Original Article

Prediction of Oral Cancer Survival Utilizing Micro RNA 21 and Clinicopathological Variables

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ABSTRACT

Objective: To investigate the impact of various socio demographic, clinical/pathological and novel miRNA biomarkers on oral cancer survival.

Study Design: Prospective cohort study

Place and Duration of Study: This study was conducted at the Department of Pathology, Ziauddin Hospital, Karachi, Pakistan from January 2014 to January 2020.

Materials and Methods: The data were obtained from 146 consecutive biopsy proven oral squamous cell carcinoma patients falling in an age bracket of 20 to 80 years. Association of variables with the survival was made through student's t test for continuous variables and chi square test for qualitative variables. Survival analysis was done via cox regression. Survival curves were plotted via Kaplan-Meier method using the Log-rank test. All statistical analysis was carried out on SPSS version 24.

Results: Overall survival was 43.8%. Cox regression analysis demonstrated miRNA 21 overexpression was linked to poor survival with a Hazard risk (HR) of 0.929, P<0.005. Other significant predictors included tumor grade (HR of 1.77, P< 0.001), nodal metastasis (HR of 9.4, p<0.01), advance stage (HR of 2.8, P<0.001 and age in years (HR of 1.02, p<0.01)

Conclusion: In this cohort we observed overexpression miRNA-21 was an independent prognostic factor suggesting it as a potential biomarker predicting poor survival. Furthermore, advancing age, nodal metastasis, poor grade, and advanced stage impacted poor survival in Oral Squamous cell carcinoma.

Key Words: Oral cancer, survival analysis, prognostic factors, miR-21

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INTRODUCTION

Oral cancer is one of the frequently diagnosed malignancies in Southeast Asia with around 66 % of global burden borne by developing countries (Gupta et al, 2014).[1] Being the most common cancer amongst men and second only to breast cancer amongst women it remains a significant contributor of cancer associated morbidity and mortality in Pakistan. A significant rise is observed in incidence of oral cancer from 12761(8.6%) in 2012 to 18,881(12.9%) in 2020 (IARC Globocan., 2020). [2]

Despite maintaining a stable rank as second most common cause of cancer related deaths in Pakistan, proportion of people dying of it is increasing as evident by IARC GLOBOCAN report endorsing a rise from 7.2.% (7266) in 2012 to 9.1% (13351) in 2020.[2,3]

To improve survival, advances in treatment strategies have been introduced but despite all these efforts survival remains unchanged for several decades.[4] Achieving a high cure rate in any cancer with minimal side effects of treatment is a very challenging task. This is especially true for oral cancers where surgeries can be extremely disfiguring owing to the anatomical location while chemo and radiotherapy is also not free of toxicities. Moreover, oral cancers are more common in low socioeconomic group patients and majority of them find it very difficult to bear the cost of treatment. On an average the cost of Cancer treatment exceeds the monthly income of these patients hence exploring the prognostic factors will help in streamlining the treatment guidelines for individual patient.[5] Tumor staging using TNM is well established prognostic indicator however its utility becomes limited as majority of patients present at advance stage[6]

It can be concluded from researches carried out in other countries that socio demographic and Clinicopathological factors including old age, poor socioeconomic status, tobacco smoking, T stage, nodal metastasis, local invasion , positive tumor margins, and presence of extra capsular spread of tumor cells have been observed to impart poor survival.[7] Inflammation in

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tumor microenvironment has been linked to survival as it contributes to local invasion reflected by rising levels of proinflammatory cytokines.[8]

This study is the first step taken to determine overall survival and impact of various factors on survival in subjects with oral cancer in Karachi, Pakistan. Sizeable research is published on risk factors, but research specific to survival analysis and prognostic factors is sparse.

MATERIALS AND METHODS

This prospective cohort study was carried out with the approval of the Ethics Research Committee of Ziauddin University, Karachi, Pakistan. Subjects were selected via Purposive nonprobability sampling. Sample size of 144 was calculated through raosoft sample size calculator with an expected margin of error of 8%.[9] Patient recruitment was started in Jan 2014. During this period, we recruited 146 biopsy proven cases of Squamous cell carcinoma of Oral cavity. Cases with a history of any other malignancy were excluded. Data was collected on age, gender, ethnicity, chewing habits, smoking, alcohol intake and family history of oral cancer through a structured questionnaire. Pathological features including tumor site, nodal status, tumor recurrences and histologic classification were recorded after physical examination and careful review of patient's file. Recurrences were detected by history, physical examinations and imaging and confirmed by histopathological examinations.

Tumors were classified according to American Joint Committee on Cancer (AJCC) seventh edition system. Survival was taken as the primary outcome. End were death related to oral cancer or January 2020 considered as last day of follow up. Overall survival (OS) was defined as the time between the date of diagnosis of primary OSCC and the date of death due to any cause or date of the last follow-up or date of end of study which was 1st January 2020.

For miRNA21 expression, miRNA was extracted, CDNA was synthesized, and qRT-PCR was carried out to quantitatively detect miR-21 expression. miR-16 was used as a normalizer detailed methodology explained by Mahmood and colleagues.[10.]

Statistical analysis was performed using the SPSS software package SPSS (Version 24.0; SPSS Inc. Chicago, IL, USA). Chi- square was used for comparison of nominal and ordinal variables between the groups. Continuous variables were examined using Students t test. Survival estimates were calculated for the period Jan 2014– Jan 2020. Survival curves were generated through Kaplan-Meier and compared by log-rank test. For multivariate analysis of significant variables ($p< 0.05$) Cox regression models were created. Statistical significance was set at $P<0.05$.

RESULTS

A total of 146 cases fulfilled our criteria. Subjects included fell in an age range of 20 to 80 years with a mean of 43.7± 11.9 years. Most of the patients, 126(86%) were diagnosed in the age bracket of 15 to 54 years. When subjects were distributed according to International Cancer Survival Standards (ICSS) age groups there were 76(52%) subjects between 15-44 years, 50(34.2%) between 45-54 years, 14 (9.6%) between 55-64 years,4 (2.7%) between 65-74 years and only 2(1.4%) above 75.[9] There were 107 (73%) males and 39(26.7%) females making a male to female ratio of 2.7 :1. A positive family history of oral cancer was reported in 9 (6.2%) subjects whereas remaining 137 (93.8%) did not report any family history of oral cancer.

Majority of patients 121(82.9%) had a tumor in oral cavity, tongue was the second commonest site with 15 cases (10.3%) and 10(6.8%) subjects had a lip cancer. Majority of subjects 94 (64.4%) had a moderately differentiated tumor whereas in 29(19.9%) subjects a well differentiated histology was observed and 23(15.8%) reported poorly differentiated histology. Distant metastasis was seen in 8(5.5%) subjects. A tumor recurrence was observed in 7(4.8%) cases however 139(95.2%) did not report any recurrence.

To compare the variables subjects were allocated into survivors and non survivors. miRNA 21 expression was checked in 100 subjects including 56 who did not survive and 44 alive or censored. Circulating miR21 was up regulated in the non-survivor group (27.6± 5.8 vs. 30.8± 4.3, p-0.002 Figure 1. Moreover, a significant difference in the two groups was observed in age at presentation, tumor grade, nodal involvement, and stage. In contrast we did not observe any difference between the two groups in gender, ethnicity, site of tumor and tumor recurrence (Table 1).

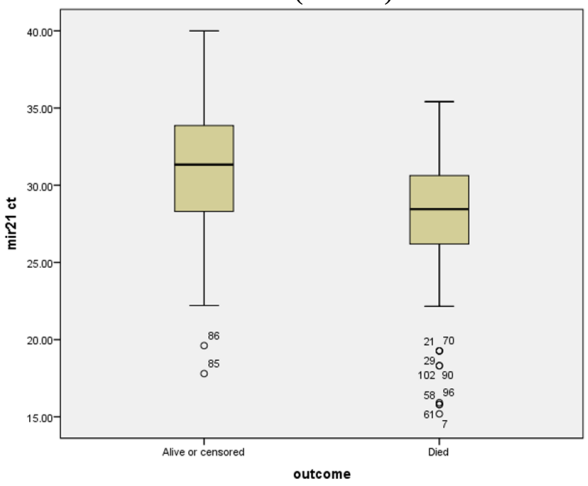

Figure No.1: miRNA 21 expression between survivors and non survivors

Table No.1: Comparison of Sociodemographic and clinic Pathologic variables between Survivors and Non-Survivors.

Variable	Category	Survival Status		*P* value
		Survivors (N= 64)	**Non-Survivors (N=82)**	
Age in years	< 40 years	35 (55.6%)	28(44.4%)	**0.013***
	>40 years	29 (34.9%)	54 (65.1%)	
Gender	Male	44(41%)	63(58.9%)	0.27
	Female	20(51.3%)	19(48.7%)	
Anatomic site	Tongue	5(33%)	10(66.7%)	0.17
	Lip	7(70%)	3(30%)	
	Buccal cavity	52(43%)	69(57%)	
Histological Differentiation	I	20(69%)	9(31%)	**0.009****
	II	36(38.3%)	58(61.7%)	
	III	8(34.8%)	15(65.2%)	
Recurrence	Yes	1(14.3%)	6(85.7%)	0.108
	No	63(45.3%)	76(54.7%)	
Nodal Metastasis	No	18(85.7%)	3(14.3%)	**<0.0001****
	Yes	46(36.8%)	79(63.2%)	
Stage	I	2(100%)	0(0%)	**0.001****
	II	13(81.2%)	3(18.8%)	
	III	19(48.7%)	20(51.3%)	
	IV	30(33.7%	59(66.3%)	

N; Number of cases, *; p<0.05, **; p<0.01

Table No.2: Cox regression analysis between variables and survival

Variable	HR	95%CI		*P* value
		Lower	Upper	
Age	0.72	0.571	0.910	.006*
Ethnicity	0.957	0.822	1.113	0.566
Site	1.09	0.755	1.558	.667
Recurrence	0.306	0.131	0.718	0.007
Grade	1.77	1.770	2.5	0.001**
Local Invasion	0.709	0.409	1.227	0.219
Tumor size	1.52	1.187	1.970	.001**
Nodal status	2.3	1.609	3.26	<0.001**
Mets	0.5	0.216	1.154	.104
Stage	2.28	1.583	3.283	<0.001**
miR-21	0.929	0.887	0.973	<0.001**

HR; hazard ratio, SE; Standard error, *; p<0.05, **; p<0.01

Survival Analysis: The Overall survival was 43.8% at the end of study. Median survival was 37 months with 95 % CI (32.2-41.7). At 20 months of follow up 112 (77%) patients were alive which dropped to 42(31%) at 40 months. Table 2 shows cox regression analysis between variables and overall survival. A significantly higher Hazard risk was observed among subjects above 40 years, having poor tumor grade, nodal involvement and advancing stage. Figure 2 shows survival curves for different tumor grades. Subjects with poor grade had poor overall survival, Log rank value of 13.1, p<0.01.

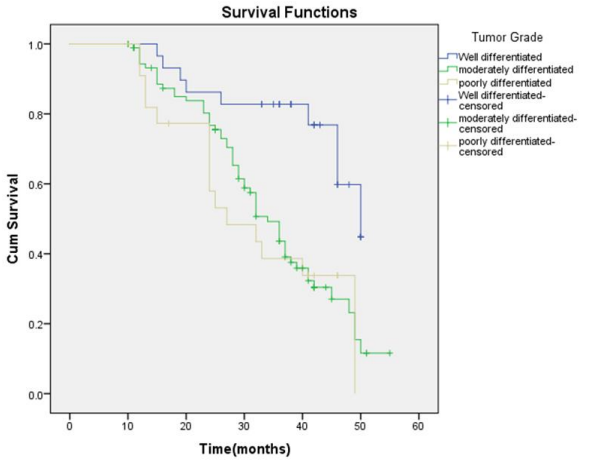

Figure No.2: Survival of subjects among different grades, Kaplan-Meier test was applied (Log rank= 13.1, p<0.01)

DISCUSSION

Despite being the second most common cancer in Pakistan research targeted at exploring potential predictors is scarce. Only two studies have reported contributors of survival in oral cancer patients. Abbas et al conducted a retrospective study to explore predictors of survival among oral cancer patients in a tertiary care hospital of Karachi and Hussain et al studied survival cancer among Tongue squamous cell carcinoma at Shaukat Khanum cancer hospital.[11,12.]

We report an overall survival of 43% in our subjects. Matching to our findings Hussain et al observed an overall survival of 50% in Stage III &IV Tongue cancers recruited at Shaukat Khanum Cancer Hospital. Selection of subjects from a cancer hospital makes it likely to recruit advanced cases and hence a poor survival. However, in contrast to our study they only followed T1 and T2 Tongue cancer. [12]

Our findings are in agreement with the findings of Centeless et al who recorded a survival rate of 44%.[13] Rao et al reported a 5 year survival of 38-42% in India.[14] Listl et al also reported a 5 year survival of 51.7% for tumors of oral cavity.[15] Whereas, a survival rate of 28.7% was reported by komolmalai et al in their subjects in Thailand.[16] Rich lymphovascular supply of oral cavity allows early nodal involvement and hence advanced stage irrespective of tumor size or grade.

We observed a better overall survival in our younger patients which agrees with other reports. A decline in survival from 61.1% in 15-44 years old to 43.9% in 65-74 years old was observed by Listl et al in German subjects.[15] The observed difference may be the result of more aggressive adherence to treatment and regular follow ups by younger patients.

We observed a poor survival in our subjects with up regulation of miRNA-21. These findings agreed with the report by Hedback et al who also observed a higher miRNA 21 expression as an independent factor predicting survival. They checked expression on tissue samples and found an up regulation of miR-21 in tumor stroma. After adjusting for clinical variables, multivariate regression analysis confirmed role of miRNA-21 in predicting disease free survival. [17]

We observed a significant association between tumor grade and survival. Analogous findings are reported by Listl et al; they observed an association of tumor grade with survival and reported a drop in survival from 65.2% to in Grade 1 cancers to 41.1% in Grade III& IV.[15] Liu et al observed a progressive decline in survival from 67.63% in well differentiated to 63% in moderately differentiated and 51.66% in poorly differentiated tumors.[18] Thomas et al also observed a strong impact of Tumor grade on survival. They found an association of poorly differentiated histology with increased risk of death in only Stage I and II subjects. Moreover, they observed increased death risk with moderately differentiated histology only in subjects who were older than 65 years.[19]It is quite possible that the observed increased risk in moderately differentiated group could have been attributed by the advancing age and not the tumor grade.

We found lymph node metastasis to impact poor survival among our subjects. Lymphatic involvement is linked to advanced stage and thus poor survival. Like us Abbas et al also observed poor survival among the subjects having lymph node involvement.[11]

We did not observe an effect of tumor location on overall survival which agrees with findings of Liu et al who also did not observe any association between tumor location and survival.[20]

No statistically significant difference was observed in different socioeconomic groups. Majority of our subjects were from low or low middle socioeconomic group, so it was not possible to evaluate its impact on overall survival. Komolamai et al observed a poor prognosis in their subjects from a low socioeconomic status.[16]

CONCLUSION

In conclusion, advancing age, poor histological grade, nodal metastasis, advanced stage, and up regulation of miR-21 predict poor prognosis. Future multicenter studies validating these findings might enable the development of prognostic scores and thus treatment guidelines.

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Author's Contribution:

Concept & Design of Study:	Nosheen Mehmood
Drafting:	Humera Akhlaq, Qamar Jamal
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Final Approval of version:	Nosheen Mehmood

Conflict of Interest: The study has no conflict of interest to declare by any author.

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