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"Clinicopathological and Prognostic Significance of Her-2/Neu Immunoexpression in Head and Neck Squamous Cell Carcinomas and its Association with Survival in Indian Population"

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Abstract
Background: *Despite advanced surgical techniques and treatment modalities, head and neck squamous cell carcinoma (HNSCC) has been a global challenge because of the high rates of disease recurrence and its advanced stage at the time of diagnosis. The increase in morbidity and mortality of HNSCC even after definitive therapies has prompted substantial research efforts in the field of early detection and prevention of the disease by identifying tumor biomarker accuracy. Human epidermal growth factor receptor 2 (Her-2/neu) have been associated with advanced disease, metastasis, poor clinical outcome and survival in various carcinomas. Overexpression of Her-2/neu leads to increased basal tyrosine kinase activity and transform cells by persistently stimulating signal transduction pathway. Therefore, it is required to affirm the prognostic significance of Her-2/neu in HNSCC which might open new avenues in the treatment approaches.*
Aim and objectives: *The purpose of current study was to observe Her-2/neu immunoexpression in HNSCC and to evaluate its prognostic relevance with certain clinicopathological variables, disease free survival and overall survival in Indian population.*
Materials & Methods: *Expressions of Her-2/neu oncoprotein in hundred histopathologically diagnosed HNSCC cases were assessed by immunohistochemical technique. Statistical analysis was done by using Pearson chi-square test / Fisher exact test to find out the association of Her-2/neu staining with included clinicopathological parameters. After one year of follow-up, survival analysis was done by using Kaplan-Meier log rank test in HNSCC patients.*
Results and Conclusion: *Membranous expression for Her-2/neu was positive in 58% of HNSCC cases. 41% HNSCC patients died and 16% had disease recurrence (DR) within one year of follow-up period. There was no apparent correlation in HNSCC Her-2/neu level with clinicopathological variables except early and advanced tumor stage (p-value=0.017). Survival analysis in HNSCC indicated that Her-2/neu oncoprotein expression was not significantly correlated with overall survival (p-value=0.475) and disease free survival (p-value=0.676). Thus, our findings suggest that Her-2/neu is not a valid prognostic marker in HNSCC. The prognostic significance and clinical implications of Her-2/neu expression in HNSCC warrants further investigations and evaluations with standard immunohistochemistry staining protocols.*

Keywords: *Head and neck squamous cell carcinoma, Her-2/neu, Disease recurrence, Disease free survival, Overall survival.*

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I. Introduction

Head and neck squamous cell carcinoma (HNSCC) is sixth most common neoplasm in the world and rising as a leading health problem in India. Worldwide, 6% of all cancer cases account for HNSCC and responsible for approximately 1-2% of tumor deaths[1]. The estimated prevalence of head and neck cancer (HNC) in Asia accounts for 57.5% of global incidences, with approximately 30-35% cases in India only[2]. The disproportionately higher prevalence of HNC in relation to other malignancies in India may be due to diverse demographic profiles, risk factors (tobacco and alcohol consumption), poor dietary habits, low socioeconomic conditions, poor hygiene, viral infections and family history[3,4]. The rapid increase in Incidence of HNC is a major clinical challenge as it is associated with high morbidity and mortality.

HNC is the most common cancer in males and the fifth most frequent cancer in females for Indian population[5]. These malignancies in India accounted for 30% of all cancers in males and in 11-16% of all cancers in females. Males are affected significantly more than females, with a ratio ranging from 2:1 to 4:1[2]. The true burden of HNC in Indian population may not be reflected by the existing literature and what comes into the picture is only the "tip of the iceberg" condition[6]. However, the annual estimate for HNC cases in India was 181606, given by Indian council of Medical Research (ICMR) in 2013[7]. Approximately 90% of all these HNCs are squamous cell carcinomas (SCC), which are derived from mucosal linings of upper areodigestive tract[8]. Moreover, 60-80% of patients presented with advanced disease (stage III and IV) in India as compared to 40% in developed countries[2]. The five year survival rate of HNSCC patients is approximately 40-60% [9], with a high rate of disease recurrence possibly due to the advanced stage of tumor at the time of diagnosis.

Since the last two decades, significant advancements have been done in the field of immunoexpression of various tumor markers and their roles in the growth and progression of HNCs. Several established factors exist and many more are being investigated for this neoplasia for their prognostic implications and clinicopathological associations with survival. Despite of all the technical improvements of therapeutic approaches, HNCs are still categorized amongst the top ten malignancies globally[1]. The successful treatment and survival of these HNSCC patients depends on early detection and appropriate therapy[10]. Alteration in immunoexpressions may take place before the development of malignancy, raising the probability of developing tumor markers to detect very early stage lesions in head and neck[11].

Growth Factors (GF) play essential roles in development, growth, embryonic tissue induction, differentiation, cell proliferation, cell migration, cell survival, apoptosis and homeostasis[12,13]. The epidermal growth factor receptor tyrosine kinase superfamily (ErbBs) consists of four members: EGFR (ErbB1, HER-1), HER-2/neu (ErbB2, EGFR2, Neu), HER-3 (ErbB3) and HER-4 (ErbB4)[12]. Tyrosine kinase (TK) represents a major portion of all oncoproteins that play a transforming role in plethora of cancer as they are chief mediators of signal transduction pathways in response to both external and internal stimuli[14]. Dysregulation of the ErbB receptors may activate various human diseases, especially cancer, as they are linked to several features of malignant tumors, including loss of cell cycle control, resistance to apoptotic stimuli, invasiveness, chemo resistance, and the induction of angiogenesis[15].

The Her-2/neu oncogene encodes a Mr 185000 glycoprotein, p185, located on the short arm of chromosome 17 (17q12)[16]. Her-2/neu oncogene was discovered by a group of scientists at Massachusetts Institute of Technology, Rockefeller and Harvard University[17,18]. Structurally, these receptors are single chain transmembrane glycoproteins composed of an extracellular ligand binding domain, a hydrophobic transmembrane segment and an intracellular tyrosine kinase domain[19]. Her-2/neu can be activated by heterooligomerization with the other members of ErbB protein family, which triggers autophosphorylation of their intracellular tyrosine kinase domains and initiate the signaling cascade[20]. The expressions of Her-2/neu oncoprotein and its correlation with poor prognosis, advanced disease, metastasis and overall survival (OS) have been reported in various previous studies[21,22,23,24].

Tumor, node and metastasis (TNM) staging and grading are the fundamental parameters for HNSCC classification and predicting prognosis. Though, patients with same tumor stage do not have similar disease progression, response to therapy, rate of disease recurrence and survival[25]. Molecular identification in biopsy specimens may not only identify the patients at high risk for developing HNSCC but it may also help to select the patients who need more aggressive treatment modalities, thus improvise disease free survival (DFS)[26]. Prognostic significance of Her-2/neu and its correlation with clinical parameters in HNSCC are still controversial[16,27,28], whether Her-2/neu oncoprotein could be considered as a useful prognostic tumor marker for HNCs or not. The aim of the study was to evaluate the immunoexpression of Her-2/neu in HNSCC patients and its associations with incorporated clinicopathological prognostic factors, DFS and OS in Indian population. The prognostic association of Her-2/neu in HNSCC might provide new therapeutic designs and management of the disease for better patients' outcome.

II. Patients & Methods

***Patient selection and tissue samples*:** The prospective study was performed on hundred patients suffered from HNSCC referred to surgery in Oncology and Ear, Nose & Throat (ENT) department of Base Hospital and Army Hospital Research & Referral, New Delhi, India, from 2013 to 2015. All these cases were histopathologically proven diagnosed cases of primary HNSCC without any kind of anticancer therapy before surgery (92 males & 8 females). The age of the patients ranged from 32 to 80 years. Patients who have undergone radiotherapy (RT) or chemotherapy (CT) for HNSCC before surgical resection, presented with metastatic disease and secondary SCCs of head and neck region were not been included in the study. All subjects were informed about the research work and agreed to participate in the study. Consent from all the patients and control subjects were obtained by sign in accord with guidelines set by the institutional ethics review board.

Tissue sample procurement obtained from surgical resection specimen of HNSCC for block formation using standardized procedure for grossing given by American Joint Committee on Cancer (AJCC). Received biopsy specimens were fixed in 10% formalin and processed by paraffin embedding histopathological technique. Paraffin embedded tissue samples were obtained from the pathology department of the hospital for immunohistochemical analysis (IHC). The slides from these paraffin blocks were again reviewed and diagnosis was confirmed with Hematoxylin & Eosin (H&E) staining. Covariables including demographics, staging, clinical, pathological and treatment parameters of these HNSCC patients were extracted from their medical records of concerned departments. All patients were staged clinically according to AJCC TNM classification system[29] and were graded according to Broder's criteria into well, moderate and poorly differentiated SCC[30].

***Follow up*:** All these HNSCC patients underwent regular follow up for one year after surgery and their outcomes at the time of review were analyzed. In present study only four patients were lost to follow up, hence censored. The OS was defined as the interval between the date of surgery to the date of death or to the last information for censored observations[31]. DFS was the specific period from tumor resection until the first evidence of tumor recurrence or of a new developed primary tumor seen in head and neck region[32].

***Immunohistochemical Analysis*:** IHC was performed on 4μm thick tissue sections prepared from formalin fixed paraffin embedded (FFPE) tissue from the resected primary tumors of HNSCC. The sections were taken on poly-L-lysine coated slides and fixed in an incubator at $600^{\circ}C$ for 20 minutes. Sections were deparaffinised in xylene and hydrated in graded alcohols. Antigen retrieval was conducted by heating slides using citrate buffer (pH - 6.0, low pH retrieval) in pressure cooker for 15 minutes and then cooled at room temperature for approximately 30 minutes. Endogenous peroxidase activity was blocked with the pre-treatment of 3% hydrogen peroxide (H2O2) in Tris buffered saline (TBS) (pH - 7.4) for 10-15 min. After washing with TBS, the slides were incubated in a humidifier chamber with primary antibody Her-2/neu (DAKO, Denmark). After three wash with TBS for 10 minutes each, the Streotavidin - Horse radish peroxidise complex (secondary antibody, HRP) (Dako, Denmark) was applied to the slides for approximately 30 minutes at room temperature. After three TBS washing, labelling and visualization were performed with 3, 3'- diaminobanzidine (DAB) solution (chromogenic substrate) for 3-10 minutes. These slides were put under running tap water for five minutes and then counterstained with Mayer's Hematoxylin. Finally, mounting of the slides were done by Dibutyl phathalate distyrene xylene (DPX) and glass coverslips. Appropriate positive and negative controls were also prepared in the same manner and used to validate each run of IHC. Positive controls were sections of breast carcinoma with strong membranous immunoexpression for Her-2/neu (Figure 1). Negative controls were sections of the same sample where primary antibody incubation was substituted with buffered saline. Membrane staining scoring pattern for evaluation of the slides followed the guidelines of clinical trial assay recommendations for breast carcinoma (0, 1+, 2+, 3+)[33]. Tumor cells with complete absence of staining were scored as 0, those with faint incomplete membranous staining of < 10% of tumor cells were classified as 1+, those with moderate, complete membranous staining of >10% of tumor cells were classified as 2+ and those with complete intense membrane staining of >10% were classified as 3+ [34]. Score 0, 1+ were considered as negative staining and score 2+, 3+ were positive staining for Her-2/neu in our study.

***Statistical Analysis*:** The Data for Her-2/neu oncoprotein expressions and all the potential prognostic variables of HNSCC of current study (sex, age, TNM stages, tumor grades, alcohol & tobacco consumption habits, family history, tumor sites, lymph node involvement, post-operative RT & CT) were gathered and analysed by using SPSS (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp). Pearson chi-square test / Fisher exact test was used to ascertain the clinicopathological association with Her-2/neu staining. OS and DFS were analysed by using Kaplan Meier method with log rank test. P-value < 0.05 has been considered to be statistically significant.

III. Results

The series comprised of hundred HNSCC patients (92 male & 8 female) with mean age of 57±11.2 (32 to 80) years. The histopathological diagnosis was established in every case according to the standard guidelines for H&E staining and IHC procedure. None of the patients had distant metastases at the time of diagnosis. Considering a cut-off point for positivity between 1+ and 2+, Her-2/neu oncoprotein membranous expression was positive in 58% of HNSCC cases (Figure 1**).** Statistical association between clinicopathological variables and Her-2/neu oncoprotein expression of HNSCC patients are summarized in Table 1. P-value was calculated by Pearson Chi-square test / Fisher exact test.

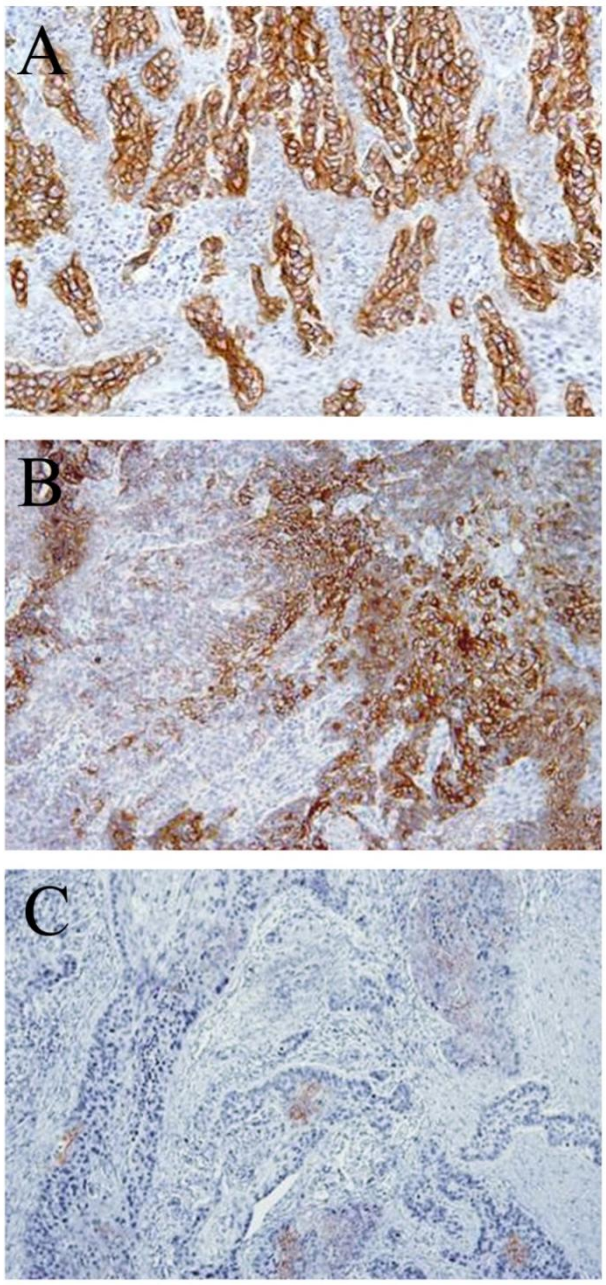


Figure 1: Representative immunohistochemical staining (IHC) for Her-2/neu in HNSCC (A) Control tissue (Invasive ductal carcinoma of breast, Intense positive membranous staining) (B) Her-2/neu Positive (Moderate, complete membranous staining of >10% of tumor cells) (C) Her-2/neu Negative (Incomplete, faint membranous staining of < 10 % of tumor cells).

Table 1: Statistical association of clinicopathological variables and Her-2/neu immunoexpression in HNSCC patients.

Prognostic variables	Subgroup	No. of patients n=100	Her-2/neu staining		p-value
			Negative (0, 1+) n (%)	Positive (2+, 3+) n (%)	
Sex	Male	92	38 (41.3)	54 (58.7)	0.633
	Female	8	4 (50.0)	4 (50.0)	
Age (years)	Below 40	12	3 (25.0)	9 (75.0)	0.325
	40-60	47	19 (40.4)	28 (59.6)	
	Above 61	41	20 (48.8)	21 (51.2)	
Tumor Stage	Stage I	12	6 (50.0)	6 (50.0)	0.088
	Stage II	18	12 (66.7)	6 (33.3)	
	Stage III	18	6 (33.3)	12 (66.7)	
	Stage IV	52	18 (34.6)	34 (65.4)	
Tumor Stage	Early (I & II)	30	18 (60.0)	12 (40.0)	**0.017***
	Advanced (III & IV)	70	24 (34.3)	46 (65.7)	
Tumor grades of differentiation	WDSCC	43	21 (48.8)	22 (51.2)	0.239
	MDSCC	47	19 (40.4)	28 (59.6)	
	PDSCC	10	2 (20.0)	8 (80.0)	
Tobacco Consumption	Yes	84	38 (45.2)	46 (54.8)	0.133
	No	16	4 (25.0)	12 (75.0)	
Alcohol Consumption	Yes	67	27 (40.3)	40 (59.7)	0.623
	No	33	15 (45.5)	18 (54.5)	
Both (Tobacco + Alcohol Consumption)	None	9	2 (22.2)	7 (77.8)	0.267
	Any one	29	15 (51.7)	14 (48.3)	
	Both	62	25 (40.3)	37 (59.7)	
Family History	Positive	14	5 (35.7)	9 (64.3)	0.607
	Negative	86	37 (43.0)	49 (57.0)	
Tumor Sites	Larynx	20	9 (45.0)	11 (55.0)	0.494
	Tongue	20	10 (50.0)	10 (50.0)	
	Buccal Mucosa	13	5 (38.5)	8 (61.5)	
	Oral cavity	12	5 (41.7)	7 (58.3)	
	Alveolus	11	3 (27.3)	8 (72.7)	
	Hypopharynx	11	3 (27.3)	8 (72.7)	
	Oropharynx	8	6 (75.0)	2 (25.0)	
	Floor of Mouth	4	1 (25.0)	3 (75.0)	
	Lips	1	0 (0.00)	1 (100.0)	
Lymph nodes involvement	No	26	10 (38.5)	16 (61.5)	0.888
	Level I	26	12 (46.2)	14 (53.8)	
	Level II	39	17 (43.6)	22 (56.4)	
	Level III	9	3 (33.3)	6 (66.7)	
Treatment	S	33	14 (42.4)	19 (57.6)	0.984
	S + RT	28	11 (39.3)	17 (60.7)	
	S + CT	5	2 (40.0)	3 (60.0)	
	S +RT +CT	34	15 (44.1)	19 (55.9)	
Disease Recurrence	Yes	16	7 (43.8)	9 (56.3)	0.877
	No	84	35 (41.7)	49 (58.3)	

*Significant p-value

Abbreviations: WDSCC – Well Differentiated Squamous Cell Carcinoma, MDSCC – Moderately Differentiated Squamous Cell Carcinoma, PDSCC – Poorly Differentiated Squamous Cell Carcinoma, S – Surgery, RT – Radiotherapy, CT – Chemotherapy.

The most affected site was larynx and tongue (20% each) followed by buccal mucosa (13%), oral cavity (12%), alveolus (11%) and rest of included cases from pharynx, floor of mouth and lips. 12% HNSCC patients had stage I, 18% had stage II and stage III each, and 52% (more than half) had stage IV tumor as per TNM staging[29]. Thus, 30% cases had early stage (stage I & II) and 70% patients had advanced stage (stage III & IV) of disease at the time of diagnosis (Table 1). As per histological grading of differentiation, 43% patients had well differentiated SCC (WDSCC), 47% patients had moderately differentiated SCC (MDSCC) and only 10% had poorly differentiated SCC (PDSCC). Out of 100 HNSCC patients, 84% were tobacco consumers (smokers and tobacco chewers) and 67% patients had habit of alcohol consumption. 62% patients were using both tobacco and alcohol simultaneously. However, 9% patients of HNSCC had no history of any of these habits. Family history for cancer was positive in 14% of our cases. 26% of cases were not having lymph nodes involvement at the time of diagnosis. All HNSCC cases included in the study underwent surgery for treatment. 28% of these cases had RT, 5% had CT and 34% had both RT and CT as adjuvant treatment after surgery. No significant association was noted between HNSCC Her-2/neu expression and clinicopathological parameters included in

the study except the tumor stage (early and advanced) (p-value = 0.017) (Table 1). Therefore, tumor stages were the only prognostic clinical parameter associated with Her-2/neu staining in HNSCC.

During one year of follow up, 16 patients had disease recurrence and 41 died. Of these 41 patients, 13 died with disease recurrence and 28 died without disease recurrence. Of 59 patients, four were lost to follow up, hence censored. Rest 55 patients survived after one year of surgery. Of these surviving patients, 3 were alive with disease recurrence and 52 were disease free survivors at the end (Figure 2). Though, disease recurrence was not significantly associated with Her-2/neu immunoexpression in HNSCC (Table 1).

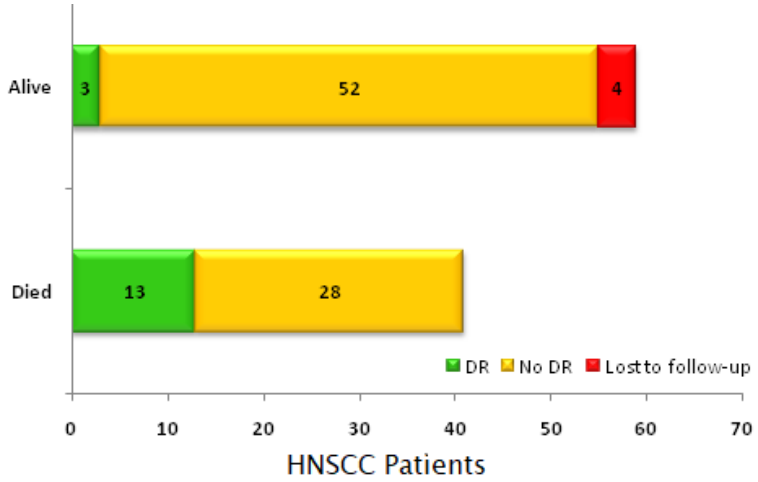


Figure 2: One year follow up for HNSCC patients.

Table 2: Survival analysis with Kaplan-Meier log rank test for overall survival (OS) and Disease free survival (DFS) in HNSCC patients.

Survival outcome	Total Sample (N)	No. of Events	Censored (n)	Mean DFS time (Days)	Median DFS time (Days)	One year survival rate
Overall Survival (OS)						
Her-2/neu Positive	58	23	35	307.4	-	60.7
Her-2/neu Negative	42	18	24	273.9	-	55.5
Overall	100	41	59	293.3	-	57.5
Outcome of event of interest = Death, Log Rank (Mantel-Cox) value = 0.511, df = 1, p-value = 0.475						
Disease free Survival (DFS)						
Her-2/neu Positive	58	9	49	342.9	-	80.0
Her-2/neu Negative	42	7	35	324.6	-	80.2
Overall	100	16	84	334.6	-	79.6
Outcome of event of interest = Disease recurrence, Log Rank (Mantel-Cox) value = 0.174, df = 1, p-value = 0.676						

The survival outcome calculated with Kaplan-Meier log rank test indicates that one year OS rate was higher in Her-2/neu positive cases (60.7%) versus Her-2/neu negative (55.5%). However, result was not statistically significant in present study (p-value = 0.475) (Table 2) (Figure 3). The DFS was not specifying any significant difference with Her-2/neu status (p-value = 0.676) (Table 2) (Figure 4).

Figure 3: Survival curves showing Overall survival (OS) in HNSCC patients with Her-2/neu immunoexpression.

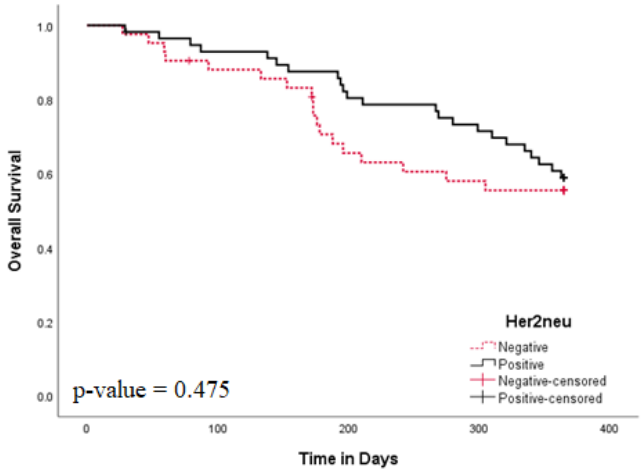
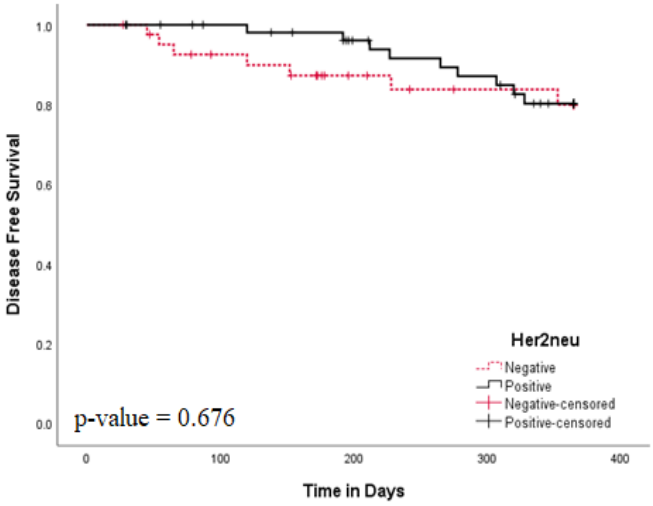


Figure 4: Survival curves showing Disease free survival (DFS) in HNSCC patients with Her-2/neu immunoexpression.



IV. Discussion

HNC is the second most common cancer related to Indian ethnicity[35]. The conventional treatment of HNSCC by surgery and adjuvant RT with or without CT has failed to improve survival beyond a certain limit. Poor survival is usually related to delayed diagnosis and frequent disease recurrence. Though clinical staging and grading does help in treatment planning and prognosis estimation, still it fails to predict information at the onset of disease for early detection. Therefore, the search for more reliable prognostic markers is still required[36]. Worldwide, extensive research is being done on potential molecular markers which can serve as therapeutic agents and improve cancer patient's survival. Her-2/neu oncoprotein enhances the metastatic potential of tumor, by promoting invasion through several stages and the metastatic cascade, thus, plays an important role in carcinogenesis[37]. Her-2/neu receptors are well established in breast and gastric carcinomas as they are involved in tumor development and have therapeutic implications[13,38]. The role of Her-2/neu in HNSCC has been investigated extensively but its clinical relevance has led to conflicting results[16,27,28]. Hence, there is uncertainty over its prognostic relevance and utility as a target of new therapy for HNSCC.

The amplification and overexpression of Her-2neu oncoprotein and its association with tumorogenesis was first reported by Schechler et al (1985) in neuroblastomas of rats[39]. Wilkman et al (1998) observed an increase in Her-2/neu expression during the sequence from normal mucosa to hyperkeratosis and to dysplasia and finally HNSCC[40]. Few studies observed elevated Her-2/neu expression between 39 to 76% [34,41], whereas

others have reported no prognostic implication of Her-2/neu with a lack of significant expression[42,43]. In present study, expression of Her-2/neu oncoprotein was analyzed by IHC technique. Considering a cut-off point for positivity at 10%, Her-2/neu expression was positive in 58% cases of present HNSCC series.

The discrepancies in results for Her-2/neu positivity in previous studies may be attributed to different methods used for IHC (direct / indirect), type of antibody (clone c-erbB-2 / monoclonal / polyclonal), no specific criteria for positive staining of Her-2/neu oncoprotein (membranous and / or cytoplasmic), different techniques (IHC, radioimmunoassay, immunosorbent assay), different interpretation methods, different locations and gender of the patients with HNSCC[44]. Another possible reason for variations of Her-2/neu expression in HNSCC may be due to the initial lack of standardization of assay methods[34]. An additional issue regarding disagreements between the IHC analyses on HNSCC were the cut-off points established for Her-2/neu positivity. Different cut-off may lead to qualitative and quantitative differences in outcomes. The American Society of Clinical Oncology (ASCO) published guidelines for Her-2/neu assessment in breast carcinomas via IHC and Fluorescence in situ hybridization (FISH) methods[45]. Though Her-2/neu testing protocols have also been established in gastric cancer, but no specific report exist for HNSCC[46]. As a result, pathologists apply the IHC / FISH scoring techniques for breast carcinomas to HNSCC, which is fundamentally a different carcinoma.

Her-2/neu immunoexpression have been reported purely membranous[47,48] or purely cytoplasmic[41,42,49] or mixed (membranous and cytoplasmic both)[43,50,51,52] in literature. In current study, Her-2/neu expression was observed purely membranous. Although, cytoplasmic staining in SCC has been extensively reported but its interpretation is yet not clear. It has been doubtful that cytoplasmic staining of Her-2/neu may be a technical artifact due to cross reactive antibodies possibly due to keratin or antigen retrieval[22]. On the other hand, Ibrahim et al stated that it may be true protein overexpression probably due to incomplete receptor degradation[53]. Cytoplasmic staining of Her-2/neu was earlier considered to be predictive of Her-2 gene amplification in breast carcinomas[54]. Some of studies suggest that pure cytoplasmic stained samples should be designated as negatives[55]. However, the importance of cytoplasmic staining for Her-2/neu expression in HNSCC is still uncertain whether or not it may be evaluated.

Giatromanolaki et al observed 89 HNSCC cases with cytoplasmic staining but failed to exhibit any correlation between Her-2/neu expression, clinicopathological parameters and poor prognosis[49]. Fong et al reported that Her-2/neu expression was significantly higher in advanced stage IV cases than the stage I-III cases[44]. There was no significant correlation between Her-2/neu expression and prognostic clinicopathological parameters in HNSCC patients[43]. In present study, the Her-2/neu staining was not significantly associated with included clinicopathological parameters (sex, age, individual TNM stages, tumor grades, alcohol & tobacco consumption habits, family history, tumor sites, lymph node involvement, post-operative RT & CT). Interestingly, it is observed that early (stage I and II) and advanced (stage III and IV) stage of HNSCC were significantly associated with the immunoexpression of Her-2/neu in this series.

Elevated Her-2/neu expression has been reportedly associated with worse prognosis, increased DR and decreased OS in HNSCC[56,57]. Cavalot et al reported that frequency of Her-2/neu overexpression was significantly higher in patients having more aggressive disease with metastatic lymph nodes. Additionally, the 5 years OS and DFS probability were significantly lower for Her-2/neu positive patients compared to Her-2/neu negative individuals[34]. Quon et al reported that high expression levels of EGFR and Her-2/neu as prognostic markers in HNSCC which were correlated with poor clinical outcomes[58]. On the contrary, a case review study indicated that Her-2/neu was associated with longer survival in node positive patients[59]. Xia et al reported that Her-2/neu is one of the most significant factors in predicting disease outcome as they found strong correlation between Her-2/neu overexpression and overall survival in oral SCC[50]. On the other hand, few reports have shown that Her-2/neu is not an independent prognostic marker as no correlation was found between Her-2/neu expression and patients' outcome[16,42,60,61]. Our observations are also in agreement with this as results revealed that Her-2/neu expression in HNSCC and their one year OS and DFS outcomes were not significantly associated. Hence, our findings conclude that Her-2/neu is not having any prognostic significance and clinical implications in HNSCC patients.

V. Conclusion

Despite decades of intensive clinical investigations, the outcome of patients presenting with advanced stage of HNSCC is still poor. Although staging and grading of cancer helps in treatment planning and prognostication, but these factors often fail to identify lesions more likely to recur or result in death attributable to the disease. Role of Her-2/neu oncoprotein has received wide attention in HNSCC as a potential targets for new therapies from last few years. The current study in HNSCC cases found that there was no significant association between Her-2/neu expression and the aforementioned prognostic parameters, overall survival and disease free survival in Indian patient population. Thus, the use of Her-2/neu as a predictive prognostic tumor

marker in HNSCC is not recommended. However, the small sample size limits the conclusiveness of this analysis. Further studies warranted for the evaluation of Her-2/neu as a routine diagnostic marker in HNSCC.

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