Journal of Complementary Medicine Research, ISSN: 2146-8397 Vol. 14, No. 3, 2023 (pp. 77-80)

RESEARCH ARTICLE WWW.JOCMR.COM

Comparison Of Histomorphology, Angiogenesis and Proliferation in Kappa and Lambda Restricted Plasma Cell Myelomas with Its Effect on Prognosis

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ABSTRACT

Aims Of the Study: To study the type of light chain restriction and correlate it with the morphological parameters, proliferation index, angiogenesis and clinical parameters.

Materials And Methods: The study includes 46 cases of myeloma diagnosed over the period of five years. The histomorphological features like plasma cell morphology, percentage of plasma cells and pattern of infiltration were studied. Immunohistochemistry for kappa and lambda light chains was done. Angiogenesis was assessed by calculating the microvessel density (MVD) using anti CD34 immunohistochemistry. Proliferation was assessed immunohistochemically using Ki67 by comparing with anti CD38 which was used to highlight the plasma cells.

Results: The cases with kappa light chain restriction had significantly less of poorly differentiated morphology (7.4% vs 36.8%; p=0.02) and diffuse pattern of infiltration (22% vs 63%; p=0.01) compared to lambda. The kappa group also had lower MVD (9.9 vs 16.5; p=0.02) and proliferation index (4.7 vs 94; p=0.04) compared to the lambda.

Conclusion: Therefore, the type of light chain restriction is also a prognostic factor in plasma cell myeloma.

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How to cite this article: Priyadarshini S G, Basu D, Kar R, Dutta T.K. (2023), Comparison of Histomorphology, Angiogenesis and Proliferation in Kappa and Lambda Restricted Plasma Cell Myelomas with Its Effect on Prognosis. Journal of Complementary Medicine Research, Vol. 14, No. 3, 2023 (pp. 77-80)

KEYWORDS:
Comparison
Effect
Cases
Parameters

ARTICLE HISTORY:
Received: Jan 20, 2023
Accepted: Mar 24, 2023
Published: May 28, 2023

DOI:
10.5455/jcmr.2023.14.03.13

INTRODUCTION

Plasma cell myeloma is a neoplastic disorder of plasma cells. It has various prognostic factors like staging by Durie and salmon staging system and International staging system (ISS) (1)(2).There are many other factors which determine the prognosis in myeloma like age, performance status, serum ß2 microglobulin, serum creatinine, serum IL-6, S-CRP, S-HGF, serum albumin, physical functioning, cognitive functioning, clinical stage, plasma cell labelling index and plasma cell proliferation (3). The type of light chain restriction also has a role in prognosis of patients. Few studies have also shown that kappa myeloma has better prognosis compared to lambda, in terms of better response to treatment and overall survival (4). Currently there are no studies showing the difference in the angiogenesis and proliferation of kappa and lambda myelomas. This is study aims to bring out the difference between the two and its role in prognosticating myeloma patients.

MATERIALS AND METHODS

This study was done in a tertiary care teaching hospital in south India. Forty-eight cases of multiple myeloma diagnosed over period of six years were enrolled after approval by the hospital Ethics committee. Bone marrow aspiration and biopsy was performed in all the cases. The bone marrow biopsies were fixed in 10% neutral buffered formalin and decalcified using EDTA. Sections were stained with Hematoxylin and Eosin (H&E). The bone marrow aspirate slides were stained with Giemsa and Leishman stain.

The bone marrow aspirates and biopsies were studied to determine the differentiation of the neoplastic plasma cells and the pattern of infiltration in the marrow. The grading of plasma cells into well, intermediate and poorly differentiated (Fig I) groups was done based on Sailer et al criteria (7) using both the aspirates and biopsies. The 'well differentiated' group included cases with more than 90% mature plasma cell morphology. The 'poorly differentiated' group included the cases with more than 30% of plasmablasts and anaplastic morphology. The 'intermediate' group included cases which did not fall in either of the criteria. This included those having plasma cells with less eccentric nucleus, less cytoplasm and not so prominent perinuclear hof.

The pattern of neoplastic growth was studied in the bone marrow trephine biopsy. The 'interstitial' pattern showed infiltration of the plasma cells in an otherwise preserved marrow space. The 'nodular' pattern showed aggregate of more than 10 plasma cells in a preserved marrow space. The 'diffuse' pattern showed replacement of marrow space by plasma cells with loss of fat spaces (8).

Immunohistochemical staining with Kappa, Lambda, CD34, CD38 and Ki67 was done in all cases. After deparaffinisation and rehydration the sections were treated with 3% hydrogen peroxide. The antigen retieval was done in Decloaking chamber at a temperature of 125˚C for 40 seconds followed by 90˚C for 10 seconds. After washing with Tris buffer saline (TBS), the primary antibodies were added and incubated for 1 hour. The primary antibodies used were anti kappa, lambda, CD34 (EP88), CD38 (EP135) and Ki67 (MIB-1), all from Dako. After second wash with TBS, the polymer (Dako envision K5007) was added and incubated for 40 minutes. The sections were stained with the chromogen (DAB) and then counterstained with hematoxylin.

The proliferation index was calculated as the percentage of plasma cells which were stained with Ki67. In cases with interstitial or scattered plasma cells, IHC with CD38 was cross-checked to identify the plasma cells on a separate slide. For calculating the microvessel density 'Hot spots' were selected, after staining with anti CD34, by examining under 10x and defined as areas showing maximum vascularisation. Both the vessels as well as the individual cells stained with anti-CD34 were counted in 10 high power fields (HPF) under 40x objective with a 20 mm eyepiece diameter and the average per HPF was taken as the 'Micro vessel density' (MVD). The MVD was expressed as number of vessels per HPF. Thick-walled blood vessels were not counted.

The statistical analysis was done using GraphPad InStat (GraphPad InStat version 3.06 for Windows 7, GraphPad Software, San Diego California USA) and a p value less than 0.05 was taken as statistically significant. The hematological and biochemical parameters between the kappa and lambda restricted myelomas were compared using Fisher's Exact test. The histomorphological features like plasma cell morphology and pattern of infiltration were compared in the kappa and lambda group of myelomas using Chi-square test. The proliferation index and MVD were compared between the two groups using Mann Whitney test.

RESULTS

Kappa light chain restriction was seen in 27 cases (58.6%) and lambda in 19 cases (41.4%). The mean age of patients in the kappa and lambda groups were 52 and 53 years respectively. The male female ratio in the two groups were 1.7:1 and 2:1. The various haematological and biochemical parameters were compared between the two groups. No statistically significant difference was found between them (Table 1). The light chain restriction was compared to the plasma cell morphology and pattern of infiltration. The kappa light chain restriction was associated with less of poorly differentiated morphology (7.4% vs 36.8%) compared to lambda (p=0.02). The diffuse pattern was also less in the kappa group compared to the lambda group (22% vs 63%) (p=0.01) (Fig 1). Both these associations were statistically significant (Table 2). The mean Ki67 and MVD were also compared between the two groups. The mean Ki67 in the kappa group was 4.7 (SD 6.7; CI 2-7.4) compared to 9.4 (SD 11.5; CI 3.8-15) in the lambda group (p=0.04) (Table 3). The kappa group also had a lower mean MVD of 9.9 (SD 9.5; CI 6.2-13.7) in contrast 16.5 (SD 11.2; CI 10.9-22.1) in the lambda group (p=0.02) (Table 3). The type of light chain restriction was also compared with the clinical staging. In the kappa group 4 cases (14.8%) were in stage I, 5 cases (18.5%) in stage II and 18 cases (66.7%) in stage III. In the lambda group there were no cases (0%) in stage I, 7 cases (36.8%) in stage II and 12 cases (63.2%) in stage III (p=0.1). Out of the 46 cases 15 cases had follow up. 2 patients succumbed to the illness and both had lambda light chain restriction.

DISCUSSION

The difference in the prognosis of kappa and lambda restricted myelomas has been previously documented but has been imputed to various factors. Cornell et al had also studied a difference in the treatment response between the kappa and lambda excretors. The treatment response rate was found to higher in the kappa excretors compared to lambda. The reason for this difference could not be satisfactorily explained. They had attributed it to the difference in the physical and chemical nature of the kappa and lambda light chains (4).

Shustik et al studied the difference in the survival and clinical manifestations of kappa and lambda light chain diseases. A significantly longer survival was found in cases with kappa restriction compared to lambda and difference in the clinical outcome could not be attributed to any of the other prognostic features like anemia or azotemia (5).

Drayson et al also observed a better survival in IgG/IgA kappa light chain type compared to the IgG/IgA lambda light chain. The median survival was 2.4 and 2 years in the kappa and lambda group respectively. This was attributed to higher free light chain excretion in the lambda group (6).

During the B cell development from the progenitors to pre-B cells heavy chain rearrangement occurs, resulting in expression

of μ heavy chain. This μ chain joins with the surrogate light chain to form pre-B cell receptor (pBCR). As the pre-B cells develop into immature B cells light chain rearrangement occurs, leading to the formation of membrane form immunoglobin (mIgM). The light chain rearrangement occurs first in the kappa locus and if it fails then there will be subsequent rearrangement of the lambda locus. The immunoglobulin gene rearrangement is mediated by VDJ recombinase which remains active till the immature B cell stage and later becomes reactivated again in the germinal centre during the receptor editing process. VDJ recombinase mediated chromosomal aberrations have been implicated during these stages. Studies have shown that these aberrations involving the immunoglobulin locus takes place during its normal physiological process of gene rearrangement. As the mature B cells migrate to the germinal centre they undergo antigenic selection and activation resulting in somatic hypermutation and IgH switching. The switching is mediated by switch recombinase and similar to VDJ recombinase they also contribute to chromosomal aberrations (7).

In the molecular pathogenesis of myeloma 'Switch translocations' involving the switch region of heavy chain gene locus have been reported in 50-75% of cases, as an early oncogenic event. These could be the consequence of the aberrations in the normal physiologic process of 'Isotype switch recombination'. Some of the translocations involving the J region of heavy chain have also been described in a few cases of myeloma. Both the translocation place an oncogene next to the enhancer in the heavy chain gene resulting in overexpression. Chromosomal aberrations involving the heavy chain gene locus has been widely studied in myeloma, unlike those involving the light chain gene locus (8).

Turkmen et al studied the light chain gene aberrations in myeloma patients and identified them in 27% of cases. The aberrations include gene rearrangement, deletion and gain of either or the light chain genes (9).

Barwick et al studied the translocations involving the lambda gene and its effects on prognosis. They had concluded that patients with lambda gene translocations are associated with poor overall survival and progression free survival. The results also showed that these patients did not benefit from conventional therapy involving immunomodulatory agents (10).

In the present study kappa light chain restriction was significantly less associated with other markers which has been shown to have prognostic significance in a previous study done by us (11). There was a significant difference in the proliferation and angiogenesis between the kappa and lambda restricted myeloma groups. This suggests an innate difference in the properties of the kappa and lambda restricted myeloma cells. Though numerous studies are being done in understanding the genetic basis of myeloma, further studies are needed in understanding the effects of light chain gene rearrangements on the pathogenesis and progression of myeloma.

CONCLUSION

Hence light chain restriction pattern may have a prognostic significance in plasma cell myeloma.

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Table 1: Comparison between the kappa and lambda group

Parameters	kappa	lambda	P value
Hypercalcemia	13.6	20	0.6
Serum creatinine >1.2mg/dl	54.5	61.6	0.7
Hemogloin<10 gm/dl	70.3	89.4	0.1
Serum albumin <3.5 gm/dl	63.1	37.5	0.4
Bone Lytic lesion	24	42	0.3

Table 2: Comparison of the type light chain restriction with the plasma cell morphology and pattern of infiltration

Monoclonality	Plasma cell morphology			
	Well differentiated (n=30)	Intermediate (n=7)	Poorly differentiated (n=9)	P=0.02
Kappa	19 (70.4%)	6 (22.2%)	2 (7.4%)	
Lambda	11 (57.9%)	1 (5.3%)	7 (36.8%)	
	Pattern of infiltration			
	Interstitial (n=18)	Nodular (n=10)	Diffuse (n=18)	P=0.01
Kappa	14 (51.9%)	7 (25.9%)	6 (22.2%)	
Lambda	4 (21.1%)	3 (15.8%)	12 (63.1%)	

Table 3: Comparison of the type light chain restriction with the mean Ki67% and MVD

Parameter	Kappa	Lambda	P value
Mean Ki67	4.7	9.4	0.04
Mean MVD	9.9	16.5	0.02