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ORIGINAL ARTICLE

Clinical Significance of Cyclin D1 Expression on Trephine Bone Marrow Biopsies in Plasma Cell Myeloma Patients an Egyptian Study

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ABSTRACT

Background: One predictive factor for patients with Mantle cell lymphoma has been identified as cyclin D1 expression. Nonetheless, there is ongoing discussion on the prognostic significance of Cycline D1 overexpression in multiple myeloma. As far as we are aware, this work has never been completed at Zagazig University's Faculty of Medicine. The purpose of this research was to assess the clinical relevance of Cyclin D1 expression in multiple myeloma.

Methods: This cohort study was conducted on newly diagnosed plasma cell myeloma (PCM) patients in clinical pathology Department and Internal Medicine Department–Hematology unit at Zagazig University Hospitals and included 52 cases diagnosed according to clinical, pathological and morphological criteria. Follow up patients was carried out for 3 months from initiation of treatment to assess response to therapy.

Results: Cyclin D1 is a stable Immunohistochemical marker with strong expression on plasma cells and there was highly statistically significant strong positive correlation between Cyclin D1 expression and increasing percentage of plasma cells, increasing CD138 expression on BMB. As regard response to treatment there was a statistical significant increase in Cyclin D1 expression percent among cases with progressive disease compared to cases with complete and partial response.

Conclusion: Cyclin D1 had a clinical significance in PCM patients and could be used as a prognostic marker in addition to traditional markers.

Keywords: Cyclin D1, Clonal plasma cell, Multiple myeloma.

INTRODUCTION

Clonal plasma cell (PC) cancer of lymphocytes is known as multiple myeloma (plasma cell myeloma). PCs travel to the bone marrow (BM) after maturing in lymph nodes, where they undergo malignant alterations that lead to the development of the disease [1].

The skeletal system becomes extensively involved when PCs proliferate abnormally, resulting in soft tissue plasmacytomas, anemia, osteopenia/osteolytic lesions, and hypercalcemia[2].

Monoclonal gammopathy of unknown significance (MGUS), a premalignant syndrome that can progress to MM symptoms with end organ

destruction, may often precede it. The worldwide myeloma working group has reduced the common clinical signs of symptomatic MM (hypercalcaemia, renal impairment, anemia, and bone disease) into the acronym CRAB [3].

About 10% to 15% of hematopoietic neoplasms, 1% of malignant tumors, and 20% of hematological malignancy-related mortality are caused by plasma cell myeloma. With a male to female ratio of 1.1:1, it is more prevalent in men than in women. As patients age, the incidence of plasma cell myeloma gradually rises. The median age at diagnosis is 70 years old, and 90% of cases include patients over 50 [4].

Although cyclin D1 plays a role in both neoplasia and the regular control of the cell cycle, there is ongoing discussion on the predictive significance of cyclin D1 overexpression in MM[5].

The gene for cyclin D1 (CCND1) is found on chromosome 11q13 cyclin D1 is implicated in both normal cell cycle control and neoplasia, and it is a crucial modulator of G1 progression to S phase. When cells must survive the cell cycle, overexpression of the CCND1 protein causes them to lose their normal regulation, impedes their maturation, and encourages the development of malignant phenotypes [6].

In cell biology, cyclin D1 is essential for controlling cell migration, mitochondrial activity, DNA repair, and proliferation and growth.

Numerous molecular mechanisms, such as amplification, chromosomal translocations, mutations, and activation of the pathways involved in cyclin D1 expression, frequently alter the CCND1 gene and its protein cyclin D1. These changes seem to be crucial in the development of human cancers, including thyroid, overian, lung, acute lymphoblastic leukemia, multiple myeloma, mantle cell lymphoma, and hairy cell leukemia. [8].

One of the common unifying pathogenic events in MM is the dysregulation of at least one of the cycline D (CCND) genes (CCND1, CCND2, CCND3). Some researchers have found that higher CCND1 levels in myeloma are linked to a better prognosis. However, negative associations with CCND1 have been identified in other research. As a result, it has been unclear whether CCND1 dysgranulation in plasma cell myeloma has any clinical importance [7].

This study aimed to use IHC on trephine BMB to identify Cyclin D1 expression in MM patients and connect it with clinical information and therapy response.

METHODS

This Cohort study was conducted on newly diagnosed plasma cell myeloma patients in clinical pathology Department at Internal Medicine Department-Hematology unit at Zagazig University Hospitals. It included 52cases (32 males and 20 females) with a male to female ratio of 1.6:1 . Mean age of cases was 57.71 years. Patients were followed up for three months later after initiation of treatment . The study carried out in the period between May 2023 to March 2024.

This study was ethically approved from Institutional Reviewer Board (IRB# 10755) in Faculty of Medicine, Zagazig University Hospital and a written consent from every patient participating in the study was taken. The study followed the Helsinki Declaration, which is the World Medical Association's guideline of ethics for research involving human subjects.

Newly diagnosed patients with plasma cell myeloma were included in the study. Patients who had any of the following conditions: plasma cell myeloma patients under treatment and follow up and other malignancy or autoimmune diseases were excluded.

Every patient had their whole medical history taken, including their age and sex. symptoms that point to infections, bone lesions, and anemia. affection for other systems (kidney and neurological system). Through clinical examination, which includes checking all body systems and detecting pallor, tacycardia, hemorrhage, and infections. Skeletal bone inspection, radiological examination, PET/CT scan, and plain X-ray. Standard laboratory procedures, such as complete blood counts (CBCs)

utilizing Sysmex XP Japan, and analysis of Leishman stained PB smears for variations in leucocyte count and cell morphology are included in laboratory examinations. An automated analyzer called the "Cobas 8000 platform-702c module, Germary" was used to measure the levels of serum calcium, liver, kidney function tests, and LDH (lactate dehydrogenase). Additionally, the automated analyzer "VISION–B, China" was used to quantify the ESR (erythrocyte sedimentation rate).

Myeloma specific tests such as serum protein Electrophoresis and Immunofixation, serum B2 microglobulin, Bence Jones protein in urine, bone marrow aspiration and morphological examination of Leishman stained smears to detect the percentage of plasma cells. Bone marrow biopsy and examination of H&E stained film. Cyclin D1 and CD 138 were stained by immunohistochemistery on (BMB) and were examined.

Detection of cyclin D1 (CCND1)(VENTANA Benchmark gx, USA) by using IHC staining of BMB specimens of PCM patients:

Samling; Trephine biopsy needles (Jamshidi needles) were used to acquire the BM biopsy from the posterior superior iliac spine (PSIS). The BMB sample had at least five intertrabicular gaps and was sufficient (1.5 cm). After that, 10% formalin was used to preserve the core.

Bone marrow biopsy processing:

Procedure:

Tissue processing for a whole day, the samples were fixed in 10% formalin. Formic acid (150 ml formic acid + 850 ml formalin 10%) was used for two days to decalcify the tissue samples. For fourteen hours, processing was carried out in an automated tissue processor (Tissue-Tek VIP6, Japan). After that, the slides were ready for H&E staining and light microscopy examination.

Cyclin D1 immunohistochemistry staining

Tissue processing, the microtome-sectioned tissue was placed on five charged glass slides and staining was performed using a VENTANA Benchmark Gx, USA. Reagents are incubated for specified periods of time at particular temperatures as part of a multi-stepstainingprocedure.

A rabbit monoclonal antibody is present in Ventana anti-Cyclin D1 antibody. Ultra View The universal detection kit 3, 3'-diaminobenzidine (DAB) technique finds certain primary antibodies from mice and rabbits attached to an antigen in a BMB sample. After that, the complex is detected using a hydrogen peroxide substrate and a detection chromogen for 3,3'-diaminobenzidine tetra hydrochloride, which results in a brown precipitate that is easily visible under a light microscope. The BenchMark IHC device cleans the sections to get rid of any loose material at the conclusion of each incubation phase. Hematoxylin II solution is put to the slide and mixed throughout the specimen region to counterstain it. When a mordant dye complex binds to heterochromatin's nucleic acids and histone proteins, hematoxylin II turns nuclei blue. Following a 5-minute rinse in distilled water, the slides were subjected to 80%, 90%, and 100% ethyl alcohol, followed by three 5-minute xylene changes. D.P.X. fixed the slide cover on the slide for inspection. To evaluate the results, a light microscope (Axiostar, Germany) was used.

Staining interpretation:

Positive anti Cyclin D1 antibody exhibiting brown nuclear staining pattern. A cutoff value ≥10% immunopositive cells were considered positive.

Treatment protocol :

Chemotherapy is administered in three cycles to all PCM patients in our research. For each cycle, they receive VRd (bortezomib, lenalidomide, and dexamethasone), with bortezomib administered on days 1, 8, 15, and 22, lenalidomide administered on days 1–21 (one week off), and dexamethasone administered on days 1, 2, 8, 9, 15, 16, 22, and 23. After that, they begin a new cycle. Most patients who have bone lesions are treated with biphosphate treatment (zometa). Cases are re-evaluated following the third cycle.

Statistical analysis:

The collected data were computerized and statistically analyzed using SPSS program

(Statistical Package for Social Science) version 27.0 (IBM, 2020). Independent T test, Mann Whiteny (MW), kruskal Wallis test, pearson's, spearman's correlation coefficient, Receiver operating characteristic (ROC) curve analysis were used.

RESULTS

This study was carried out on 52 patient were divided into 32 males and 20 females.

The age of the studied cases ranged from 42 to 82 years with mean± SD of age 57.71±9.69 years. 11.5% of the studied cases had enlarged liver, 7.7% had enlarged spleen, 3.8% had enlarged LN. Regarding Osteolytic bone lesion, 67.3% of patients had multiple lesions (Table 1).

The laboratory findings showed that, the mean±SD of TLC among the PCM patients was (6.11±2.17) $x10^3$/µL while mean± SD of Hb was 9.34±1.64 gm/dL, mean± SD of platelets count was(173.23±84.23) $x10^3$/µL, mean± SD of protein and albumin were8.88±2.71 &3.37±1.1 gm/dl respectively. Mean ±SD of LDH was 173.69 ±84.75 µ/L & of creatinine was 2.41±2.16 mg/dl, mean±SD of Ca was 9.26±1.16 mg/dl and finally mean± SD of B2M among PCM was 6.52±3.56mg/L (Table 2).

The mean±SD of BMA & BMB plasma cell percent were 38.27±22.71, 53.65±19.05 respectively. While mean±SD of CD 138(%) on BMB by IHC was 57.89±18.77. The most frequent M protein founded among PCM patients was IG G Kappa (55.8%) followed by IG G Lambda (21.1%), IG A Kappa (13.5%), lastely IG A Lambda (9.6%) (Table 3, Figure 1 A,B,C).

Cyclin D1 expression by IHC showed that 30.8% of the studied cases were positive for Cyclin D1 by IHC on BMB, while 69.2% were negative. The studied cases had Cyclin D1 expression ranged from 10 to 80% with mean±SD was 13.12±21.73. A cut off value of positivity of Cyclin D1 is ≥10% (Table 4 , figure 2).

As regarded to response to treatment, 26.9% of the patients had complete response to treatment, while 57.7% had partial response and 15.4% had a progressive disease(No response to ttt) (Table 5).

There were no statistical significant differences between negative & positive Cyclin D1 cases as regard age, sex distribution or clinical findings (Liver, spleen, L.N enlargement and osteolytic bone lesion). There were no statistical significant differences between negative & positive Cyclin D1 cases as regard TLC, Hb, platelets, protein, albumin, LDH, creatinine , Ca level nor B2 microglobulin level. There were no statistical significant differences between negative & positive Cyclin D1 cases as regard plasma cell percentage by BMA, BMB, CD 138 or M protein by electrophoresis and immunofixation. There were no statistical significance differences between negative & positive Cyclin D1 cases as regard plasma cell & M protein by electrophoresis and immunofixation. There was a highly statistical significant increase of progressive disease among positive cyclin D1 cases compared to negative cases (Table 6).

There was a statistically significant positive correlation between Cyclin D1 expression , percent of plasma cells and CD138 immunostaining of BMB among plasma cell myeloma patients with positive Cyclin D1 (Table 7).

There was a statistical significant increase in Cyclin D1 expression percent among cases with progressive disease compared to cases with CR & PR (Table 8).

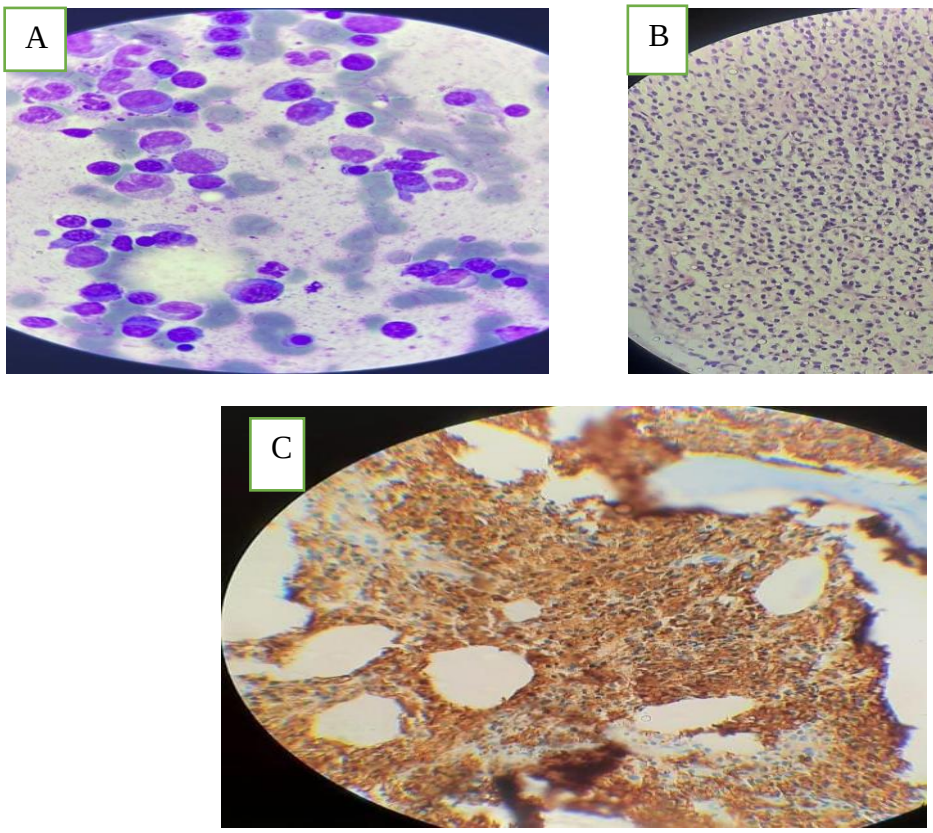

Figure 1: Showed A; B.M.A infilterated by plasma cells, B; B.M.B infilterated by plasma cells, C; +Ve CD138 on B.M.B in PCM patients

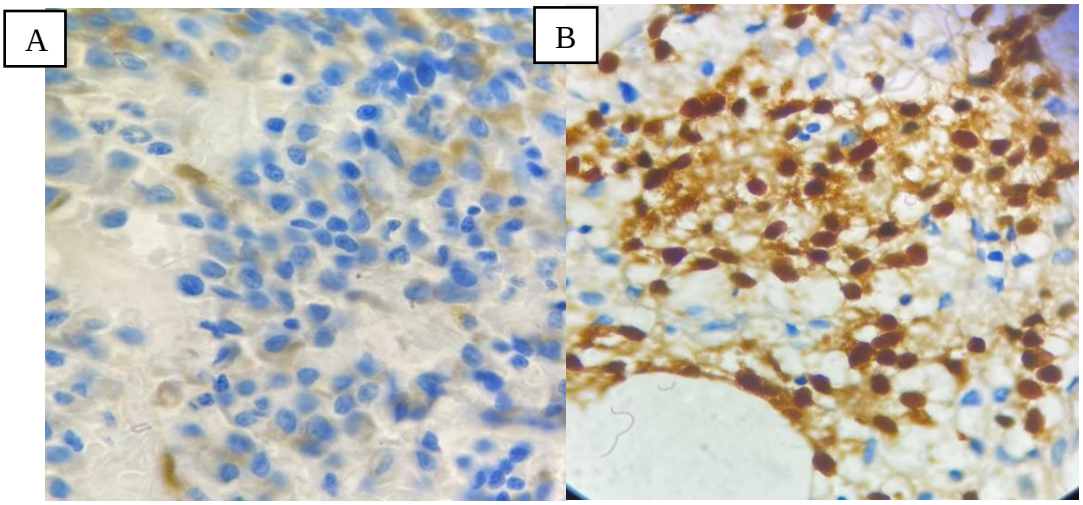

Figure 2: A: Shows CyclinD1 –ve on B.M.B by immunohistochemistery.
B: Shows Cyclin D1 +ve on B.M.B by immunohistochemistery .

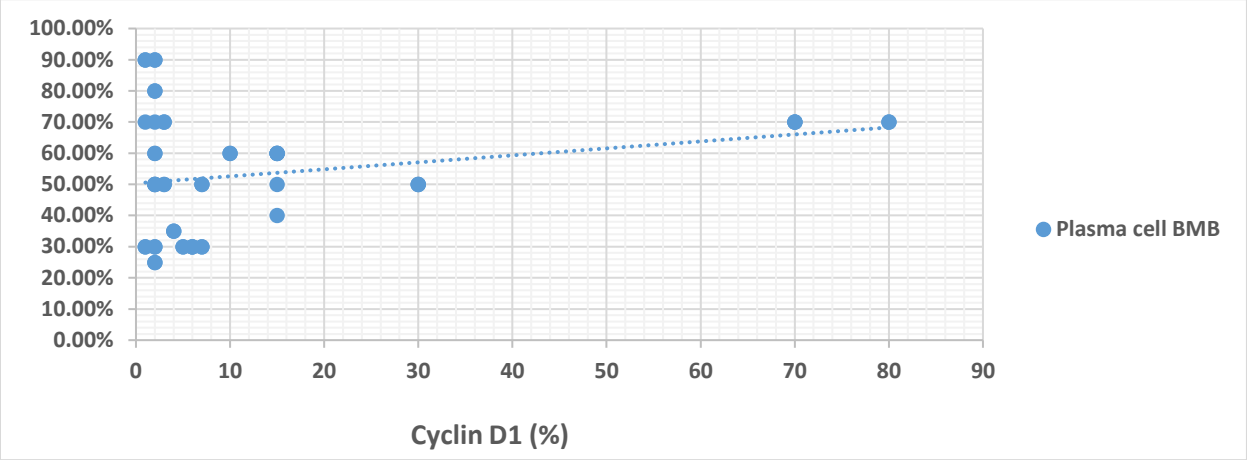

Figure 3: Correlation between Cyclin D1 value and BMB plasma cell % among the studied +ve cyclin 1D cases.

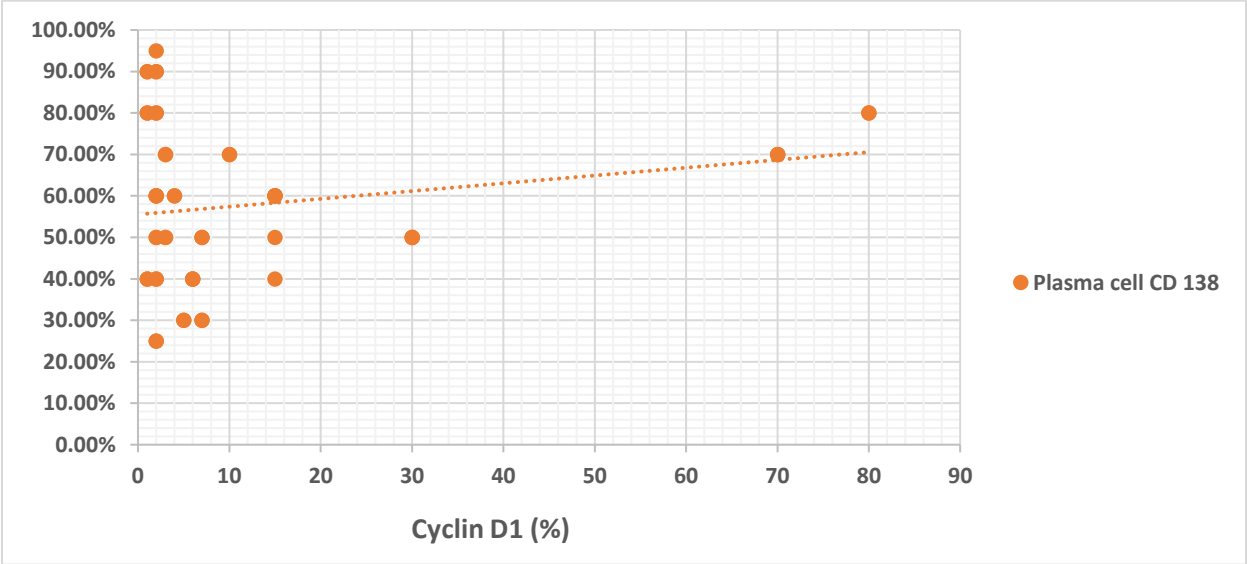

Figure 4: Correlation between Cyclin D1 value and plasma cell CD 138% among the studied +ve cyclin 1D cases.

Table 1: Relation between Cyclin D1 expression % & response to ttt among plasma cell myeloma patients :

Variable		Cyclin D1		KW	P
		Median	Range		
Response to ttt:	**CR** **PR** **PD**	**2** **4.5** **70**	**1-7** **1-15** **30-80**	**25.30**	**<0.001****

KW: Kruskal Wallis test ****: Highly significant (P<0.001)**
CR: Complete response
PR: Partial response
PD: Progressive disease

DISCUSSION

Clonal plasma cell (PC) cancer is known as multiple myeloma (plasma cell myeloma). PCs travel to the bone marrow (BM) after maturing in lymph nodes, where they undergo malignant alterations that lead to the development of the disease**[1].**

The term "cyclins" refers to a family of proteins that exhibit quantitative fluctuations throughout the cell cycle and cyclic expression.

Determining cyclin D1 expression in patients with plasma cell myeloma and comparing it to clinical information and therapy response were the goals of this study.

The majority of PCM patients (61.5%) were male, with a male to female ratio of 1.6:1, and their mean age ± SD was 57.71±9.69 years, according to the current study. Furthermore, **Pojero et al. [9]** discovered that the majority of PCM patients (56%) were male, with a male to female ratio of 1.2:1, and that the mean age of these patients was 60 years. However, in the research conducted by **Singh et al. [10]** the mean age of cases was 64.40 years with a male to female ratio 2.07:1

The percentage of Cyclin D1 expression and clinical variables (spleenomgally, hepatomegaly, and L.N. enlargement) in the current study do not statistically significantly correlate, and there is no statistically significant correlation between osteolytic bone lesions and Cyclin D1 expression.

These findings are in concordance with **Padhi et al. [7]** they discovered that there was no correlation between Cyclin D1 expression and any clinical factors, but **Nazarovs et al. [11]** discovered that in their investigation, there was a positive correlation between cyclin D1 expression and more osteolytic lesions.

In terms of laboratory variables, there was no statistically significant correlation between Cyclin D1 expression and TLC, Hb, platelets, TP, albumin, LDH, calcium, creatinine, and B2 microglobulin.

While **Padhi et al. [7]** claimed that the hemoglobin level was substantially lower in the cyclin D1 positive group (P value = 0.03). Our lab's findings may be comparable to those published by **Abobakr et al. [12]** who discovered that, in his investigation, there was no correlation between Cyclin D1 expression and laboratory variables, with the exception of serum B2microglobulin and TP, which are significantly elevated when Cyclin D1 expression is positive.

Cyclin D1 expression was substantially correlated with hypercalcemia (p=0.042), according to **Mengich et al. [13].** The percentages of BM plasma cells and CD138, which are expressed on BMB plasma cells, were connected with Cyclin D1 expression in the current investigation. Although the research published by **Padhi et al. [7]** they discovered that there was no correlation between the percentages of BM plasma cells and Cyclin D1 expression.

Regarding M protein, the current study found that 55.8% of the patients had IgG Kappa, 21.1% had IgG lambda, 13.5% had IgA Kappa, and 9.6% had IgA lambda. This was comparable to the study conducted by **Aita et al. [14].** They discovered that IgG kappa was more common in MM patients. Additionally, the research conducted by **Mengich et al. [13]** corresponds with our research, which found that IgG was the most prevalent immunoglobulin isotype (55.8%), followed by IgA (25.6%), and that kappa was the most common light chain involved (60.4%).

Our study's cutoff value for cyclin D1 expression to distinguish between positive and negative cases was ≥10, whereas the study conducted by **Padhi et al. [7].**

Cyclin D1 and its expression were examined in connection with treatment response in order to further highlight the prognostic significance and the role that Cyclin D1 plays in deciding outcome. Cyclin D1 expression percentage and treatment response were highly statistically significantly correlated (p<0.001).

For Cyclin D1 expression, the patients are split into 16 positive and 36 negative groups. Cyclin D1 is highly expressed in 16 individuals, 8 of whom have progressive disease (PD) and 8 of whom have partial response (PR). These patients had a substantially poor response to treatment and a poor prognosis (p<0.001).

Concurring this result with **Sewify et al. [5]** who claimed that a higher proportion of plasma cells in the BM and more aggressive osteolytic lesions in cyclin D1 positive patients are linked to cyclin D1

gene amplification, disease severity, and a poorer overall survival. Additionally, **Nazarovs et al. [11]** discovered that his patient's bad prognosis was linked to elevated cyclin D1 expression.

Jiang et al. [15] showed that cyclin D1 may deserve as a valuable prognostic biomarker for MM. MM patients overexpressing CCND1 treated with bortezomib or receiving ASCT are more likely to have better prognoses. Besides, the increased expression of CCND1 in relapsed and refractory MM patients probably be associated with survival benefits.

According to our research, individuals with MM who tested positive for cyclin D1 had a significantly higher chance of having a worse prognosis than those who tested negative for the protein, as evidenced by the percentage of plasma cells, CD138 expression on BMB, and therapy response.

Cyclin D1 is regarded as a good IHC marker and may be employed as one of the prognostic factors of MM patients since its detection by IHC is seen to be a practical approach, easily accessible, and less expensive than molecular techniques.

Conclusion:

For MM, cyclin D1 might be a useful prognostic biomarker. Cyclin D1 is a persistent immunohistochemical marker that is strongly expressed on plasma cells, according to the current study. Additionally, there was a highly significant positive association between Cyclin D1 expression, the percentage of plasma cells, and CD138 expression. This demonstrated that it can be utilized as a marker in addition to conventional markers and that it is a predictive sign of PCM.

Because there was a highly statistically significant correlation between Cyclin D1 expression and response to treatment, low expression of Cyclin D1 was linked to a significantly better response to treatment and a good prognosis, while high expression of Cyclin D1 was linked to a poor response to treatment and a bad prognosis. This suggests the significance of Cyclin D1 in evaluating PCM patients' response to treatment.

Recommendations:

In order to confirm the predictive impact of Cyclin D1 expression by examining its association with disease free survival (DFS) and overall survival (OS), a longer follow-up period and a greater number of patients are advised for PCM patients.

Conflict of Interest: None

Financial Disclosures: None

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Citation

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