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ORIGINAL ARTICLE

To Study the Association of Cyclin D1 Expression with Fuhrman's Nuclear Grading in Renal Cell Carcinoma

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ABSTRACT

Background: In adults, renal cell cancer (RCC) consists of tumors arising from the renal parenchyma and renal pelvis. Renal parenchyma cancer is mainly renal cancer i.e. adenocarcinoma. Renal pelvis malignancy is mainly transitional cell type. Presently, prognostic markers for renal cell cancer are tumor stage and nuclear grade.
Aim: To observe the expression of cyclinD1 in different variants of RCC and to associate cyclinD1 expression with Fuhrman nuclear grades of RCC.
Results: Out of 93 patients, 54(58.1%) were of more than 40 years of age, with M: F ratio of 1: 1. Clear cell RCC was the most common histological type i.e. 72out of 93(77.4%) biopsies. Low grades RCC were 50 out of 93(53.8%) and High grade RCC were in 43 out of 93(46.6%) biopsies and out of these 43 high grade RCC 23(24.7%) were found to invade surrounding tissue. As far as CyclinD1 expression score was concerned, 60 out of 93(64.4%) biopsies were found to have score 1 and 2 with no and upto30% +ve cells and 33(35.5%) biopsies were of score 3 and 4 with 30-60% and >60% positive cells respectively. In this study, 28 out of 93(30.2%) biopsies of low Fuhrman's nuclear grade showed cyclin D1 score 3 and 4 respectively.
Conclusion: In our study cyclin D1 protein was over expressed in low grade RCC showing that high protein levels may be related with the disease progression in patients of RCC. Cyclin D1 protein expression in future can be used as adjuvant to Fuhrman's nuclear grading and will increase the predictive accuracy of the current disease progression models for RCC.
Keywords: CyclinD1, RCC, Low and High Fuhrman nuclear grade

INTRODUCTION

In visceral cancers of human being, 1-3% is renal cell carcinoma (RCC). It represents the sixth most frequently diagnosed cancer in men and tenth in women. There are 2% of deaths occur due to this cancer in the population. Incidence of renal cancer has been increasing in every aspect of the world. Annually, the incidence of renal cell cancer has been increased about 2%. However, it is about 1 to 3% of all cancers in adulthood. In other studies, in patients with kidney cancers, metastatic cancers are about 17% to 20% (Siegel RL et al., 2018).

A cell which is released from normal cell cycle, transformed into a cancerous cell by the over expression of cyclinD1 or may be due to amplification of gene of cyclin D1. These changes give information about the diagnosis of lymphomas of mantle type. In many cancers of the body, CCND1 has better prognostic values. In many types of cancers, role of cyclinD1 gives us a base line for scheduling a treatment of different cancers in future life. The correlation of tumor aggression and cyclinD1 over expression is documented in different types of tumors i.e. lung cancer of non-small cell type, cancer of esophagus and cancer of oral cavity and head & neck (Latic D et al., 2018, Zhu et al., 2010).

Many researches on breast carcinomas observed that cyclinD1 with moderate/strong staining was associated with improvement of overall survival as compared to the subjects having weakly cyclin D1 staining tumors. Tumors that were negative for cyclinD1 staining had bad prognosis and negative impact was seen with estrogen receptor-negative patients. Cyclin D1 protein expression and bcl-1 gene rearrangement has been seen necessary in the mantle cell lymphoma diagnosis. CyclinD1 represents a key component in proliferation of cells and it also regulates a G1/S transition and unstable protein (Mohammadizadeh F et al., 2013).

Fuhrman's (nuclear) grade: (Humphrey P et al., 2016)
G1: There are small and dark lymphocyte-like nuclei with or without visible nucleoli.
G2: Nuclei small and uniform with open, granular chromatin with inconspicuous nucleoli and seen only at 200-400x
G3: Nuclei usually mild/ moderate pleomorphic with prominent nucleoli seen at 100x
G4: Nuclei of bizarre shape with marked pleomorphism. Nucleoli are multiple.

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METHODOLOGY

This descriptive study was conducted for one year in Postgraduate Medical Institute (PGMI), Lahore General Hospital and other tertiary care hospital like Mayo hospital etc also included. Nighty three cases are calculated and confidence level is 95% with margin of errors 10%.Expected %age of subjects with positivity is 41% among the RCC. Sampling technique used was non-probability, Purposive Following formula is applied:

$$n = \frac{Z^2 P(1-P)}{d^2}$$

Where, Z at 95% C.I was 1.96, P=anticipated population proportion= 41%, d = margin of error = 10%
Inclusion criteria: Histopathologically diagnosed cases of Renal cell carcinoma of all ages and both gender.
Exclusion criteria: Patients already taking chemotherapy, radiotherapy or hormone therapy
This study was started after permission from Ethical Review Board.

RESULTS

The detail of results is given in following table.

Table: Association of Fuhrman's Grade and CyclinD1Expression Score

CyclinD1 expression Score	Fuhrman Nuclear Grades		Total
	Low	High	
1(Zero% +ve cells)	5(5.4%)	27*(29.0%)	32(34.4%)
2(30% +ve cells)	17**(18.3%)	11 (11.8%)	28(30.1%)
3(30-60% +ve cells)	18***(19.4%)	03 (3.2%)	21(22.6%)
4(>60% +ve cells)	10 (10.8%)	02(2.1%)	12(12.9%)
Total subjects	50(50%)	43(43%)	93 (100%)

Statistical Analysis: P< 0.05 (Significant) (chi square test)
*27(29%) cases were of 0% +ve cells
**17(18.3%) cases were of 30% +ve cells
***18(19.4%) cases were of 30-60% +ve cells

DISCUSSION

Despite the advances in clinical treatment, immunotherapy, targeted cancer therapies, and chemo and radiotherapy, the prognosis of RCC patients is still very poor. Renal tumorigenesis is a complex multistep process which also involves aberrations in cell cycle control through cyclins and their regulators (Casimiro et al., 2013).

RCC is an exceptionally heterogeneous and complex neoplasia with a fluctuating outcome. Different RCC subtypes showed different intensity and type of stains for cyclinD1. Papillary RCC indicated less positive cells when comparing with clear cell RCC (Escudier et al., 2016).

In this study, 18 out of 93(19.4%) biopsies having low Fuhrman grade were significantly showing to have high cyclinD1 expression (score 3) with 30% - 60% positive cells. The difference is highly significant showing inverse association between Fuhrman grade and cyclin D1 expression. As the Fuhrman grades decreased, cyclin D1 expression scores increased significantly ($p<0.05$). There were 10(10.8%) biopsies having low Fuhrman grades showed increased cyclin D1 expression score 4 with > 60% positive cells showing inverse association between Fuhrman grades and cyclinD1 expression in RCC. This study is consistent and comparable with the results of Latic D et al (2019) & Lima et al (2013) who also observed same results in their studies.

In this study, low Fuhrman grade tumors were more frequent i.e. 44 out of 93(47.3%) when comparing other grades and difference was found to be significant statistically ($p<0.05$).In this study, cyclinD1 expression of score 1with zero% +ve cells was found in low grade 05(5.4%) and high grade 27(29%) .

When comparing Fuhrman nuclear grade and cyclinD1 expression score, there is significant inverse association. In this study, high Fuhrman grades showed decreased cyclinD1 expression score with zero % +ve cells in 27 out of 93(29%) biopsies. 17 out of 93(18.3%) biopsies show low Fuhrman grade with cyclinD1 expression of score 2 with upto 30% +ve cells .

In another study, it is shown that cyclin D1 levels within the tumor i.e. intratumoralcyclinD1were associated with the disease progression for many cancers. In breast carcinoma, the over expression of cyclinD1 was correlated with positive steroid receptors of sex, favorable histological features and good prognosis (Guo et al., 2010).

In another study, it is observed that in 80 patients having RCC, there were increased cyclinD1levels in most cases. Association of longer survival is with high protein contents of cyclinD1. CyclinD1 was an important protein for clear cell RCC after multivariate analysis (Latic et al., 2019; Hedberg et al., 2004).

By the addition of cyclinD1, staging and nuclear grades were used as clinical and pathological indicator in different analysis. A study also showed that use of cyclinD1 is early detector of metastasis especially in clear cell RCC. The reason for this is that cyclinD1 expression decreased as there is progression of tumor. In early tumor growth, there is an increase in cyclinD1. This showed the association between over expression of cyclinD1 and absence of spread or with small sized tumor. This association was observed by (Dragana L et al., 2019).

In a variety of kidney tumors, cyclinD1 expression demonstrated diffuse positivity in clear cell kidney sarcoma (Mirkovicet al., 2015).

Regarding the tumor features, the study of Dragana L et al., (2019), found that low cyclin D1 protein level in the RCC was associated with high nuclear grade and large tumor size.

Cyclin-D1 levels in renal cell cancer appeared to be associated with progression and aggression of tumor, since cases of score 3 and 4 cyclinD1expression have survival longer than those with score 1 and 2 cyclinD1 expression. In cancer of breast, better results were seen in high cyclinD1 expression (Zhao et al., 2015; Dragana L et al., 2019).

CONCLUSION

Our study shows that the cyclin D1 protein is overexpressed in RCC and that high protein levels may be related with the disease progression in patients of RCC. Cyclin D1 protein expression in future will increase the predictive accuracy of the current disease progression models for RCC in adjuvant to Fuhrman's nuclear grading. Further studies and clinical trials are needed to establish the role of Cyclin D1 in targeted therapy of renal cell carcinoma.

Conflict of interest: Nil

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