10.1053.paor.1999.0166 available online at http://www.idealibrary.com on IDEAL

ARTICLE

Histopathologic Features and Expression of Bcl-2 and p53 Proteins in Primary Gastric Lymphomas

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The aim of this study is to present a histopathologic and immunohistochemical analysis of primary gastric lymphomas which were reclassified according to the concept of mucosa associated lymphoid tissue (MALT). The resected specimens from 41 patients with primary gastric lymphoma were investigated retrospectively. Immunohistochemical study was done to analyze the immunophenotype and bcl-2 and p53 proteins expression. Twenty three of the cases had tumors mainly located in the antrum. Histologically, 12 were low grade and 20 were high grade B-cell lymphoma of MALT, 9 other B-cell nonHodgkin's lymphomas. Helicobacter pylori was identified in 72% of the cases. According to Musshoff's modification, most of the MALT lymphoma cases had stage I or II disease. There was significant difference between low and high grade cases, in respect to depth of invasion in gastric wall. Immunohistochemically, the neoplastic cells in all MALT lymphomas expressed B-cell phenotype. Bcl-2 protein was found to be expressed in 59% and p53 protein expression was detected in 72% of cases. Among the B-cell lymphoma of MALT, bcl-2 positivity decreased and p53 positivity increased significantly as the histological grade advanced. So, an inverse correlation was observed between the expression of bcl-2 and p53. In conclusion, most primary gastric lymphomas are low or high grade B-cell MALT lymphomas and appear to arise in MALT acquired as a reaction to Helicobacter pylori infection. Expression of bcl-2 and p53 in gastric lymphomas may be associated with transformation from low-grade to high-grade disease. (Pathology Oncology Research Vol 5, No 1, 36–40, 1999)

Key words: lymphoma, MALT, stomach, histopathology, bcl-2, p53

Introduction

The gastrointestinal tract is the most common site of the primary extranodal lymphomas and gastric lymphomas are the majority of the cases.[9] However gastric lymphoma accounts for only 1–10% of all gastric malignant neoplasms.[13]

Received: Dec 12, 1998; *accepted:* Febr 2, 1999

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This research was partly supported by Reseach Fund of University of Istanbul. Project No: Ö-424/110398

Abbreviations: CCL – centrocyte-like cells; HG – high grade; Hp – Helicobacter pylori; LEL – lymphoepithelial lesion; LF – lymphoid follicle; LG – low grade; MALT – mucosa associated lymphoid tissue; NHL – non-Hodgkin's lymphoma; PC – plasma cell

Since the first publication of Isaacson and Wright,[6] the concept of low grade (LG) B-cell lymphoma of mucosa-associated lymphoid tissue (MALT) seems to have become accepted widely and it has been incorporated into the new classification scheme (REAL) as extranodal marginal zone B-cell lymphoma.[4] In addition, transformation to high grade (HG) lymphomas from LG MALT lesions has been described by many authors.[1,5,13,17]

Acquired MALT accumulates in the stomach after Helicobacter pylori (Hp) infection, and Hp can be found in most of the cases. More recently, eradication of Hp has been added to the potentially effective stomach-conserving therapies for LG-MALT-lymphomas.[3,15,20,21]

Compared with node-based lymphomas, these MALT lymphomas of the stomach is recognized as a distinct disease entity with a characteristic presentation, histological spectrum and clinical behavior,[7] they differ in at least three instances: (a) in localized stages, they behave as

 1219-4956/99/010036+05 $ 12.00/0

focal tumors and may, therefore, be curable by radical resection; (b) most tumors of LG malignancy have a distinct morphology and (c) relapse of the disease may occur exclusively within the gastrointestinal tract, even long after remission.[1]

The over expression of p53 protein has been recently reported in HG gastric lymphomas.[2,14] An inverse relationship between bcl-2 protein expression and p53 expression in primary gastric lymphomas has been reported and bcl-2 positivity was found to decrease whereas p53 positivity increased significantly as the histologic grade advances.[14]

Although gastric lymphomas are not rare in our country, there are few detailed studies in this subject. The aim of this retrospective study is to reinvestigate primary gastric lymphomas and characterize them histopathologically and immunophenotypically. The expression of bcl-2 oncoprotein and p53 oncogene were evaluated in the same cases, using immunohistochemical methods.

Material and Methods

During the period from 1992 to 1997, 41 surgically resected specimens with gastric lymphomas which were diagnosed in the Department of Pathology, Cerrahpaşa Faculty of Medicine, University of Istanbul, were available for this study.

Histologic sections prepared from paraffin blocks were routinely stained with hematoxylin and eosin (HE) and were reexamined using a standard light microscope. The cases were cathegorized in 3 basic groups, according to Isaacson's criteria for MALT lymphomas: LG lymphoma of MALT (composed of centocyte like cells, lymphoid follicles and plasma cell differentiation), HG lymphoma of MALT (composed of sheets or clusters of large blastic cells), and other than MALT lymphomas. In LG group, there were 9 cases of lymphoma with focal HG component and in HG group, all the cases were HG lymphoma with focal LG component, as it was difficult to diagnose HG MALT lymphoma, without detecting LG component histopathologically.[1,8,9]

Modified Giemsa (MG) staining was performed using a mixture of 60 ml distilled water and 2.6 ml MG solution. After the treatment of sections with this solution, they were left overnight and then treated with 1% acetic acid.

An immunohistochemical study was performed by using a Labeled Strepavidin Biotin (LSAB) Universal kit (DAKO) according to the instructions of manufacturer and AEC was used as chromogen. The primary antibodies used are prediluted forms of anti-bcl-2 protein, p53 oncoprotein, CD20, and CD3 antibodies. 5 micron thick paraffin sections were left overnight in 37°C in autoclave for overnight. After deparaffinization for 7 minutes, they were treated with 800 KW antigen retrival. The immunostained slides were examined by light microscopy. The staining was considered positive where more than 10% of the lymphoma and neoplastic plasma cells were stained strongly. When less than 10% of the cells were stained positivly, it was scored as weak positivity.

Statistical studies were performed using Fisher's exact test.

Results

The 41 patients had an age range from 18 to 78, with a median age of 55 years. The median age of HG tumors was higher than LG cases (60 and 55 years respectively). There were 12 women and 29 men. The predominance of men was statistically significant (p=0.0003)

Most of the cases had tumors located mainly in antrum of the stomach (23 cases). Body and fundus location was seen in 10 cases. The exact location was unknown in 8 cases because following revision of slides or the paraffin blocks, insufficient information about the patients was available. Tumor size ranged from 0.5 to 14 cm. Again tumor size was not known in 15 cases.

When the classification suggested by Isaacson[8,9] for gastrointestinal lymphomas was applied, of the 41 cases, 12 were classified as LG MALT, 20 cases as HG MALT, and 9 cases as other than MALT lymphomas. In 9 of 12 LG cases, focal HG component was seen. In all HG MALT lymphomas, there was focal LG component, as it was difficult to diagnose HG MALT lymphoma histolopathologically without seeing LG component.

Macroscopically, 10 of 20 HG MALT and 6 of 12 LG MALT lymphomas were classified as diffuse ulcerous type, 9 HG MALT lymphomas as mass forming type, where none of LG cases had mass *(Table 1)*.

Table 1. **Macroscopic Types and Histopathologic Features of Gastric Lymphomas**

Macroscopic Types	*LG MALT*	*HG MALT*	*Others*
Diffuse/ulcerous	6	10	2
Superficial	4	1	0
Mass	0	9	0
Unknown	2	2	7
Total	12	20	9
Histopathologic features			
CCL	12	17	3
LF	12	20	4
PC	12	20	1
LEL	10	18	1
Blasts	9	20	8
Total	12	20	9

CCL: Centrocyte like cells, LF: lymphoid follicles, PC: plasma cells, LEL: lymphoepithelial lesion

Table 2. **Depth of invasion, lymph node involvement and staging in gastric lymphomas**

Depth of invasion	*LG MALT*	*HG MALT*	*Others*
Submucosa	5	3	0
Muscularis propria	5	4	4
Serosa	1	9	1
Beyond serosa	1	4	4
Lymph Nodes			
Positive lymph nodes	4	11	3
Negative lymph nodes	8	8	0
Unknown lymph nodes	0	1	6
Stage			
I	8	8	0
II	4	10	2
III	0	0	0
IV	0	1	1
Unknown	0	1	6
Total	12	20	9

On histopathologic examination, centrocyte like cell (CCL), lymphoid follicles (LF), plasma cell differentiation (PC) and lymphoepithelial lesion (LEL) were seen in all or most LG and HG MALT lymphoma cases. All of the HG MALT lymphomas were composed of sheets of blastic cells *(Table 1)*. Occasional large cells were present in 9 cases, they were less than 20% of the lymphoma cells and did not form clusters or sheets.

The depth of the invasion into the gastric wall was usually submucosa and muscularis propria in LG MALT cases (10 cases) where it was serosa and beyond serosa in most of HG MALT lymphomas (13 cases). The difference with respect to depth of invasion between LG and HG cases was statistically significant (Fisher's exact test, p=0.0118) *(Table 2)*. The gastric lymph nodes were involved in 4 of 12 LG and 11 of 20 HG MALT lymphomas *(Table 2)*.

According to Musshoff's modification of Ann Arbor staging system,[16] all of LG MALT lymphomas and most of HG MALT lymphoma cases (18/20) were in stage I or II. *(Table 2)*. Stage could not be determined in 7 cases because of insufficient clinical information.

In both HE and MG stained slides, Hp was positive in 9 of 12 LG and 14 of 20 HG MALT lymphomas *(Table 3)*.

All of the LG and HG MALT lymphomas revealed B-cell phenotype as immunohistochemistry showed CD20 expression in all MALT cases. 5 HG cases showed CD3 coexpression on B-cells *(Table 3)*. In most of LG cases, CD3 positive T cells were seen among lymphoma cells while they were rare or absent in HG cases.

Of the 29 specimens examined, bcl-2 protein was expressed in 17 cases (59%). In 4 LG cases, it was strongly positive in neoplastic CCLs, where in 2 other LG cases, it was weakly positive. bcl-2 was expressed in 7 of 13 HG cases. The expression of p53 protein was observed in 21 of 29 specimens (72%) examined. Among MALT lymphomas, p53 was positive in 6 of 11 LG and 12 of 13 HG tumors. The difference of p53 positivity was found statistically significant between LG and HG cases (p= 0.0302 for overall positivity, p=0.0036 for only strong positivity) *(Table 3)*. In HG cases, when strong positivity of bcl-2 and p53 oncogens were compared, p53 expression was found higher than bcl-2 expression and the difference between the positivity of these oncogens in HG cases, was significant (p= 0.0422). There was no statistically significant difference between their expression in LG tumors.

Discussion

Gastric lymphomas account for the majority of the extranodal lymphomas and until the 1980s, all of the NHL classifications were unavailable for gastrointestinal lymphomas.[13] A new classification for these lymphomas according to LG MALT concept, was proposed by Isaacson et al in 1983[6] and since then, this classification has become widely used. Later, transformation from LG lesions to HG lymphoma was described by some authors.[1,8,13] In addition, others described mixed grade (LG B-cell lymphoma of MALT with a focal HG component).[11] HG gastric MALT lymphomas were reported to be more frequent than LG lesions.[1,8,18] In Nakamura's study, among 233 cases, 43% of the cases were HG, 30% were LG, 12% were LG with focal HG, 6% other B, 6% other T-cell lymphomas.[13] In Chan's series, 12 of the 48 cases were LG, 26 were HG and 10 were mixed LG and HG.[1] However, Hsi et al found LG MALT lymphomas more. (22 of 48 cases).[5] By contrast, some investigators reported that not MALT type, but the diffuse large cell type of lymphoma, was highest in incidence among primary gastric lymphomas in Japan.[16] In

Table 3. **Immunohistochemistry results and Helicobacter pylori staining in gastric lymphomas**

Antibody	*LG MALT*	*HG MALT*	*Others*
CD20	10/10	19/19	4/5
CD3	0/10	5/9	0/5
Bcl-2 positive	4/11	3/13	1/5
Bcl-2 weak positive	2/11	4/13	3/5
Bcl-2 negative	5/11	6/13	1/5
P53 positive	1/11	9/13	1/5
P53 weak positive	5/11	3/13	2/5
P53 negative	5/11	1/13	2/5
Helicobacter pylori			
Positive	9	14	2
Negative	2	1	5
Unknown	1	5	2

our study HG MALT lymphomas with focal LG component were the most frequent group.

The distribution of our cases according to age, sex and location, was in correlation with previous reports.[5,8,13]

In the study of Nakamura, among macroscopic types, most of the tumors that appeared as mass forming type proved to be HG tumors.[13] In our study, most HG-MALT and 50% of LG MALT lymphomas were classified as diffuse ulcerous type. 9 of the HG cases were mass forming but none of the LG tumors. There is no correlation between macroscopic type and histologic grade.

In our study, characteristic features of LG MALT lymphoma,[7-9] CCL, LF, PC differentiation and LEL were seen in all or most of the LG and HG MALT lymphoma cases, but they were rare in other types. All of the HG MALT lymphomas were composed of sheets of blastic cells.

For the staging of gastrointestinal lymphomas, Musshoff's modification of Ann Arbor classification is used.[8,13,18] As the gastric lymphomas are localized tumors, most of the LG and HG cases were in stage I and II.[13,18] In Nakamura's report, 121 of 233 patients had stage I disease. In our study, although there was no correlation between stage and grade, most of the cases had stage I or II disease. However, histologic grade of MALT lymphoma correlated with depth of tumor invasion.

As the MALT accumulates in stomach after Hp infection, Hp can be found in most of the lymphoma cases.[7,20,21] In another study of Nakamura, Hp was detected in 61% of patients with gastric lymphoma, and was found higher in LG lesions.[15] In our study, Hp was positive in 72% of all MALT lymphoma cases without any difference between LG and HG lymphomas.

The great majority of primary gastric lymphomas are B-cell tumors. T-cell lymphomas are very rare.[1,5-8,11-13,16] In the immunohistochemical study of our cases all of the LG and HG MALT lymphomas showed B-cell phenotype as they were stained positive with CD20. This findings supports the B-cell origin of MALT lymphomas.

The bcl-2 proto-oncogene, which was cloned from the break-point region of t(14;18) chromosomal translocation is frequently observed in the follicular lymphoma and the expression of bcl-2 protein has been detected in various nodal lymphomas. However, few articles have evaluated the expression of bcl-2 protein by immunohistochemical technique in primary gastric lymphomas. The frequency of bcl-2 positivity in these studies is different according to grade.[14,17] In the largest series, bcl-2 protein expression was detectable in 68% of primary gastric lymphoma cases. The authors confirm that the frequency of bcl-2 positivity in MALT lymphoma significantly decreases as the grade advances and this finding supports the possibility that the bcl-2 protein dissappears during the transition from LG to HG tumors.[14]

The overexpression of p53 protein either with or without a gene mutation has been reported in various tumors including nodal lymphomas. However, immunohistochemical analysis of the p53 expression in gastric lymphomas has been done in a few studies.[2,14] p53 protein positivity was found in 20% of the cases in a recent study. In contrast to bcl-2, the p53 expression in MALT lymphomas in this study increased significantly as the grade advanced.[12] The positivity of p53 protein was 51% and bcl-2 protein was 40% among our cases. As in the study, which showed inverse relationship between bcl-2 and p53 expression, p53 positivity increased and bcl-2 positivity decreased as the histologic grade advanced.

In conclusion, primary gastric lymphomas comprise a group of distinctive clinicopathologic entities. Most of LG B-cell gastric lymphomas are of MALT type and appear to arise in MALT acquired as a reaction to Hp infection. LG MALT NHL may undergo HG transformation, and LG component can be shown in HG MALT lesions. There is an inverse correlation between the expression pf bcl-2 and p53 proteins in gastric lymphomas. p53 oncoprotein positivity increases where bcl-2 oncoprotein positivity decreases as the histologic grade advances. This result suggests that the expression of bcl-2 and p53 may be associated with a transition from LG to HG tumors.

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