ORIGINAL ARTICLE

Prognostic Value of Cyclin-D1 Expression in Head-a nd-Neck Squamous Cell Carcinoma: A Half-Decade Follow-Up

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ABSTRACT

Objective: To check the expression pattern of cyclin-D1 in stage I head and neck squamous cell carcinoma (H.N.S.C.C.) cases (evaluated based on history, clinical, medical, and radiological records) in our setting and evaluate the role of cyclin-D1 as a prognostic marker of H.N.S.C.C. (considering recurrence within five years and metastasis).

Study Design: Retrospective cohort study.

Place and Duration of Study: The study was carried out at Armed Forces Institute of Pathology (A.F.I.P.) Rawalpindi working in conjunction with the Department of Radiation Oncology, Rawalpindi. The duration of study was from 7th March 2022-14th October 2022.

Materials and Methods: The study comprised 92 patients from year 2015-16 of stage I diagnosed with squamous cell carcinoma of head and neck with complete five-year follow-up after fulfilling the inclusion and exclusion criteria. After collecting and matching the data on follow-up with record of the patients from Department of Radiation Oncology, C.M.H., Rawalpindi; paraffin-embedded blocks of these patients from year 2015/2016 were retrieved. Fresh *Hematoxylin and Eosin (*H&E) slides were prepared, and diagnosis was reconfirmed, vimentin stain was applied to check the antigenicity of the tissue followed by application of cyclin-D1 immuno-histochemical marker (I.H.C.) marker with controls to check the expression pattern.

Results: In our study we included a total of 92 diagnosed cases of stage I H.N.S.C.C. with a complete 5-year follow-up. Out of 92 cases male to female ratio was almost 3:1 with males comprising a total of 68 (73.9%) and females comprising 24 (26.1%). Mean age of patients was 62.48± 11.439 years. Larynx was the most frequently involved site 34.8% (n=32), followed by tongue 23.9% (n=22) and buccal mucosa 13.0% (n=12). Cyclin-D1 expression and tumor grade were revealed to have a statistically significant clinicopathological relationship (p value 0.001). A statistically significant association between the expression of cyclin-D1 and tumor recurrence (p value < 0.001) and duration of tumor recurrence (p value 0.003) was seen. The cyclin-D1 expression was also compared with metastasis which was also statistically significant (p value 0.003).

Conclusion: An increased risk of metastasis and recurrence is linked to increased levels of cyclin-D1 expression in the early years. Hence it is valuable to include cyclin-D1 expression in the initial diagnostic work up of H.N.S.C.C. for an early prognostic assessment. Further use of these results could be made for targeted treatment of head and neck malignancies.

Key Words*: Cyclin-D1, Five-Year Follow-Up, Head-and-Neck Squamous Cell Carcinoma, Immuno-Histochemical Expression, Prognosis*.

Introduction

Among the top ten types of human cancer, head and neck malignancies are a diverse category of tumors with distinct clinical and biological characteristics, causing an estimated 17,960 deaths worldwide each year.[1] H.N.S.C.C. has a substantial risk of both metastases and local recurrence, so aggressive treatments are typically performed to maximize the likelihood of long-term control.[2] These treatments may include surgery, radiotherapy, and/or chemotherapy. The high prevalence of this cancer requires special concern to devise authentic treatment protocols that are least invasive and show better outcomes.[3] Despite advancements in cancer prevention and treatment, the five-year survival rate of a patient with head and neck squamous cell

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carcinoma remains lower than that of other malignancies, such as colorectal, breast and cervical.[4] In conventional T.N.M. classification systems, the molecular heterogeneity of squamous cell carcinoma of the head and neck (H.N.S.C.C.) is not taken into account.[5] Hence, it is imperative to understand how recent molecular markers that have been identified correlate with prognosis for H.N.S.C.C., as they may offer new methods for early diagnosis, evaluation of prognosis and treatment. Cyclin-D1 a gene found on chromosome 11q13 plays a major role in activation of cell-cycle progression.[6]

Cyclin-D1 overexpression has been proposed by various international studies as a good marker for predicting poor prognosis in diagnosed cases of H.N.S.C.C.,[6,7,8,9] but these studies show the lack of proper follow-up, the uncertainty of such results in oral squamous cell carcinoma (O.S.C.C.), and smaller overall sample size.[10] This study's objective was to assess the expression of cyclin-D1 on a larger sample of stage I H.N.S.C.C. cases with a documented complete five-year follow-up to determine its prognostic significance. So that if the association is proved then cyclin-D1 marker should be made part of the diagnostic protocol. Patients with strong expression of cyclin-D1 on premier biopsy should be kept at close follow-up and treatment should be planned accordingly (targeted therapy can be given to patients such as cyclin-D inhibitors). If on the contrary this association is disapproved then we should stop investing time and money on this marker and evaluate better prognostic markers for the improvement in the treatment of patients with H.N.S.C.C. Information in regard to this study was collected on data collection questionnaire in the form of variables, statistical analysis was performed using S.P.S.S. version 26.0, Microsoft Excel 2013 was used for diagrammatic representations, qualitative / categorical variables were presented as frequency and percentages, to compare various parameters Chi-square test was performed and p-value of 0.05 was considered to be the essential level of significance.

Materials and Methods

A retrospective cohort study was carried out at Armed Forces Institute of Pathology (A.F.I.P.). This study had 92 patients in total. The approach of non-probability convenient sampling was applied. The study was conducted after the approval of Ethical Review Committee (Letter number: MP-OMP20-1/READ-IRB/21/781, dated: 30th-December-2021). Contact details of all cases diagnosed from Armed Forces Institute of Pathology (A.F.I.P.) of squamous cell carcinoma of head and neck region from January 1, 2015, to December 31, 2016 were taken from archives of Histopathology Department at Armed Forces Institute of Pathology (A.F.I.P.), Rawalpindi, Pakistan. The diagnosed patients of stage 1 H.N.S.C.C. were contacted for consent to participate in the research and five-year follow-up i.e., till December 31, 2021, and onwards. The data was matched with the record of these patients kept in the Department of Radiation Oncology, C.M.H. Rawalpindi. After collecting the data on follow-up of these patients, paraffin-embedded blocks of these patients from year 2015 to 2016 were retrieved. Confounding factors were excluded by firmly following the exclusion criteria [patients lost to follow-up or could not be contacted, poorly fixed specimens, very scanty tissue specimens, extensive necrosis or the retrieved blocks failed to take vimentin stain to check the antigenicity of tissue, patients dying within the study period, patients who had irretrievably taken away paraffin sections, cases diagnosed with H.N.S.C.C. of conjunctiva or skin or cases diagnosed with any immuno-morphological subtype of squamous cell carcinoma or mixed forms (with more than one component) i.e. adeno-squamous carcinoma] and inclusion criterion [all stage I H.N.S.C.C. cases with a documented complete five-year follow-up (evaluated on the basis of history, clinical, medical and radiological records) in our setting at A.F.I.P. during the calendar year January 1, 2015- December 31, 2016 (irrespective of age, gender or ethnicity)].H&E slides were prepared freshly for already diagnosed cases, but to eliminate bias the slides were viewed by three investigators separately. After confirmation of diagnosis on freshly prepared H&E slides, vimentin stain was applied to check the antigenicity of the tissue, followed by the application of cyclin-D1 immunohistochemical marker by Leica Microsystem (Germany). Lymph node tissue (mantle cell lymphoma) was used as positive control and appendix tissue was used as negative controls for cyclin-D1. When distinct brown nuclear staining was observed in ≥1% of the cells, the

tissue samples was considered positive for cyclin-D1. Cytoplasmic staining was not considered. The quantification criteria by *Dhingra et al.,* was used to quantify cyclin-D1 expression.[9] The sections with an inter-observer difference of more than 10% were re-examined by means of a multi-headed light microscope to reach consensus. Information was collected on data collection questionnaire in the form of variables, statistical analysis was performed using S.P.S.S. version 26.0, Microsoft Excel 2013 was used for diagrammatic representations, qualitative / categorical variables were presented as frequency and percentages, to compare various parameters chi-square test was performed and p-value of 0.05 was the essential level of significance.

Results

A total of 92 diagnosed cases of stage I H.N.S.C.C. with a complete 5-year follow-up were included in the study. Out of 92 cases male to female ratio was almost 3:1 with males comprising a total of 68 (73.9%) and females comprising 24 (26.1%). Mean age of patients was 62.48± 11.439 years. Larynx was the most frequently involved site 34.8% (n=32), followed by tongue 23.9% (n=22) and buccal mucosa 13.0% (n=12). The chi-square test revealed a statistically significant clinico-pathological relationship between cyclin-D1 expression and tumor grade (p value 0.001) Table I.

The cyclin-D1 expression was also compared with recurrence within five years by using chi-square test which was statistically significant (p value < 0.001) Table II and a statistically significant association was also seen between cyclin-D1 expression and duration of tumor recurrence (p value 0.003) Table III.

The cyclin-D1 expression was also compared with metastasis within five years by using chi-square test which was also statistically significant (p value = 0.003) Table IV

Table I: Case Distribution Based on Overall Cyclin-D1 Expression Score and Tumor Grade (n=92)

Expression of cyclin D1	Diagnostic Grade			Total
	Well differentiated. (%)	Moderately differentiated (%)	Poorly differentiated (%)	
Weak + (1-4)	10 (33)	28 (50)	2 (33)	40
Moderate ++ (5-8)	18 (60)	16 (29)	0 (0)	34
Strong +++ (9-12)	2 (7)	12 (21)	4 (67)	18
Total	**30**	**56**	**6**	**92**

***p*-value = 0.001**

Table II: Case Distribution Based on Overall Cyclin-D1 Expression Score with Recurrence (n=92)

Recurrence	Expression of Cyclin D1			Total
	Weak + (1-4)	Moderate ++ (5-8)	Strong +++ (9-12)	
Absent	40	32	4	76
Present	0	2	14	16
Total	**40**	**34**	**18**	**92**

***p*-value = 0.001**

Table III: Case Distribution Based on Overall Cyclin-D1 Expression Score With Recurrence as Per Follow up Duration (N=92)

Recurrence	Cyclin-D1 Expression			Total
	Weak + (1-4)	Moderate ++ (5-8)	Strong +++ (9-12)	
First year	0	0	4	4
Second year	0	0	6	6
Third year	0	0	2	2
Fourth year	0	2	0	2
Fifth year	0	0	2	2
Total	0	2	14	16

***p*-value = 0.003**

Table IV: Case Distribution Based on Overall Cyclin-D1 Expression Score with Metastasis (n=92)

Metastasis	Cyclin-D1 Expression			Total
	Weak + (1-4)	Moderate ++ (9-12)	Strong +++ (9-12)	
Absent	38	32	12	82
Present	2	2	6	10
Total	40	34	18	92

***p*-value = 0.003**

Discussion

Cyclin-D1 is a gene located on chromosome 11q13 and plays a major role in activation of cell-cycle progression. Cyclin-D is a cyclin-dependent kinase (C.D.K.) 4 and C.D.K. 6-binding cell cycle regulator. The stimulation of D.N.A. synthesis and the transition of cells between the G1/S phases are both significantly influenced by this cyclin-CDK complex, [11] rendering it a genuine target during carcinogenesis.[12] As a result, it has been determined that 30% of head and neck S.C.C. have cyclin-D1 amplification.[13]

In our investigation, it was discovered that cyclin-D1 expression positively associated with tumor grade. When the histopathological grade increased, from well differentiated to poorly differentiated

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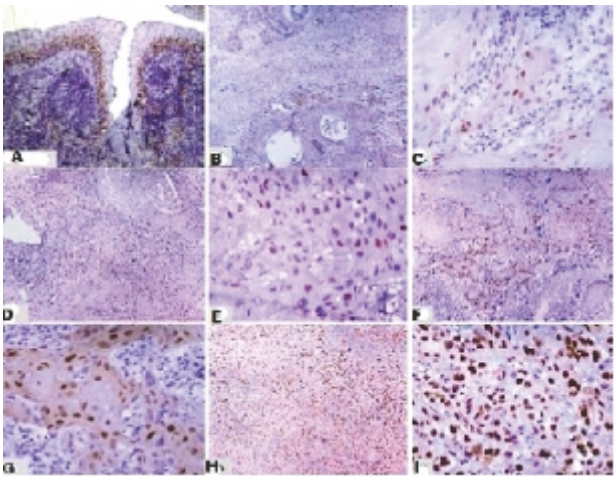
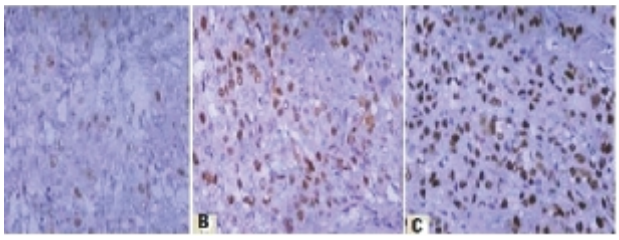


This figure is showing expression score as per quantification criteria.



This figure is showing intensity score as per quantification criteria

Figure 1: Figure Showing Total Score of Cyclin-D1 Expression (Product of Expression Score and Intensity Score)

H.N.S.C.C., the percentage of cyclin-D1 expression increased as well. In various studies, higher cyclin-D1 expression has been linked to poor histological grade.[8] According to Chinnathambi et al., in year 2021cyclin-D1 immuno-expression was discovered in all patients, and it was substantially correlated with deteriorating tumor grade and positive lymph node disease. Nevertheless, several authors discovered no clinical correlation. Batool et al. in 2020 in her study described similar findings that the histological grade of H.N.S.C.C. was not substantially correlated with cyclin-D reactivity.[14] Zand et al. in 2020 and Nazar et al. in year 2020 also described that the results of their study demonstrated no link between expression of cyclin-D1 and grade of the disease.[15,16]

The main finding of our research was that the stage I diagnosed cases of H.N.S.C.C. showing an elevated expression of cyclin-D1 at the time of diagnosis were at a greater risk of developing recurrence in the earlier years of five-year period of follow-up as compared to those cases showing a weak or no expression of cyclin-D1 immuno-histochemical stain. Another important finding of this study was that cyclin-D1 expression was found to positively correlate with tumor metastasis. An increase in cyclin-D1 expression at the time of diagnosis of stage I cases of H.N.S.C.C. was found to show an increased risk of developing tumor metastasis within the five year of diagnosis of tumor.

Various studies have been conducted on this molecular marker suggesting that strong positivity of cyclin-D1 expression in H.N.S.C.C. is related to metastasis and recurrence in operable cases of H.N.S.C.C.[9] Sharada et al., in year 2018 and Patel et al., in year 2017 described similar results in their studies that overexpression of cyclin-D1 has been seen to associate with an increased risk of metastasis of lymph nodes and increased risk of recurrence.[17,18]

Our research had this limitation that five year survival of the cases could not be included in the study to determine prognostic value of cyclin-D1 as it was a retrospective study and confounders could not be removed for those who died within five years; as exact cause of death could not be determined for those dying within study period (five years of diagnosis of H.N.S.C.C.).Hence, prospective follow-up study is recommended including five year survival of cases as a variable to determine prognostic value of cyclin-D1 thus minimizing confounders by notifying exact cause of death of participants within the study period.

Conclusion

This study showed a statistically strong association between recurrence, duration of developing recurrence and metastasis with increasing cyclin-D1 expression. Therefore, an increased risk of metastasis and recurrence is linked to increased levels of cyclin-D1 expression in the early years. Hence it is valuable to include cyclin-D1 expression in the initial diagnostic work up of H.N.S.C.C. for an early prognostic assessment. . Further use of these results could be made for targeted treatment of head and neck malignancies.

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CONFLICT OF INTEREST

Authors declared no conflicts of Interest.

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DATA SHARING STATMENT

The data that support the findings of this study are available from the corresponding author upon request.