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Pathology

Cyclin D1 Expression by Immunohistochemistry in Patients with Multiple Myeloma and Its Correlation with Clinical Parameters

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Original Research Article

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Abstract: Multiple myeloma is a hematological malignancy characterized by the monoclonal proliferation of neoplastic plasma and cytogenetic studies have shown numerous chromosomal abnormalities that play crucial roles in disease progression in myeloma patients. Studies have shown cyclin D1 expression to be associated with advanced clinical stage, and poor prognosis. The study aims to assess the cyclin D1 expression in newly diagnosed multiple myeloma using immunohistochemistry, and correlating it with the clinical parameters. A total of 50 newly diagnosed cases of multiple myeloma were included in the study. Cyclin D1 expression was assessed by immunohistochemistry, with mantle cell lymphoma taken as positive control. Expression of cyclin D1 was correlated with clinical parameters. SPSS statistical software was used for all statistical analysis. Majority of the patients were male (60%), with median age of 59.96 years at diagnosis. Seventy two percent of patients had anemia and 90% had bone involvement at presentation. Sixty percent of the patients presented with ISS stage 3. The predominant pattern of marrow infiltration by plasma cells was found to be diffuse (64%), followed by an interstitial pattern. Twenty eight patients (56%) showed cyclin D1 over expression with 14(28%) showing strong expression (2+/3+). A significant association between the cyclin D1 expression and a higher histological grade was found (p value= 0.05). Expression of cyclin D1 could not be correlated with a higher ISS stage in the present study. Cyclin D1 positivity is found in 56% of patients with newly diagnosed myeloma and its expression correlated with a higher histological grade.
Keywords: Cyclin D1; immunohistochemistry; multiple myeloma.

INTRODUCTION

Cyclin D1 dys-regulation has been suggested to play a key role in the pathogenesis of multiple myeloma [1-5]. However its role as a prognostic marker still remains uncertain. Cytogenetic studies have shown cyclin D1 expression to be associated with advanced clinical stage, higher histological stage and grade, and increased proliferative index. However reverse transcriptase-polymerase chain reaction (RT-PCR), fluorescence in Situ hybridization (FISH), cannot be done on archived samples, and is usually expensive [6-11]. The aim of this study was to assess the cyclin D1 expression in newly diagnosed multiple myeloma using immunohistochemistry. The correlation between cyclin D1 expression and clinical parameters was also assessed.

METHODS

Between February 2015 to July 2016, 50 newly diagnosed cases of multiple myeloma were included in the study. Previously treated patients or those with inadequate biopsy samples or other coexisting malignancies were excluded from the study. The primary end point was to study the expression of Cyclin D1 in multiple myeloma by immunohistochemically study in bone marrow trephine biopsies. The secondary endpoint included correlation between the clinical parameters and Cyclin D1 expression. The study was approved by Institute Ethics Committee and informed written consent was obtained from all patients before starting the study.

Base line clinical details were obtained as per the clinical performa. Investigations including complete blood counts, liver and kidney function tests, urine bence jonce protein, serum beta 2 macroglobulin, serum electrophoresis, immunofixation electrophoresis, serum immunoglobulin assay, skeletal survey, and light chain assay, 24 hour urine protein, bone marrow aspiration and biopsy was done for all patients. Patients were staged as per the international staging system.

Bone marrow trephine biopsies received in the department of pathology were fixed in 10% neutral buffered formalin, decalcified, processed and paraffin embedded, sectioned, stained by routine Haematoxylin

and Eosin and studied. The biopsy sections were assessed for pattern of marrow infiltration, histological grading based on cell morphology, and presence of marrow fibrosis.

Cyclin D1 estimation

Appropriate blocks were selected and sections of 3 micron thickness were taken onto slides. Antigen retrieval was done by the heat induced epitope retrieval method and immunohistochemically staining using rabbit monoclonal CyclinD1 antibody, was done for all 50 cases. A section of a case with mantle cell lymphoma was taken as positive control. These were then counterstained with hematoxylin and analyzed for the expression of cyclin D1. Nuclear positivity, in at least 10% of myeloma cells was considered as positive. The positivity was then graded semi quantitatively as 1+ (10-19%positivity), 2+ (20-50% positivity) and 3+ (more than 50% positivity).

STATISTICAL ANALYSIS

Data was recorded on a pre designed performa and entered on an excel spreadsheet. Categorical variables were summarized by frequency (%) and quantitative variables were summarized as median and range. Chi square test was used to find association between 2 categorical variables. A p value of <0.05 was taken as significant. SPSS v16 (SPSS for Windows, Version 16.0. Chicago, SPSS Inc.) Was used for all statistical analysis.

RESULTS

The majority of the patients were male (n=30), and with a median age at diagnosis of 59 years. Of the 50 cases, 36 patients (72%) had hemoglobin levels less than 10 g/dL, of which 21(42%) cases had less than 8.5g/dL. Mild leucopenia was noted in 2 cases, and lactate dehydrogenase levels were elevated above the normal range in 27 cases. Lytic lesions of the bones were noted in 45 patients, and serum β2microglobulin was elevated above normal levels in 46 cases, with 30 cases having levels above 5.5µg/ml. Corrected serum calcium was raised in 16 (32%) of the patients.

Serum protein electrophoresis was positive for M band in 49 of the cases, with only one case as negative that was confirmed as non-secretory myeloma. The immunoglobulin assay determined majority of the M protein to be composed of immunoglobulin G (n=38), while 4 had immunoglobulin A, and 7 cases had pure light chain myeloma. Only one case had immunoglobulin E myeloma.

Thirty two patients showed a diffuse pattern of plasma cell infiltrates in the bone marrow, while other patterns noted included, focal nodular and interstitial patterns (Figure 1). High histological grade with a plasmablastic morphology was noted in the majority of cases, with 36 cases showing grade 3, 7 cases showing grade 2 (intermediate, immature morphology) and 7 showing grade1 (small mature, lymphoplasmacytoid morphology) (figure 2). 30 patients were of ISS stage 3, 16 were of ISS stage 2 and only 4 were of ISS stage 1. Myelofibrosis was noted in 12 cases, with a majority (n=7) having grade 2, while only one case had grade 3 myelofibrosis and 4 showed grade 1 fibrosis. Patient characteristics are summarized in table 1.

Among the 50 cases studied, 28 showed cyclin D1 over expression. 14 patients had grade 1 intensity (with 10-19% expression), 11 had grade 2 (with 20-50% expression) and 3 had grade 3 positivity (with more than 50% expression) (Figure 3). On correlating cyclin D1 over expression with clinical parameters, a significant association (p value= 0.05) between the cyclin D1 expression and a higher histological grade than compared to those with a negative cyclin D1 expression was seen. No significant association was noted between cyclin D1 over expression and Beta 2 microglobulin levels, Serum LDH, M protein type, bone marrow infiltration pattern, or ISS staging.

Table-1: Patient Characteristics

Parameters	Results
Age (median)	59 years
Sex: Male Female	30/50 20/50
Hemoglobin: <8.5g/dL 8.5-10 g/dL	21/50 29/50
Bone lesions	Present 45/50
β2 microglobulin levels: >5.5µg/ml 3.5-5.5µg/ml	30/50 20/50
LDH: >500U/L <500U/L	38/50 12/50
Corrected Serum Calcium : <10mg/L >10mg/L	44/50 16/50
Serum Creatinine <1.1mg/L: 1.1-4mg/L: >4mg/L:	10/50 33/50 7/50
A: G ratio reversal : Present	68%
Immunoglobulin : Ig G Ig A Ig E Pure light chain	38/50 4/50 1/50 7/50
Pattern of marrow infiltration: Diffuse Focal nodular Interstitial	 32/50 6/50 12/50
Histological stage: stage 1: stage 2 : stage 3:	0/50 19/50 31/50
Histological grade: Grade 1: Grade 2: Grade 3:	7/50 7/50 36/50
ISS staging: Stage I Stage II Stage III	4/50 17/50 29/50
Myelofibrosis: Grade 0: Grade 1: Grade 2: Grade 3:	38/50 4/50 7/50 1/50
Immunohistochemistry: Grade 1 positivity Grade 2 positivity Grade 3 positivity	14/50 11/50 3/50

Table-2: Summary of cyclin D1 expression from various studies

	Number of patients	Technique Used	Cyclin D1 positive cases
Padhi *et al.*	14	IHC	57%
Cook *et al*.	20	IHC, FISH	50%
Vollmar *et al*.	50	FISH	40%
Athanisiou *et al*.	71	ISH, IHC	24%
Hoyer *et al*.	24	IHC	79%
Pruneri *et al*.	48	IHC, FISH	25%
Present study	50	IHC	56%

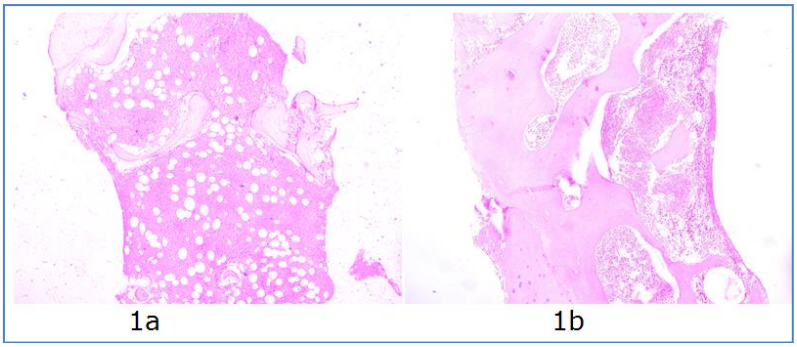

Fig-01: Patterns of plasma cell infiltration in bone marrow (a) diffuse pattern (b) focal nodular, (magnification 10x)

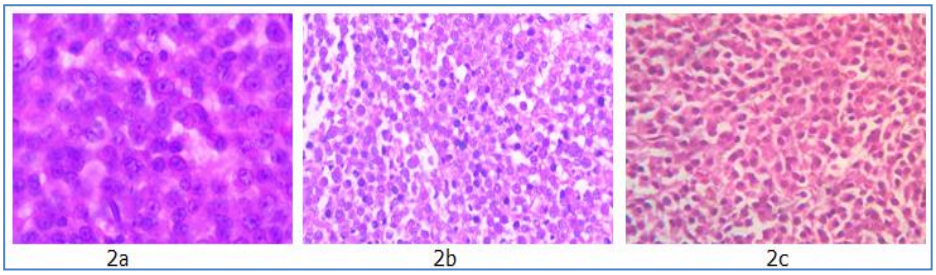

Fig-02 (a): Plasma cell infiltrate with histological grade 3 morphology,(magnification 100x), (b) histological grade 2 morphology; (c) histological grade 1 morphology (magnification 20x)

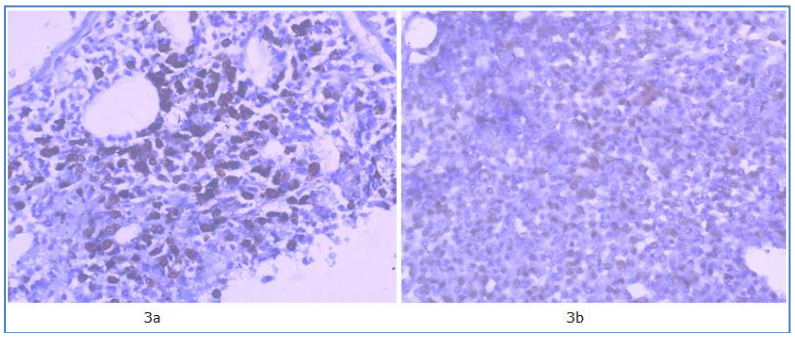

Fig-03: Photomicrograph (40x) (a) Immunohistochemistry of CyclinD1 on bone marrow trephine sections with grade 3 positivity (>50%cells showing positive nuclear staining); (b) IHC of cyclinD1 with grade 2 positivity (20-50% cell showing nuclear positivity)

DISCUSSION

Cyclin D1 dysregulation has been suggested to play a key role in the pathogenesis of multiple myeloma [1]. However its role as a prognostic marker still remains uncertain. Although several studies have shown an association between cyclin D1 over expression and various poor prognostic factors, and poor response to conventional chemotherapy, others have found its expression to be associated with better overall survival in response to modified chemotherapy and stem cell transplantation and prolonged disease free interval[6-11]. In the present study, the cyclin D1 over expression among 50 newly diagnosed cases of multiple myeloma were assessed and correlated with its clinical, radiological and biochemical parameters.

The cyclin D1 over expression in bone marrow samples of multiple myeloma patients detected by immunohistochemistry was found to be positive in 28 out of 50 cases (56%). These results are comparable with that of studies conducted by Padhi *et al*. Cook *et al*. and Kohima *et al*. [12-14]. Some other studies have showed a lower percentage of cases with cyclinD1 over expression, by IHC, with the exception of the study conducted by Hoyer *et al.* [15]. These differences in detection may be as a result of variation in the type of antibody used, methods of antigen retrieval, tissue processing and sample selection. Comparison of cyclin D1 expression from various studies is summarized in table 2.

The histological staging among the positive cases were analyzed and compared with the findings in other studies. The majority of the cases (14/28 positive cases) in the present study had a histological stage 3(>50% plasma cell infiltrate), which was consistent with the findings of others. In the present study, only the histological grading was found to have a statistically significant association with the Cyclin D1 overexpression (p value=0.05). Studies conducted by

Athanasiou *et al*. and Vollmar *et al*. found similar association of cyclin D1 expression with the histological grade , along with the extent of plasma cell infiltration in the marrow, plasma cell labeling index, and serum B2- microglobulin and C-reactive protein levels. While Padhi *et al*. found a significant association only with the hemoglobin concentration among the cases [7,16,17].

CONCLUSIONS

The present study detected used immunohistochemistry to detect cyclin D1 positivity in 50 cases of newly diagnosed multiple myeloma and showed positivity in 56%, which was comparable to the findings of most of the other studies. A significant association between the cyclin D1 expression and a higher histological was found (p value= 0.05) although expression of cyclin D1 could not be correlated other clinical parameters.

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