoptosis [12]. The SNP has also been reported to cause more aggressive tumour progression and decreased sensitivity to *EGFR* targeted therapies like cetuximab. For instance, in HNSCC and colorectal cancer cells carrying this SNP have been shown to have reduced sensitivity to receptor inhibitors. Therefore, the presence of *EGFR R497K* variant may be a functional marker associated with poor clinical outcomes and decreased therapeutic efficacy, which is in line with the survival patterns found in our study [13].

Single nucleotide polymorphisms are associated with cause, response to treatment, and progression of various diseases including cancer. In the current study, we screened for *R497K* (rs2227983) polymorphism of the *EGFR* gene in the Indian population of HNSCC patients to determine the relationship between the susceptibility to HNSCC and response to treatment of the patients with the variant gene. Out of a total of 50 patients included in the study, 84% of them (42 out of 50) carried the *R497K* variant, of which 3 (6%) were homozygous (Lys/Lys), and 39 (78%) were heterozygous (Lys/Arg) and rest 8 (16%) were found to be wild type (Arg/Arg). The prevalence of this gene variant in HNSCC patients differs across the geographical location. In Spain, it was found to be 12% homozygous, 35% heterozygous and 53% were wild type [11] In the Iranian population of oral cancer patients, 62.50% carried heterozygous polymorphism, and the rest (47.5%) were wild type [14]. In the German population of HNSCC patients out of 45, 17 (37.7%) patients carried the variant either in homozygous or heterozygous condition [8]. Thus,

by comparing these studies, our cohort consisted of the highest percentage (84%) of HNSCC patients carrying the *R497K* variant. Hence, we can conclude that people with the *EGFR R497K* variant gene are susceptible to HNSCC, which is supported by a study done by Nagalakshmi et. al. [15]. To strengthen the hypothesis further, we compared the percentage of the non-cancerous Indian population carrying the variant gene. As per the data available in the database (Indigen public database), this gene variant was present in only 31.6% of the population either in homozygous or heterozygous form (we had to use the data from Indigen as obtaining healthy control tissue samples was not feasible due to institutional and ethical limitations in accessing non-cancerous tissues of HNSCC patients from consenting individuals. Additionally, due to ethical restrains, sample collection was only restricted to patients undergoing surgical resection for HNSCC). A similar study carried out in patients with oral squamous cell carcinoma (OSCC) also concluded that individuals with the *EGFR R497K* variant gene are common in the cancer patients compared to that of control individuals, suggesting individuals with *EGFR R497K* variant gene are related to OSCC susceptibility [14].

Disease progression and response to treatment are reflected by employing analysing overall survival (OS) and progression-free survival (PFS), hence OS and PFS were calculated for HNSCC patients carrying Arg/Arg, Arg/Lys, and Lys/Lys variants of *EGFR R497K* gene. Compared to the Arg/Arg variant, the presence of the Lys variant either in homozygous condition or heterozygous condition decreased both OS and PFS. OS for Arg/Arg, Arg/Lys, and Lys/Lys was 41.1, 34.7, and 30.8 months respectively; PFS for Arg/Arg, Arg/Lys, and Lys/Lys were 40.4, 33.5 and 30.0 months respectively.

In other cohorts with HNSCC patients, it is reported that even the heterozygous form of the gene variant had significantly less prognosis in patients treated with *EGFR* inhibitor cetuximab [8, 13]. A poorer survival rate was also observed in colorectal cancer patients with *EGFR R497K* variant [16], supporting our observation that HNSCC patients with *EGFR R497K* gene variant have a poor prognosis.

Even in recent years the prevalence of this SNP remains poorly investigated, hence this study was conducted to further learn the prevalence of *EGFR R497K* in the HNSCC population.

Limitations

The sample size is small, and low incidence rates have caused challenges in achieving statistically significant results. Therefore, these findings should be considered exploratory and a study of a larger population cohort is needed. The method we use in our study is PCR-RFLP, which provides for rapid and easy way to screen for gene mutations but this technique focuses only on the restriction enzyme recognition sequences. The use of next-generation DNA sequencing technique would have provided global mutations in the whole genome of the patients. Also, the status of mutation in heavily pre-treated or progressive HNSCC couldn't be checked, since the sampling of this study is completed and more than 70% of patients in the cohort are no more. Hence it was not possible to check and report further molecular changes that have occurred in the tumor during the disease's progression or response to treatment protocol.

In conclusion, our study concludes that the SNP *R497K* (rs2227983) of the *EGFR* gene has a higher prevalence in the Indian cohort compared to studies done in other cohort populations. When we correlated the variant status to the survival rates of the patients it was found, that homozygous and heterozygous variants have declining rates of OS and PFS compared to wild-type patients. Our findings conclude that the *R497K* (rs2227983) SNP of the *EGFR* gene has a negative effect on HNSCC patients. Hence, we suggest that this polymorphism can act as a biomarker for clinical treatment outcomes and disease-related mortality in patients.

Screening for this SNP can help distinguish patients based on genetic risk and prognosis, and enable more precise treatment planning. For example, patients carrying this variant can be closely monitored by clinicians, and intervention strategies can be implemented if found they respond less effectively to *EGFR*-targeted therapies like cetuximab. Furthermore, incorporating screening of *EGFR R497K* in routine diagnosis can improve risk assessment and provide a better therapeutic approach based on individual genetic profiling.

Author Contribution Statement

Anbalagan Moorthy contributed in conceptualization and reviewing and correcting the article. Arjita Ghosh contributed in writing and performing the experiment and data analysis of the article. All authors contributed equally to the preparation of this article.

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Ethical Committee

Informed consent forms were obtained from all the patients and the institute's human ethical committee approved this study (IEC/IRB No: IECH/2013/Dec18-006). This study was done in accordance with the ethical standards laid down by the 1964 Declaration of Helsinki and its later amendments.

Scientific Body Approval

The research was conducted as part of the first author's Ph.D. dissertation work under the Department of Integrative Biology, School of Biosciences and Technology, Vellore Institute of Technology, India.

Data Availability

The data used in this work can be made available by requesting the authors of this manuscript.

Registration Data set

This study is a retrospective observational analysis

of archival tumor samples and was not registered in any clinical trial or meta-analysis registry.

Competing Conflict of Interest

The authors report there is no competing conflict of interest to declare.

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