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Clinicopathological and Immunohistochemical Evaluation of Mantle Cell Lymphoma Variants

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Abstract

Introduction: Mantle cell lymphoma (MCL) is an intermediate grade lymphoma characterized by reciprocal translocation *t*(11;14)(q13;q32) which results in cyclin D1 overexpression. Several variant forms of MCL are recognized, namely, classical, centrocytic, blastoid & pleomorphic type. The former two are considered non aggressive & the later two show aggressive behaviour. The purpose of the study was to study and compare the clinicopathological and immunohistochemical (IHC) features of MCL and its variants.

Materials and Methods: All cases diagnosed as MCL in our institution over a period of 4½ years were included in this study. Histoppathology was reviewed and a panel of IHC comprising CD3, CD5, CD10, CD20, CD23, cyclin D1, Bcl2, Ki67 and TDT was done on all cases.

Results: 15 cases of MCL were identified: 2 small cell, 4 centrocytic, seven blastoid & two pleomorphic, variant. They were grouped into non-aggressive (classical and small cell variant) and aggressive (blastoid and pleomorphic variant) groups. The aggressive group had a higher mitotic rate, Ki67 proliferative index and poor prognosis. All cases show strong positivity for cyclin D1 & CD20 and variable expression of CD5 & CD10 with complete negativity for CD23 & TDT.

Conclusions: Our study highlights that MCL can have different histological appearances which can lead to diagnostic confusion with various other lymphomas. Since aberrant IHC expressions are also frequent, a cautious approach using a panel of IHC markers is essential for a correct diagnosis. Blastoid and pleomorphic subtypes may strongly express Ki67 and have a poor outcome.

Key words: Mantle cell lymphoma, Centrocytic, Blastoid, Pleomorphic, Cyclin D1, Ki67

1. Introduction

Mantle cell lymphoma (MCL) is an intermediate grade B-cell lymphoma and comprises 2-10% of all non-Hodgkin lymphomas (NHL). It is characterized by reciprocal translocation *t*(11;14)(q13;q32) between the *CCND1* and the immunoglobulin heavy chain (*IgH*) genes which results in cyclin D1 overexpression.[1]

MCL has several architectural and cytologic patterns that differ in their biologic behavior. .[2] Two main variants are recognized- typical, or classic, and blastoid, or blastic when assessed cytologically. [3] The typical MCL variant is composed of small to medium-sized lymphocytes with scanty cytoplasm, irregular nuclei, and condensed chromatin. [4] In a minority of cases, the atypical lymphocytes may have round nuclei with little atypia, mimicking chronic lymphocytic lymphoma, or abundant pale cytoplasm, mimicking marginal zone lymphoma. [5] The blastoid MCL variant has two subgroups are recognized-classic blastoid and pleomorphic blastoid. The classic blastoid variant is characterized by medium-sized lymphocytes with scanty cytoplasm and round nuclei with finely dispersed chromatin and high mitotic index, resembling lymphoblasts. The pleomorphic blastoid variant is composed of heterogeneous large cells with irregular cleaved nuclei, finely dispersed chromatin, and small distinct nucleoli.[6] Architecturally, three different patterns are recognized in nodes involved by MCL: mantle zone, nodular, or diffuse. The mantle zone pattern (3%-26% of cases) resembles a normal node with expansion of the mantle zone with malignant cells. This pattern is considered a low-grade subtype of MCL. The nodular pattern (13%-39% of cases) has ill-defined follicle-like nodules with neoplastic cells blending with the non neoplastic cells, and germinal centers are absent. The diffuse pattern (28%-78% of cases) is composed of small neoplastic cells replacing the node, with loss of normal architecture and absent follicles. The blastoid variant (up to 39%of cases) often causes a diffuse pattern.[7] Clinically, it is important to recognize mantle-zone-pattern MCL and blastoid MCL.The incidence of these subtypes of MCL has varied with different reports. Histologic progression between the different patterns is uncommon, although rare progression from typical MCL to the blastoid variant has been documented. [8] Bone marrow involvement is present in more than 50% of patients with MCL and may be nodular, diffuse, paratrabecular, or a combination of these patterns. [9] Neoplastic cells in MCL are related to the mature B-mantle cells of the follicular lymphoid cuff. These are monoclonal B cells expressing the B cell markers CD19, CD20, CD22, CD79a, and intense surface immunoglobulin (Ig)M ± IgD with a tendency to express more lambda light chain than kappa light chain. In addition, the neoplastic cells express CD5,CD43, Bcl-2, and cyclin D1, and lack CD10, Bcl-6, and CD23 antigens useful in differentiating from follicular lymphoma and chronic lymphocytic leukemia.[10] 10 The t(11;14)(q13;q32) is a characteristic alteration in MCL. In this translocation, the heavy-chain joining region in chromosome 14 is juxtaposed to the Bcl-1 region on 11q13.

The CCND1 gene encoding for cyclin D1 is positioned in t(11;14) chromosomal translocation adjacent to the enhancer region of the immunoglobulin heavy-chain gene, resulting in upregulation of the CCND1 gene (Bcl-1/PRAD-1) and overexpression of cyclin D1 protein.[11] Cyclin D1 protein expression is universal in MCL and can be detected by immunohistochemical staining, polymerase chain reaction (PCR) analysis, or flow cytometry.[12] Translocation (11;14) is detected in 65% of MCL cases by classic cytogenetic analysis, and in nearly all cases, by fluorescent in situ hybridization (FISH).[13] Other chromosomal changes, particularly in blastoid variants of MCL, include gains in chromosomes 3q, 8q, and 12q, and losses in chromosomes 1p, 9p, 11q, and 13q.[14] Altered apoptosis pathways with down regulation of the apoptotic genes FADD, PDCD1, and PAIDD have been detected by oligonucleotide microarray in a few MCL patients. [15] Mutations in p53 and over expression of p53 protein occur in blastoid MCL.The frequency of chromosomal imbalances and DNA amplifications are higher in blastoid MCL than in the common variant.[15] [17] Cyclin/cyclin-dependent kinase (CDK) complexes play an essential role in regulation of cell-cycle progression through various cell-cycle checkpoints (cell-cycle-positive regulators). [16] In MCL, cyclin D1 binds to CDK4 and forms cyclin/CDK complex, which binds to the retinoblastoma protein, leading to its phosphorylation and the loss of its suppressor activity on cell-cycle progression through the release of transcription factors E2F that promote cell-cycle progression into the S phase. [17] Mutations in p53 and inactivation of CDK inhibitors (p16, p18, p21, p27)-both negative regulators of the cell cycle-have been reported mostly in the aggressive variants of MCL. Proteosome activity might be responsible for the degradation of some of these CDK inhibitors. [18]

2. SUBJECTS & METHODS

2.1. SAMPLE COLLECTION

All cases diagnosed as MCL over a period of 3 years between 2014 until June 2017 in the Department of Pathology were included in this study.Out of total of 710 patients with B- NHL, 15 cases of mantle cell morphology were enrolled in this retrospective study. Also, patients refereed from private hospitals were included. The inclusion criteria were histo-pathologic diagnosis of B-NHL and the availability of clinical sheet details, laboratory investigations, imaging studies and paraffin-embedded tumor tissues for all cases. Excluded cases from the study were included minute biopsy specimens, tissues with extensive necrosis, cases with plasmacytoid differentiation. Routinely stained hematoxylin and eosin (H&E) slides were reviewed. Also special , Periodic Acid-Schiff, Giemsa, and silver impregnation by Gomori in some selected cases of lymphomas. Histopathologic subtyping & grading was performed based on the criteria described in the World Health Organization (WHO,2008), working formulation and Kiel classification with special attention to various histological features such as the pattern of infiltration (diffuse, vaguely nodular or mantle zone pattern), the size of cells & nuclei and the mitotic index (per 20 high-power field[HPF]).

2.2. IMMUNOHISTOCHEMISTRY

2.2.A- PROCEDURE AND ANTIBODIES

Serial 3-µm sections were cut from the paraffin block, mounted on positively charged slides and dried overnight in a 60°C oven. Sections were then de-paraffinized in xylene for 24 h and hydrated in a descending grades of alcohol; 100%, 90%, 85% and 70%. Antigen unmasking was performed by heat induced an epitope retrieval method by placing the slide in a plastic Coplin jar filled with citric acid buffer so that the solution covers the slides, then placing the jar in a microwave at 800Watt for 20 min (divided into 4 cycles 5 min each). The Coplin jar was then removed from the oven and allowed to cool for 15 min. Slides were placed in a humidified chamber and rinsed three times in phosphate buffer saline (PBS). Endogenous peroxidase activity was blocked by incubation of the tissue section with 3% hydrogen peroxide in water for 30 min. After washing, the tissue sections were then incubated with the primary monoclonal antibody, cyclin D1 (BLC-1 Dako CD3 (PS1, Biogenex), CD5 (4C7, Novocastra), CD10 (56C6, Biogenex), CD20 (L-26, Novocastra), CD23 (1B12, Novocastra), cyclin D1 (ERP- 224-32, Biogenex), Bcl6 (LN22, Novocastra), Bcl2 (124, Dako), Ki67 (MM1, Novocastra), c-myc (9E10, Biogenex), and p53 (DO-7, Dako). The blastoid and pleomorphic variants were also stained for TdT (Sen28, Novocastra). All the primary antibodies were pre-diluted and ready-to-use. The detection kit used was "NovoLink polymer" from Leica Biosystems, Newcastle upon Tyne, United Kingdom. Corporation at dilution 1:50) & CD 5,monoclonal mouse anti-CD23 antibody (clone 1B12) (0.1ml supernatant)(Neo Markers, Westinghouse).Immunohistochemistry was performed using an avidin-biotin-peroxidase system with DAB used as a chromogen and Mayer's haematoxlin applied as a light counterstain. The sections were cover-slipped by DPX mount media and examined & images were captured by the OLYMPUS CX21 Motorized System Microscope (Olympus Corporation, Tokyo, Japan). All cases were stained with CD20 to confirm B cell origin of non Hodgkin lymphomas.[19]

2.2.B- QUALITY CONTROL OF IHC

Appropriate negative controls for the immunostaining, consisting of histological sections of each case processed without the addition of primary antibody were prepared for each antigen, along with a positive control sections prepared with each IHC run then staining results were evaluated[20].

2.3. SCORING OF IHC

IHC evaluation was conducted, scored and estimated. The evaluation of immunostaining for Cyclin D1, CD5 and CD23 was scored for the percentage of immunopositive tumor cells. Cyclin D1 over expression was defined as positive in the nuclei of lymphoma cells with or without simultaneous weak staining of cytoplasm. Endothelial cells and histiocytes were used as internal positive control. Negative (-)=<10% of cells positive, Regional (+)=10-50% of cells positive, Diffuse (++) >50% of cells positive [21] . According to Watson et al[22] CD23 & CD5 positivity was identified by significant labeling of neoplastic cells (in the form of membranous reaction) in any area of the section. If positivity was only restricted to the dendritic reticulum cell network, the case was considered negative. Negative (-)=No individual lymphoid cells positive, Regional (+) =≤50% of cells positive, Diffuse (++) >50% of cells positive. CD20 positivity was identified by significant labeling of neoplastic cells in the form of membranous reaction) in any area of the section.

2.4. STATISTICAL ANALYSIS

The Statistical Package for Social Sciences (SPSS) for windows (version 12) computer program was used for statistical analysis. Comparison between positive cases was calculated by Chi square test. P values ≤0.05 and ≤0.01 were considered significant and highly significant, respectively, in all analyses. T-test to compare mean and SD between two groups.

3. Results

Table 1: Comparative clinical features, laboratory parameters and Ann Arbor staging of non-aggressive (Group A) and aggressive (Group B) subgroups of Mantle cell lymphoma

Parameters	Group A Non aggressive (n=6)	Group B Aggressive (n=9)
Age (year)	38-72 Median:65	42-75 Median:62
Sex	M=5 F=1	M=7 F=2
Generalized LN	3	8
Extra nodal involvement	2	5
Hepatomegaly	4	6
Splenomegaly	2	4
Complete blood spill	2	6
Bone marrow	3	6
Lactate dehydrogenaze LDH (U/L)	Meam:530	Mean:820
Stage	IV 5 - III:1	IV:8 - III:1

15 cases (2.1%) of MCL were identified out of a total of 710 cases diagnosed as lymphoma. For comparative study, classical and small cell types were grouped into non-aggressive category (Group A), while blastoid and pleomorphic types were grouped as aggressive (Group B). The age range of patients was 38-75 years & the median of 65 years & 62 for non aggressive & aggressive groups. 12 out of 15 patients were male. Generalized lymphadenopathy (LN) (11/15), hepatomegaly (10/15), splenomegaly (6/15), bone marrow (BM) involvement (9/15), peripheral blood (PB) spill (8/15), and extra nodal involvement (7/15) were frequent findings. About 13 cases had stage IV and two had stage III disease. The mean serum lactate dehydrogenase (LDH) level was 530 & 820 U/L foe non aggressive & aggressive groups respectively.

Table 2: Comparative histopathological features and Ki67 proliferative index of non-aggressive (Group A) and aggressive (Group B) subgroups of Mantle cell lymphoma

Parameters	Group A Non aggressive (n=6)	Group B Aggressive (n=9)
Pattern	Nodular +diffuse 5 Diffuse 1	Diffuse 9
Mitoses/hpf	Range: 2-12 Mean: 8	Range: 20- 30 Mean: 28
Cell morphology	Small=2 Centrocytic=4	Blastoid=7 Pleomorphic=2
Ki 67%	Range: 8-20 Mean: 15	Range: 50-80 Mean: 65

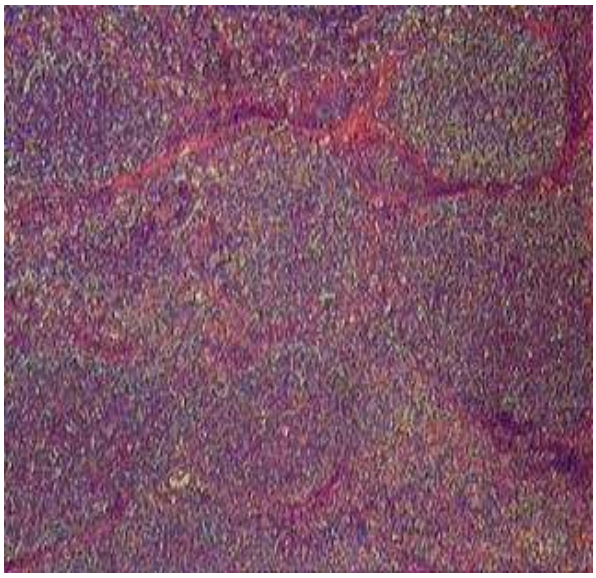
Table 2 shows 4 had centocytic morphology (case no. 1 to 4), 2 had small cell type (case no. 5 & 6) seven were blastoid (case no. 7-13) and 2 cases each were pleomorphic (case no. 14 & 15). The histopathological features of all the MCL cases. Growth pattern in the lymph nodes 8 cases had diffuse and nodular pattern in 5 cases and diffuse pattern in 10 cases. The neoplastic lymphoid cells in classical type showed centrocyte-like morphology with round indented nuclei, coarse chromatin, and inconspicuous nucleoli in 4 cases. The small cell variant showed cells with small, condensed, hyperchromatic nuclei, morphologically resembling small lymphocytic lymphoma (SLL) in 2 cases. The blastoid variants showed lymphoblast-like morphology with slightly enlarged, rounded nuclei having fine chromatin and small nucleoli. The nuclei were more enlarged and pleomorphic in the pleomorphic subtype in 7 cases. Tumor cell proliferation as assessed by mean mitotic activity was 2/12 high power field (hpf) for Group A and 20/30 hpf for Group B. The median Ki67 proliferative fraction was 15% for Group A and 65% for Group B.

Table 3: Immunohistochemical profile of 12 cases of mantle cell lymphoma

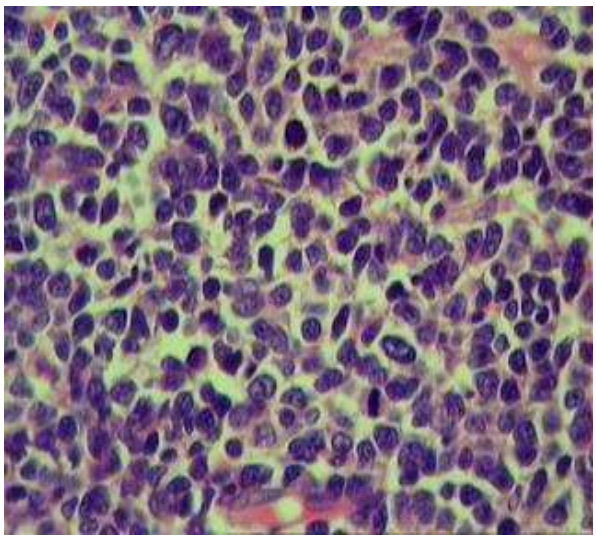
Case	Parameters	CD20	CD3	CD5	CD10	CD23	Cyclin D1	Bcl2	TDT
1	Classical	+	-	+	-	-	+	+	ND
2	Classical	+	-	+	-	-	+	+	ND
3	Classical	+	-	+	-	-	+	+	ND
4	Classical	+	-	+	-	-	+	+	ND
5	Small	+	-	-	+	-	+	+	ND
6	Small	+	-	-	+	-	+	+	-
7	Blastoid	+	-	+	-	-	+	+	-
8	Blastoid	+	-	+	-	-	+	+	-
9	Blastoid	+	-	-	+	-	+	+	-
10	Blastoid	+	-	+	-	-	+	+	-
11	Blastoid	+	-	+	-	-	+	+	-
12	Blastoid	+	-	+	-	-	+	+	-
13	Blastoid	+	-	+	-	-	+	+	-
14	Pleomorphic	+	+	+	-	-	+	+	-
15	Pleomorphic	+	-	-	-	-	+	+	-

All cases were positive for CD20, cyclin D1 and bcl2. 14 cases were positive for CD5 . The intensity of cyclin D1 nuclear staining was heterogeneous, being weaker in the blastoid cells as compared to the centrocytic. Aberrant phenotypes such as CD5 negative in (4/15), CD10 positive in (3/15) and CD3 positive in (1/15) of cases . The case with small cell histology was CD5 negative and strongly positive for CD10.

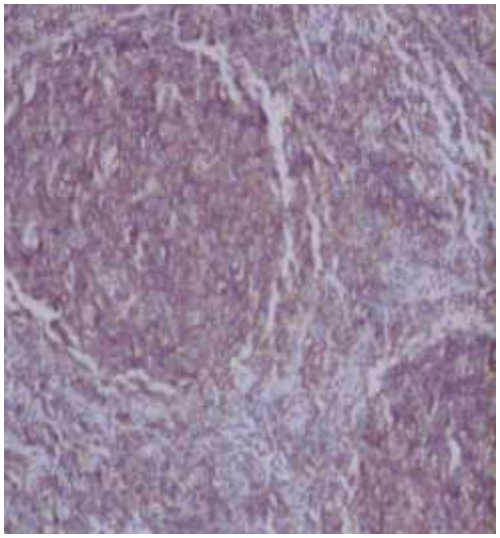
Figures:



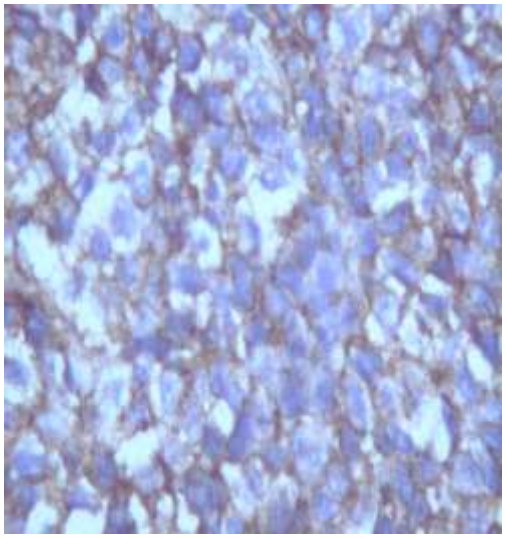
Photomicrograph (1) : Mantle cell lymphoma shows diffuse and nodular areas throughout the lymph node. (H & E X 40)



Photomicrograph (2) : Blastoid variant of Mantle cell lymphoma shows medium-sized lymphocytes with scanty cytoplasm and round nuclei with finely dispersed chromatin and scattered mitotic figures (H & E X 400)



Photomicrograph (3) : Nodular variant of Mantle cell lymphoma shows nuclear staining for cyclin D1 (immunoperoxidase staining, hematoxylin counterstain X 100)



Photomicrograph (4) : Pleomorphic variant of Mantle cell lymphoma shows membranous CD5 staining (immunoperoxidase staining, hematoxylin counterstain X 400)

4. Discussion

Mantle cell lymphoma is a type of B-cell NHL with distinctive morphologic and immunophenotypic features and a characteristic cytogenetic abnormality, the t(11;14)(q13;q32). [23] Tumor cells express BCL-1 also called as cyclin D1 and CCND1. Approximately 70% and 50% of the MCL at the cytogenetic level and genomic level, respectively, showed a specific chromosomal rearrangement, t(11;14) (q13;32), involving the BCL-1 locus on chromosome 11q13 and the immunoglobulin heavy chain gene complex on chromosome 14q32. [24] This genetic event is thought to have an important role in pathogenesis of MCL, because overexpression of cyclin D1 protein is thought to lead to deregulation of normal cell cycle, particularly at the G1-S phase transition. The histopathology of MCL is well defined in western literature[25] but there is little data available middle east . Distribution of NHL subtypes in middle east is different with those from the rest of the world. MCL is less common in middle east when compared with Europe and the USA. [26] Previous published reports from India have stated incidence of MCL to be 3.4% and 2%.[27& 28] Our incidence of MCL (2.1%) is low in comparison to published incidence of MCL (2-10%).[29&30] MCL constituted 2.1% (15 cases) from a total of 710 cases of NHL. Clinical characteristics in our series were similar to those reported elsewhere. [31] With regards to gender distribution, our results confirm that the incidence in men is higher than in woman in compatible with previous report. [32] MCL presented in stage 3 or 4, in agreement with previous report. LDH levels was higher in aggressive MCL compared to non aggressive morphological patterns, a finding similar to as described by others. [33] Bone marrow biopsy involvement in our 15 cases of MCL was 33%, & 67% in aggressive & non aggressive MCL respectively. Incidence and patterns of BM involvement is similar to as described elsewhere. [34] Hepatomegally & splenomegally was seen in ten & six cases (66.7% & 40%) out of 15 cases respectively which were similar to those reported elsewhere. [35&36] MCL has been morphologically sub-classified according to patterns (nodular, diffuse and mantle zone) and cytology (lymphocytic and blastic). [38] Clinical significance of these features is unclear. Common patterns were diffuse (10 cases, 66.7%), nodular & diffuse (5 cases, 33.3%). Similar findings have been described in previous studies. [39] However, another reports showing a high incidence of 55% of mantle zone pattern of MCL. [40] Diffuse pattern and the lymphocytic cytology was commonest subtype in our series, as seen elsewhere. [41] Clinicopathological presentation differ between patterns and cytological variants of MCL. Diffuse pattern of MCL was associated with poorer prognosis as also seen by others. [42] MCL with nodular & diffuse pattern in our series had a better prognosis as reported elsewhere. [43&44] There are other studies showing no relation with various morphological patterns. [45] All blastoid & pleomorphic MCL had a diffuse pattern of involvement as described elsewhere. [46] CD23 was a useful stain to highlight follicular dendritic cells and hence the follicles. Pink histiocytes and hyalinized blood vessels were seen in 53 (77%) and 58 (85%) cases, respectively, in concordance with as reported previously. [47] [42] Mitosis was more prominent in the blastic variants as reported previously. There are a few reports available, which examine the prognostic importance of cytology and patterns in MCL. Blastic MCL and pleomorphic MCL are reported to have very aggressive disease, while small & lymphocytic MCL have comparatively better prognosis. [48] Common differentials on morphology were SLL, FL, MZL, DLBCL and LL. IHC was required in all cases for a definitive diagnosis and showed a classical phenotype (CD20 and cyclin D1) in all cases & CD5 in 13 case(86.7%) may be due to because of poor tissue fixation[49] Cyclin D1 was expressed (nuclear stain) in all cases. All cases were negative for CD23, thus differentiating it from SLL and FL. where 3 cases were CD5-negative. Tdt was not expressed or undetermined in any of the blastic variants[50] More objective criteria such as high Ki67 proliferation index lead to more aggressive outcome. [50] Blastoid MCL constituted only 46.7% in

our series, while others have reported it to be 17%. [51] This may be due to lack of standardized criteria to diagnose this entity. Morphology & mitotic count were thus helpful in diagnosing blastoid variants of MCL. Blastoid MCL had poorer outcome in agreement with previous reports, but the number of cases was low to be statistically significant. [52]

5. Conclusion

Because of varying cytoarchitectural and morphological appearances MCL can be confused with various other lymphomas. Though cyclin D1 is a reliable marker for all forms of MCL, aberrant IHC expressions are also common. Hence, a cautious approach with a panel of IHC markers is essentially recommended for an accurate diagnosis of MCL.In cases with inconclusive IHC, molecular testing becomes necessary for confirmation of diagnosis. Strong p53 and c-myc expression by IHC along with higher Ki67 proliferative indices may be seen in the aggressive subtypes and might contribute toward poor prognosis

6. References

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