

Mantle Cell Lymphoma: A Clinicopathologic View

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ABSTRACT

Mantle cell lymphoma (MCL) is a recently recognized subtype of non-Hodgkin's lymphoma (NHL) by the REAL classification. It is characterized by chromosomal translocation t (11;14) (q13;32) resulting in overexpression of Bcl-1 (cyclin D1), the cell regulator protein. Clinically, the disease presents as a generalized form with frequent treatment failures and poor prognosis. MCL has not been documented as a specific disease entity in Egyptian material prior to its precise identification.

Owing to its important clinico-pathologic implications, retrospective analysis of 712 cases of NHL treated at the NCI during the years 1990-1995 was performed. Re-classification of 63 MCL cases (8.8%) was established with a male: female ratio of 3:1 and a median age of 55 years (range 16-65 years). Histologic variants were predominantly diffuse, mantle zone or vaguely nodular. The cytologic variants were small, pleomorphic or blastoid. Molecular and immunohistochemical markers of this group showed the following profile: Bcl-1 +ve in 89% of cases, multidrug resistance (MDR) p-glycoprotein +ve in half of the cases, and p53 occasionally +ve in the blastoid variant.

Response to anthracyclin-based chemotherapy was evaluated in 20 out of the 63 cases. Complete remission was achieved in 15 patients (75%), with a median disease-free survival time of 12 + months. These data confirm that MCL represents a distinct clinicopathologic entity and urge for including the REAL classification in the diagnosis of malignant lymphoma.

Key words: Mantle cell lymphoma - Cyclin D1 - P53 - MDR-1.

INTRODUCTION

Mantle cell lymphoma (MCL) is a newly recognized subtype of non Hodgkin Lymphoma (NHL) [1]. MCLs have not previously been identified in some lymphoma classifications. In some other classifications, they have been given different names. The Kiel classification distinguished these lymphomas as centrocytic lymphomas, whereas the term "intermediately differentiated lymphoma" has been used in the United States. The term "mantle zone lymphoma" was introduced because of the peculiar growth pattern in some cases. The International

Working Formulation (WF), which has been used extensively in the United States and served as a common language to translate between six different classification schemes, failed to recognize MCL as a distinct entity. In the WF, MCL is classified as diffuse small cleaved most frequently, but also as follicular, small cleaved cell type, small lymphocytic lymphoma, diffuse large cell type and lymphoblastic lymphoma [10]. To obtain consistency between these different classification systems, the term "mantle cell lymphoma" has been proposed [22]. R.E.A.L. classification [12] included MCL as a distinct entity and provided new important features showing an eight-year survival of 18% and a poor prognosis (five-year survival <30%) [26].

Subclassification of lymphoma is important since optimal treatment regimes differ between subtypes [29]. A lymphoma classification becomes simply a list of well defined "real" disease entities many of which are associated with distinctive clinical presentation and natural history [26].

The new clinicopathologic entity (MCL) represents 5-6% of nodal lymphomas and is a tumour derived from CD5+ virgin B-cells of the follicular mantle zone. It is characterized by a genetic marker, the chromosomal translocation t (11;14) (q13; q32), which results in overexpression of cyclin D₁. It has been progressively identified in recent years, through the demonstration of its unique, morphologic, immunophenotypic, cytogenetic and molecular features [9].

Histologically MCL is characterized by neoplastic expansion of the mantle zone, surrounding lymph node germinal centers, with a homogeneous population [10]. Three histologic patterns are observed: diffuse, nodular and mantle zone. A markedly follicular pattern is

rare, as well as the mantle zone pattern [6]. Occasional presence of nodal germinal centers and hyaline deposits around capillaries may be noticed [22].

Three different cytology variants are noticed: classic, blastoid and pleomorphic [24]. Classic cases show a uniform population of intermediate to medium sized tumour cells, irregularly shaped or indented nuclei, with moderately coarse chromatin pattern, few small nucleoli and scanty cytoplasm [22]. The blastoid variant shows tumour cells resembling lymphoblasts with finely dispersed chromatin, displaying high proliferative and apoptotic rates and a frequent starry sky pattern. Development of the MCL-blastoid variant may represent transformations of B-cells at different stages of ontogeny (pregerminal-germinal and post-germinal center) B-cell origin [17].

The pleomorphic variant shows medium to large-sized cells with predominantly cleaved nuclei resembling large centrocytes. They were classified as centrocytoid centroblastic lymphomas in the Kiel classification and need immunophenotyping cytogenetic or molecular studies [30].

As the morphologic diagnosis of MCL remains very difficult for the most experienced haematopathologist, immunophenotyping and molecular genetics have proven to be of tremendous value in corroborating the diagnosis [10]. These techniques proved, beyond doubt, that MCL is a distinct entity. Malignant lymphoid cells of MCL characteristically express monotype surface IgM and sIgD pan B-cell antigens (CD19, CD20, CD22) and the pan T cell antigen CD5 [7]. They rarely express CD10 or CD23. Immunophenotype is sIgM +, usually sIgD+ [3], λ >K, pan B marker+, CD5+, CD10-, CD23-, usually CD43+, CD11c-, bcl 2 protein+ [10]. Biology and growth enhancement of MCL B lymphocytes is affected by CD154 (CD 40 ligand) and IL-4, two signals provided by CD4+ T-cells. These specific immunologic stimuli have the potential of the possibility of their exploitation to confer susceptibility to chemotherapy agents and to develop novel therapies in this disease [3].

MCL is characterized by the chromosomal translocation t (11; 14) (q 13; q32) [9]. Its molecular counterpart, BCL-1 gene rearrangement, juxtaposes the cyclin D₁ gene (11q13) to the IgH heavy chain locus (14q 32). As a consequence, cyclin D₁ gene comes under the transcriptional control of the Ig enhancer E_μ and is constitutively expressed in B-cell lymphomas

bearing this translocation (MCL). The breakpoints in the BCL-1 locus are not all tightly clustered, although 20-40% of cases of MCL have breaks in the major translocation cluster (MTC region). All of these break points leave the cyclin D₁ coding region structurally intact and result in its transcriptional activation and increased protein expression. Thus, rearrangement of BCL gene resulting in increased expression of cyclin D₁ appears to be a specific and important step in the definition of this group of lymphomas (MCL) [22].

MCL is characterized by complex karyotypes and secondary aberrations. It shares similarities with B-cell chronic lymphocytic leukemia [2]. Mutations with overexpression of p53 have been demonstrated in a minority of MCL [11], as well as deletion and loss of expression of p16 and p21 genes. These anomalies are associated with the pleomorphic or blastoid variants which also harbour tetraploid clones and have poor prognosis [24]. This rapidly expanding knowledge in the area of molecular genetics has to identify previously unrecognized entities with distinct histopathologic and clinical features such as MCL [25].

Clinical Characteristics, Differential Diagnosis and Treatment:

Patients with MCL have a median age of approximately 60 years with a male predominance [16]. Clinically, a high percentage (90%) present with advanced stage (stage III and IV) diseases, usually with generalized lymphadenopathy and bone marrow involvement. Extranodal sites (FIT and Waldeyer's ring) are also frequently involved. The disease course is moderately aggressive and the disease is rarely curable. Median survival ranges between 3 and 4 years in large series [22]. MCLs are associated with poor prognosis [4].

MCLs are frequently misdiagnosed, the classic form has to be distinguished from atypical B-CLL/small lymphocytic lymphoma, and follicular lymphoma. The blastoid variant is frequently misdiagnosed as lymphoblastic lymphoma. The small cell variant is misdiagnosed as splenic or nodal marginal zone B-cell lymphoma or MALT lymphoma. The pleomorphic variant is misdiagnosed as diffuse or follicular large B-cell lymphoma [28].

The median survival of patients with MCL is only 36 months. The available conventional chemotherapy regimens do not appear to be curative. MCL appears to represent the worst prognostic category of all non Hodgkin Lymphomas. It lacks both the long survival of in-

dolent lymphomas and the curability potential of the aggressive lymphomas [10].

The present study aims at drawing attention to the need for precise identification and re-classification of the new entity (MCL) with its different subtypes, since this entity is not receiving due attention as a specific disease entity in Egyptian reports. The need to perform immunohistochemical and molecular marker studies is emphasized as adjuncts to routine histomorphology. The study also aims at the evaluation of prognosis and response to anthracyclin-based chemotherapeutic regimens.

MATERIAL AND METHODS

A total of 712 cases of NHL (Non Hodgkin Lymphoma), previously diagnosed according to the International Working Formulation (WF) and treated at the National Cancer Institute during the years 1990-1995, were revised retrospectively. Histomorphologic re-evaluation was performed according to the "Revised European American Classification of Lymphoma (REAL classification) by two observers independently. A total of 63 cases were provisionally reclassified as mantle cell lymphomas (MCL).

Immunohistochemical study of the 63 reclassified MCL cases was performed for the detection of cyclin D₁ overexpression, p53, and MDR-1 immunoreactivity.

Mouse monoclonal primary antibody 5D4 (Immunotech, Westbrook, Me), directed against recombinant cyclin D₁, was applied in a dilution of 1:25 to deparaffinized sections to test for overexpression of PRAD₁/cyclin D₁ protein product. Mouse monoclonal antibody for p53 oncoprotein (Do7-Dako M 7001) was applied. MDR1 gene protein product (P-glycoprotein product) was studied by applying the ready-to-use mouse monoclonal antibody (JSB-1), which recognizes a 170 Kd glycoprotein epitope on the plasma membrane (Biogenex Laboratories).

Antigen retrieval was done by microwave heating in citrate solution (Biogenex Laboratories). Secondary antibody and the peroxidase-streptavidin complex were applied (Biogenex-Neufahrn, Germany). Chromogen used was DAB (Biogenex). Counterstaining was performed using Mayer's Haematoxylin in cases immunostained for overexpression of cyclin D₁ and p53. Light Green counterstain was used in cases immunostained for MDR-1 gene protein product.

Positive reaction for p53 and cyclin D₁ over-

expression appeared as brownish nuclear staining of tumour cells. MDR-1 (P-Glycoprotein) positive immunoreactivity appeared as clear brown staining of cell membrane, with or without cytoplasm staining of neoplastic cells. Cases were considered positive for p53 immunoreactivity when at least 10% of cells showed a positive reaction [18]. Percentage of positivity for P-glycoprotein immunostaining reaction was estimated as being one of 3 groups [23].

I - Negative, less than 10%+ cells

II - Weakly positive, 11-50% +ve cells

III- Strongly positive, more than 50% +ve cells

Monoclonal antibody against cyclin D₁ protein product was also applied to deparaffinized sections of 15 other non-MCL NHL cases, to be used as negative control. These non-MCL NHL cases included:

- Small lymphocytic lymphoma
- Lymphoblastic lymphoma
- Small non-cleaved cell lymphoma
- Large cell lymphoma

Response to anthracyclin-based chemotherapy was also evaluated.

Statistical Analysis:

- Statistical Analysis was done according to Ingelfinger [14]. Chi square test for contingency table analysis was used. Results were considered:

Significant at $p \leq 0.05$

Highly significant at $p \leq 0.01$

RESULTS

The 63 cases that have been previously classified according to the WF belonged to the following subtypes:

- Diffuse, small non cleaved cell type: 34 cases
- Small lymphocytic: 14 cases
- Diffuse, mixed small and large cell type: 10 cases
- Diffuse, large cell type: 2 cases
- Diffuse, lymphoblastic: 1 case
- Lymphocytic predominance: 1 case
- Atypical reactive lymphadenitis: 1 case

These 63 patients had the following clinicopathologic features:

Age and Sex. Among the 63 cases of reclassified MCL, 84 were males and 16 were females, with a male / female ratio 3:1. Their

ages ranged from 16-65 with a median age of 55 years.

Clinical presentation. All patients presented with generalized disease. Response to anthracyclin-based chemotherapy was evaluated. Complete remission was achieved in 75% of patients, with a median disease free survival time of 12 months. Patients who did not achieve complete remission were either in partial remission, increasing disease or died during therapy.

Histopathology:

MCL exhibited a range of architectural patterns from mantle zone, nodular, to diffuse patterns. The majority (85.3%, 54/63) showed a diffuse pattern, while 7.9% of cases (5/63) showed a vague nodular pattern and 6.3% of cases (4/63) showed a mantle zone pattern (Fig. 3).

A total of 42 cases (66.7%) showed classic cytologic features, with a monotonous uniform population of intermediate sized cells with indented nuclei, irregularly shaped small nucleoli, coarse chromatin and scant cytoplasm (Fig. 1). The pleomorphic cell variant was noticed in 13 cases (20.6%), with large sized cells exhibiting cleaved nuclei. The blastoid cell variant was noticed in 8 cases (12.7%) with tumour cells resembling lymphoblasts, showing finely dispersed chromatin (Fig. 2). All blastoid cases showed a diffuse pattern.

Concerning cytological features in relation to architectural pattern, all mantle zone MCL cases (5/5) and all nodular MCL cases (4/4) showed features of the classic small cell variant. A total of 54 diffuse MCL cases showed classical cell features in 33 cases (33/54), pleomorphic cell variant in 13 cases (13/54) and blastoid cell variant in 8 cases (8/54).

Immunohistochemical Study:

a- Cyclin D1. Overexpression of cyclin D1 (PRAD1) protein corresponding to BCL1 gene locus rearrangement as a marker for chromosomal translocation t (11,14) (q13,q32) was detected in 88.8% of cases (56/63). A positive reaction was seen as granular nuclear staining of malignant cells only either as diffuse or clustered areas (Fig. 4). Pronounced variation in intensity of reaction was observed among individual tumour cells. The nuclear staining reaction was present in 50-80% of tumour cells, although staining intensity varied among cells of individual cases. Mitotic figures were negative. Moreover, a difference in overall staining intensity was noted amongst different tumours.

Staining for cyclin D₁ resulted in an impressive accentuation of the mantle zone pattern (perifollicular growth pattern), because reactive B-cells of residual germinal centers were negative for cyclin D₁. No cytoplasmic staining was detected. Benign lymphoid cells were devoid of the reaction. Residual normal lymphoid tissue of germinal centers and paracortex was totally negative. Among the 63 MCL cases, 56 showed a positive reaction for cyclin D₁ overexpression, out of which 48 cases showed a diffuse pattern (48/56). Out of the 5 nodular MCL cases, 4 cases (4/5) showed overexpression of cyclin D₁. All mantle zone MCL cases (4/4) were positive for cyclin D₁ overexpression. Overexpression was noticed in 40 cases with the classical cell variant, 10 cases with pleomorphic cell variant, and 6 cases with blastoid cell variant. All of the other 15 non-MCL NHL cases were found to be negative for cyclin D₁.

b- p53 oncoprotein. This oncoprotein was seen as sharp nuclear staining of malignant cells only, either as diffuse or clustered areas (Fig. 5). No cytoplasmic staining was detected. Benign lymphoid cells were devoid of the reaction. Cases were considered positive with a cut off value of 10% positive cells, as they may represent future clones of proliferation. A total of eleven cases (17.5%) expressed p53 oncoprotein. Out of these, 9 cases showed a diffuse pattern (9/11). A nodular pattern was noticed in one p53-positive MCL case (1/11). The mantle zone pattern was noticed in one p53 positive MCL case (1/11). The classic cell variant was noticed in four MCL cases positive for p53 oncoprotein. The pleomorphic cell variant was noticed in one MCL case positive for p53 oncoprotein. Blastoid cell variant was noticed in six MCL cases positive for p53 oncoprotein. In the majority of cases, more than 15-50% of cells exhibited positive nuclear staining. The relation between p53 immunohistochemical reactivity and histopathologic subtype of MCL proved to be statistically significant where $p = 0.04$ ($p < 0.05$) (Table 1).

c- P- Glycoprotein analysis. P-glycoprotein reaction as expression of MDR-1 gene was detected in 32 out of the 63 MCL cases (51%). Positive reaction was seen in diffuse or clustered areas. Membranous, with or without cytoplasmic, staining was evident in all positive cases (Fig. 6) No reaction was detected in normal lymphoid cells. Weak endothelial and histiocytic reaction was occasionally seen. All of the 32 p-glycoprotein positive cases, showed diffuse pattern, while all of the five nodular

MCL cases were negative for glycoprotein expression. All four mantle zone MCL cases were negative for P-glycoprotein expression. Classic cell variant was noticed in 20 MCL cases positive for P-glycoprotein expression. Blastoid cell variant was noticed in 7 cases positive for MDR-1 gene protein. Pleomorphic cell variant

was noticed in five cases positive for P-glycoprotein expression. The relationship between MDR-1 gene protein product immunohistochemical reactivity and histopathologic subtype of MCL proved to be statistically significant where $p = 0.028$ ($p < 0.05$) (Table 1).

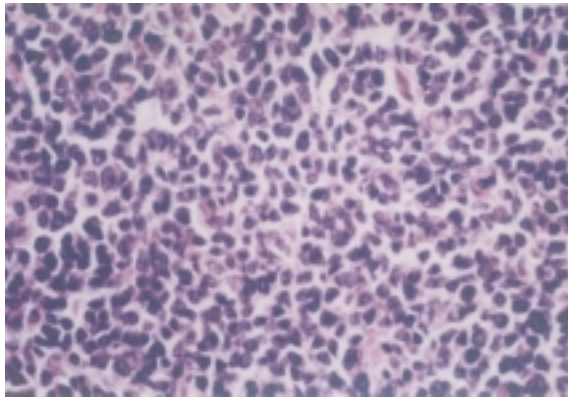


Fig. (1): Mantle cell lymphoma - Classic (Small cell variant) (Hx. and E. X 200).

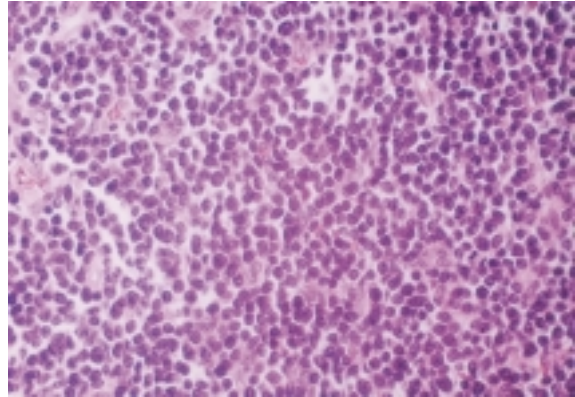


Fig. (2): Mantle cell lymphoma - Blastoid cell variant (Hx. and E. X 200).

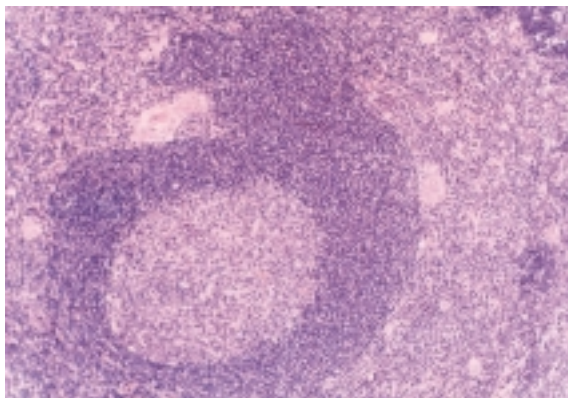


Fig. (3): Mantle cell lymphoma - mantle zone pattern (Hx. and E. X 100).

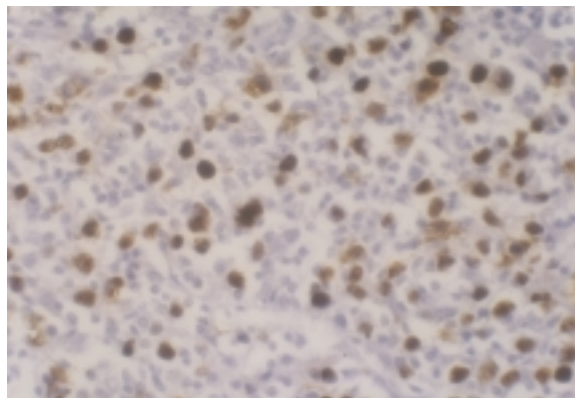


Fig. (4): Mantle cell lymphoma with positive nuclear staining for cyclin D₁ (IP-Mayers Hx. counterstain- X 400).

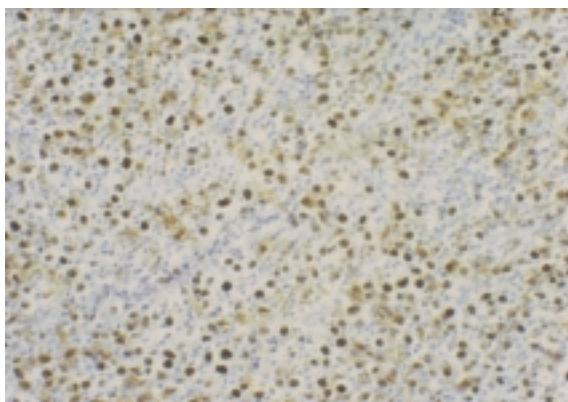


Fig. (5): Mantle cell lymphoma showing sharp positive nuclear staining for p53 (IP-Mayer's Hx. counterstain- X 200).

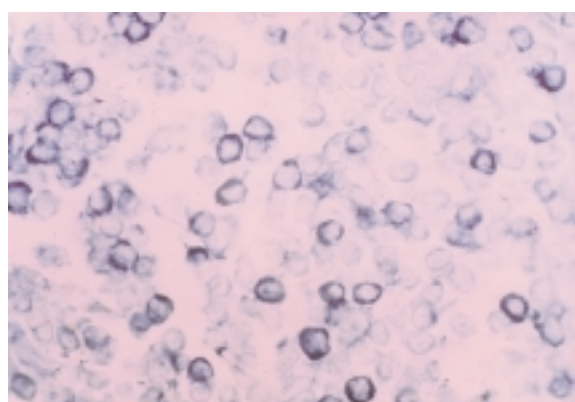


Fig. (6): Mantle cell lymphoma strongly positive for membranous staining of p-glycoprotein (IP-Light green counterstain X 400).

Table (1): Immunohistochemical reactivity for cyclin D1 overexpression, P-glycoprotein and p53 oncoproteins expression in different histopathologic types of MCL with different patterns and cell variants.

Histopathological type and pattern	N	%	Cyclin D1	p53	P-glycoprotein
<i>Diffuse:</i>					
Small	33	52.4%	32	2	20
Pleomorphic	13	20.6%	10	1	5
Blastoid	8	12.7%	6	6	7
Nodular Small	5	7.9%	4	1	-
Mantle zone small	4	6.4%	4	1	-
Total	63	100%	56	11	32

DISCUSSION

Mantle cell lymphoma is a distinct clinicopathologic entity, that is at present diagnosed in 5 to 10% of all NHLs [10]. This frequency is in accordance with the results of the present study, where MCLs constituted 8.8% of NHL seen at the NCI. This is also in agreement with De Boer et al. [6], who reported that MCL comprised 2-10% of all NHL. According to the WF, the majority of these cases are classified as WF-E or diffuse, small cleaved. Currently, in the REAL classification, they are recognized as a separate entity [12], where they are categorized as peripheral B cell neoplasms, with distinct morphologic, immunologic and genetic phenotype [10]. MCL shows a relatively poor prognosis [5].

Previous clinical studies of MCL patients have demonstrated a heterogeneous natural history, some patients expressing very aggressive disease, while the behaviour of others was similar to that of the classic indolent histology. Recent studies emphasized that the majority of patients with MCL have distinct presentations, immunologic and genetic characteristics and a very different natural history from other patients with low grade lymphomas. Patients with MCL have an overall median survival of less than 5 years, which is more similar to intermediate grade B-cell lymphomas [28]. All patients of this work presented with generalized form. A median disease free survival of 12 months was noticed, a figure confirmed by Segal et al. [27], who reported a median survival of 19 months, frequent relapses and disease progression after initial response to therapy.

It is important, therefore, to distinguish MCL from other low grade B-cell neoplasms such as small lymphocytic lymphoma/chronic lymphocytic leukaemia (SLL/CLL) and fol-

licular lymphomas. Distinction of MCL from other B-NHL was performed in this study, on the basis of histopathologic (morphologic and cytologic) evaluation in conjunction with the immunophenotypic and clinical profiles.

The present study showed a male predominance of MCL with a male/female ratio of 3: 1, thus agreeing with Gronbaek et al. [11], who reported that 81% of MCL patients were males. The median age of MCL patients in this study was 55 years with an age range from 16-65 years, thus agreeing with Laszlo et al. [17], who reported that most MCL patients were older men.

Applying the strict criteria mentioned in the unifying proposal for MCL [22] revealed striking variability. Most cases (85.3%) had a diffuse growth pattern. Vaguely nodular pattern was noticed in 7.9% of cases and the mantle zone pattern was observed in 6.3% of cases. This is in concordance with Majlis et al. [19], who classified MCL into: Diffuse 61%, nodular 13% and mantle zone 26%. Three cytologic variants were noticed in this study: Classic, pleomorphic and blastoid. The classic variant was the most frequent, and was noted in 42 cases. The pleomorphic variant was less frequent, being observed in 13 cases. The blastoid cell variant was the least frequent, being noticed in 8 cases. These findings coincide with those of other authors [6,30]. However, the prognostic significance of the various subtypes remains somewhat controversial. Most authorities believe that MZ (mantle zone) variant pursues a more benign clinical course than the other subtypes. The blastoid variant is believed to demonstrate the most aggressive clinical behaviour [24]. If MZ pattern actually behaves as an indolent lymphoma, patients with this histologic subtype may be excellent candidates for therapy with purine analogues [10].

Morphologic diagnosis of MCL remains very difficult except for the most experienced haematopathologist [10]. Immunophenotyping and molecular genetics have proved to be of tremendous value in corroborating the diagnosis. Cyclin D1 overexpression was noticed in the majority of MCL cases in this study (89%). Most MCL cases with diffuse architecture (48/56 cases) showed cyclin D1 overexpression. All four MZ MCL cases and most nodular MCL cases (4/5) were positive for cyclin D1 overexpression. Different cytologic variants of MCL showed cyclin D1 overexpression, where the majority of classic (small cell) variant (40 cases) and the majority of blastoid cell variant (6 cases) were positive for cy-

clin D1 overexpression. In all cases, immunohistochemical staining showed variation between tumour cells within the same tissue section. Heterogeneous staining of tumour cells may reflect real heterogeneity. It may also be explained on the basis of rapid proliferation, increased mitotic index, high turnover and aggression associated with MCL. These results coincide with those in other reports [4,6,7,9], which pointed out that cyclin D1 staining is useful in distinguishing MCL from other low-grade B-cell neoplasms, with special reference to bone marrow infiltration where such distinction can be difficult [28]. Thus, cyclin D1 staining can be of value in discovering minimal residual disease in bone marrow and proper staging. Overexpression of cyclin D1 is a fairly unique feature of MCLs compared with the other types of B-cell lymphomas [24]. Apart from some cases of hairy cell leukaemia and splenic lymphomas with villous lymphocytes, no other B-cell lymphoproliferative disorders show overexpression of cyclin D1 [28]. Cyclin D1 is known to be expressed mainly by epithelial cells or in some mesenchymal cells, but not in normal lymphoid cells [20]. An overexpression of cyclin D1 can be induced either by amplification of the chromosomal region of cyclin D1 gene at 11q13 as described in breast cancer or by chromosomal translocation found in NHL of MCL type. As a consequence, overexpression of cyclin D1 mRNA as well as protein, can be detected in most cases of MCL [24]. As an important further consequence of chromosomal translocation, a downregulation of cyclin D3 unrelated to proliferative activity occurs in MCL, a feature that distinguishes this entity from other B-cell lymphomas.

For routine purposes, immunohistochemical detection assay for cyclin D1 overexpression, proves to be a very useful additional marker for MCL. Routine analysis of mRNA expression is not easily applicable in most pathology laboratories. Besides, detection of genomic rearrangements is very laborious, because of the presence of at least five different breakpoint clusters within the 120 kb BCL-1 locus. However, overexpression of cyclin D1 may be present in MCL, with or without a detectable rearrangement in the BCL-1 locus. This renders overexpression of cyclin D1 gene a good candidate marker for MCL [6] particularly as overexpression of cyclin D1 in MCL can be performed using molecular probes [22].

Results of this study showed MCL to be of high biological aggression that is cytologically

indicated by the blastoid variant and immunohistochemically by the high p53 and MDR-1 gene proteins expression. The association of an aggressive behaviour and cyclin D1 overexpression may be explained by several mechanisms. Cyclin D1 is known to be associated with specific cyclin dependent kinases (mainly CDK4 and CDK 6) to become functionally active. Deregulated expression of cyclin D1 can lead to aberrant growth behaviour and uncontrolled mitotic cycling. Cyclin D1 cooperates with myc genes in the generation of MCL [22]. Mantle zone expansion by cells with the "intermediate" cytology may reflect expansion of this cellular compartment before the last step in B-lymphoid differentiation owing to an impaired ability to exit from cell cycle. Another mechanism which could be implicated in tumorigenesis of MCL by cyclin D1 is related to inactivation of Rb gene (a tumour suppressor gene), by phosphorylation [8]. This suggests that cyclin D1 overexpression may play a role in lymphomatous cell proliferation by overcoming the suppressive growth control pRb.

In this study, p53 immunoreactivity was noticed in 11/63 MCL cases, the majority of which showed diffuse architecture (9/11) and large cell variant blastoid and pleomorphic pattern (7/11). This indicates a significant relationship between p53 immunoreactivity and highly aggressive histopathologic subtypes ($p < 0.05$). P53 immunoreactivity was only noticed in one out of four nodular and one out of four MZ cases, known to have a rather indolent clinical course.

The results of the present study agree with that of Louie et al. [18], who found that all p53 +ve MCL cases were of the diffuse pattern, and Zoldan et al. [3], who reported that most MCLs with blastoid cytology showed p53 mutations. Koduru et al. [15] also reported that somatic p53 changes were present in MCL at a higher frequency than other B-NHL, thus having negative prognostic influence. This may be explained by the fact that, by inactivation of TP53 gene, the tumour may become resistant to apoptotic signals induced by radiation and DNA damaging chemotherapy, leading to poor response to therapy [18]. The results of this study come also in concordance with Chiarle et al. [5], who reported that overexpression of p53 in MCL is a prognostic marker that identifies patients at high risk.

The present study showed that 32 MCL cases (51%) were positive for P-glycoprotein and all showed diffuse pattern. All MCL cases

showing nodular and mantle zone patterns were negative for P-glycoprotein, and the relationship between immunoreactivity for MDR protein and aggressive histopathologic subtype was statistically significant ($p < 0.05$). These results suggest that P-glycoprotein expression may be only present in MCL cases known to have a poor prognosis. This is accentuated by the fact that most blastoid cases included in this study (7/8) were positive for P-glycoprotein. Lymphoid cells may acquire MDR as a result of genetic alterations occurring during lymphomatogenesis [21]. The present study suggested that MDR-1 may contribute to chemotherapy resistance in a fraction of patients with MCL, thus accounting for the relatively high failure of treatment with anthracyclins. The combined negative immunoreactivity for p53 and MDR-1 in 40% of MCL cases may indicate a relatively better prognosis in such cases.

Conclusions:

The use of molecular biologic and genetic techniques has proved to be at present mandatory for diagnostic assessment. The present data confirm that MCL (the cyclin D₁ over-expressing lymphoma) represents a distinct clinicopathologic entity and this urges for applying the REAL classification in the diagnosis and classification of malignant lymphomas.

REFERENCES

- 1- Armitage J.O. and Weisenberger D.D.: New approach to classifying Non Hodgkin's Lymphomas: Clinical features of the major histologic subtypes. *J. Clinical Oncol.*, 16 (8): 2780-2795, 1998.
- 2- Bentz M., Plesch A., Bullinger L., Stilgenbauer S., Ott G., Muller-Hermelink H.K., Barth T.F., Moller P., Lichter P. and Dohner H.: t (11;14)-positive mantle cell lymphomas exhibit complex karyotypes and share similarities with B-cell chronic lymphocytic leukemia. *Genes-Chromosomes-Cancer*, 27 (3): 285-294, 2000.
- 3- Castillo R., Mascarenhas J., Telford W., Chadburn A., Frieduman S.M. and Schattner E.J.: Proliferative response of mantle cell lymphoma cells stimulated by CD40 ligation and IL-4. *Leukemia*, 14 (2): 292-298, 2000.
- 4- Chan J.K., Miller K.D., Munson P. and Isaacson P.G.: Immunostaining for cyclin D₁ and the diagnosis of mantle cell lymphoma, is there a reliable method? *Histopathology*, 34 (3): 266-270, 1999.
- 5- Chiarle R., Budel L.M., Skolnik J., Frizzera G., Chioldi M., Corato A., Pizzolo G., Magidson J., Montagnoli A., Pagano M., Maes B., De-Wolf-Peters C. and Inghirami G.: Increased proteasome degradation of cyclin-dependent kinase inhibitor p27 is associated with a decreased overall survival in mantle cell lymphoma. *Blood*, 95 (2): 619-626, 2000.
- 6- De Boer C.J., Van Krieken J.H., Schuring E. and Klein P.M.: Bcl- 1/cyclin D₁, in Malignant Lymphomas. *Ann. Oncol.*, 8 (2): 109-117, 1997.
- 7- Deleon E.D., Alkan S., Huang J.C. and His E.D.: Usefulness of an immunohistochemical pannel in paraffin-embedded tissues for the differentiation of B-cell non Hodgkin's lymphomas of small lymphocytes. *Mod. Pathol.*, 11 (11): 1046-1051, 1998.
- 8- Dreyling M.H., Bullinger L., Ott G., Stilgenbauer S., Muller-Hermelink H.K., Bentz M., Hiddemann W. and Dohner H.: Alterations of the cyclin D₁/p16-pRB pathway in mantle cell lymphoma. *Cancer Res.*, 57: 4608-4614, 1997.
- 9- Fiel Gan M.D., Almeida L.M., Rose D.C., Takano A., Rezuze W.N., Coleman W.B., Garcia N.F. and Tsongalis G.J.: Proliferative fraction, BCL-1 gene translocation and p53 mutation status as markers in mantle cell lymphoma. *Int. J. Mol. Med.*, 3 (4): 373-379, 1999.
- 10- Fisher R.I.: Mantle cell lymphoma: Classification and therapeutic implications. *Ann. Oncol.*, 7 (Suppl. 6): S35-S39, 1996.
- 11- Gronbaek K., Nedergaard T., Andersen M.K., Thor Straten P., Guldborg P., Moller P., Zeuthen J., Ebbesen Hansen N., Hou-Jensen K. and Ralfkiaer E.: Concurrent disruption of cell cycle associated genes in mantle cell lymphoma: A genotypic and phenotypic study of cyclin D₁, p16, p15, p53 and pRb. *Leukemia*, 12 (18): 1266-1271, 1998.
- 12- Harris N.L., Jaffe E.S., Stein H., Banks P.M., Chan J.K.C., Cleary M.L., Delsol G., Peeters C.D.W., Falini B., K.C., Grogan T.M., Isaacson P.G., Knowles D.M., Mason D.Y., Muller-Hermelink H.K., Pileri S.A., Pirs M.A., Ralfkiaer E. and Warnke R.A.: A revised European American classification of lymphoid neoplasms: A proposal from the International Lymphoma Study Group. *Blood*, 84: 1361-1392, 1994.
- 13- Hiddeman W., Longo D.L., Coiffier B. et al.: Lymphoma classification: The gap between biology and clinical management is closing. *Blood*, 88: 4085-4089, 1996.
- 14- Ingelfinger J.A., Mosteller F.M., Thibodeau L.A. and Ware J.H.: Biostatistics in clinical medicine. Mc Graw-Hill Inc., New York, 112-117, 1994.
- 15- Koduru P.R., Raju V., Vadmal V., Menezes G., Shah S., Susin M., Kolitz J. and Broome J.D.: Correlation between mutation in p53, p53 expression, cytogenetics, histologic type and survival in patients with B-cell non Hodgkin's lymphoma. *Blood*, 90 (10): 4078-4091, 1997.
- 16- Kurtin P.J.: Mantle cell lymphoma. *Adv. Anat. Pathol.*, 5 (6): 376-398, 1998.
- 17- Laszlo T., Nagy M., Kelenyi G. and Matolcsy A.: Immunoglobulin V (H) gene mutational analysis suggests that blastic variant of mantle cell lymphoma derives from different stages of B-cell maturation. *Leuk Res*, 24 (1): 27-31, 2000.
- 18- Louie D.C., Offit K., Jaslow R., Parsa N.Z., Murty V.V.S., Schluger A. and Chaganti R.S.K.: P53 over-expression as a marker of poor prognosis in mantle cell lymphomas with t (11, 14) (q13, q32). *Blood*, 86 (8): 2892-2899, 1995.
- 19- Majlis A., Pugh W.C., Rodriguez M.A., Benedict W.F. and Cabanillar F.: Mantle cell lymphoma: Cor-

- relation of clinical outcome and biologic features with three histologic variants. *J. Clin. Oncol.*, 15 (4): 1664-1671, 1997.
- 20- Mokhtar N. (ed.): *Molecular Diagnosis of Lymphoma and Leukemia*. in: *Molecular Pathology of Cancer*. Published by National Cancer Institute-Cairo University: 288-333, 1998.
- 21- Mokhtar N., Khaled H., Anwar N. et al.: Biologic profile of non Hodgkin's lymphoma in Egypt: Relation to response to BECOP regimen. *CMB*, 1: 255-264, 1994.
- 22- Nakamura S., Yatabe Y. and Seto M.: Review article. Cyclin D₁ overexpression in malignant lymphoma. *Pathol. International*, 47: 421-429, 1997.
- 23- Niehans G., Jasztes W. et al.: Immunohistochemical identification of P-glycoprotein in previously untreated diffuse large cell and immunoblastic lymphoma. *Cancer Res.*, 52: 3768-3775, 1992.
- 24- Ott G., Kalla J., Hanke A., Muller J.G., Posenwald A., Katzenberger T., Kretschmar R., Kreipe H. and Muller Hermelink H.K.: The cytomorphological spectrum of mantle cell lymphoma is reflected by distinct biological features. *Leuk-Lymphoma*, 32 (1-2): 55-63, 1998.
- 25- Panayiotidis P. and kotsi P.: Genetics of small lymphocyte disorders. *Semin-Hematol.*, 36 (2): 171-177, 1999.
- 26- Pileri S.A., Milani M., Fraternali G. and Sabattini E.: Commentary from the R.E.A.L. classification to the upcoming WHO scheme: A stop toward universal categorization of lymphoma entities. *Ann. Oncol.*, 9: 607-612, 1998.
- 27- Segal G.H., Masih A.S., Fox A.C., Jorgensen T., Scott. M. and Braylan-RC CD5 expressing B-cell non Hodgkin's lymphomas with bcl-1 gene rearrangement have a relatively homogenous immunophenotype and are associated with an overall poor prognosis. *Blood*, 85 (6): 1570-1779. 1995.
- 28- Vasef M.A., Medeiros J., Mccourty C.A. and Brynes R.K.: Cyclin D₁ immunohistochemical staining is useful in distinguishing mantle cell lymphoma from other low grade B-cell neoplasms in bone marrow. *A.J.C.P.*, 108 (3): 302-307, 1997.
- 29- Watson P., Wood K.M., Lodge A., Mc Intosh G.G., Milton-1, Piggott-N.H., Proctor S.J., Taylor P.R., Smith S., Jack F., Bell H., Stewart M., Anderson J.J., Horne C.H. and Angus B.: Monoclonal antibodies recognizing CD5, CD10 and CD32 in formalin fixed, paraffin embedded tissue: Production and assessment of the value in the diagnosis of small B-cell Lymphoma. *Histopathology*, 36 (2): 145-150, 2000.
- 30- Zoldan M.C., Inghirami G., Masuda Y., Vandekerckhove F., Raphael B., Amorosi E., Hymes K. and Frizzera G.: Large cell variants of mantle cell lymphoma: Cytologic characteristics and p53 anomalies may predict poor outcome. *Br. J. Haematol.*, 93 (2): 475-486, 1996.