Original Article

Primary Gastric Lymphoma: A Study of Morphology, Immunophenotype, and Prevalence of *Helicobacter pylori* Infection

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Mohamed Elmoaket[1,2], Fatma Emaetig[3,4], Firas Abdulmalik[5], Fairouz Torjman[1,6], Nabil Enattah[7], Eric A. Van Marck[8], Adam ElZagheid[3,7]

[1]Department of Pathology, Tripoli Medical Center, [2]Judicial Expertise and Research Center, Tripoli, [7]Department of Genetic Engineering, Biotechnology Research Center, [6]Department of Pathology, Faculty of Medicine, Tripoli University, Tripoli, [3]Department of Pathology, Faculty of Medicine, Benghazi University, Benghazi, [4]Department of Pathology, Faculty of Medicine, Misurata University, [5]Department of Pathology, Misurata Cancer Center, Misurata, Libya, [8]Department of Pathology, Faculty of Medicine, Universiteit Antwerpen, Antwerpen, Belgium

Abstract

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Objectives: We aimed to study the pattern of primary gastric lymphoma in two geographically different locations, namely, Tripoli Medical Center (TMC) in Libya and Antwerp University Hospital (Universitair Ziekenhuis Antwerpen [UZA]) in Belgium. **Materials and Methods:** Twenty-four cases of primary gastric lymphoma diagnosed during an 8-year period at TMC and 20 cases diagnosed during 11-year period in UZA were studied. Immunohistochemistry lymphoma panel CD3, CD5, CD20, CD10, CD79a, CyclinD1, KI67, and pancytokeratin were applied in all cases. **Results:** Primary gastric lymphoma in UZA occurs in a slightly older age group with marked male predominance while in TMC occurs at a slightly younger age with marginal male predominance. Two-thirds of the TMC cases were of high-grade lymphoma (HGL) and one-third were low-grade lymphoma (LGL). UZA cases included nine cases of HGL with (45%) and 11 of LGL (55%). Of the TMC primary gastric lymphoma cases, 12 were infected with *Helicobacter pylori* (50%) and eight cases were mucosa-associated lymphoid tissue lymphoma (MALT-L) of which three cases had *H. pylori* infection. In the UZA cases, *H. pylori* infection was evident in seven of 20 primary gastric lymphoma cases (35%) and three of six MALT-L had *H. pylori*-infected cases (50%). **Conclusions:** This comparative study of primary gastric lymphoma in TMC (Libyan) and UZA (Belgian) studied cases showed no marked differences between the two patient populations based on the histological features and immunohistochemical phenotype and genotype and the clinical features.

Keywords: *Helicobacter pylori*, immunohistochemistry, primary gastric lymphoma

Introduction

Primary gastric lymphoma is among the extranodal types of nonHodgkin lymphomas (NHL). They represent 2%–3% of all NHL and 7% of all gastric tumors. Nonetheless, they are the most common extranodal manifestation of NHL. Most of these are of B-cell origin.[1,2] The current World Health Organization (WHO) classification considers 40% of primary gastric lymphoma as "indolent" (low-grade) and 60% as "aggressive" (high-grade) types.[1,3] The grading of gastric lymphoma is significant for both the prognosis and treatment of this disease. Indolent gastric lymphomas include mantle cell lymphoma (MCL), chronic lymphocytic leukemia, follicular lymphoma, and gastric mucosa-associated lymphoid tissue lymphoma ((MALT-L). The latter has also been termed low-grade gastric MALT lymphoma, gastric marginal B-cell lymphoma, and extranodal marginal zone B-cell lymphoma (MZBCL) of MALT type.[1,4] Gastric MALT lymphoma represents the vast majority of the three types of MZBCLs according to the Revised European American Lymphoma Classification (REAL).[1-3]

The observation that the histology of certain extranodal NHLs was related to MALT rather than that of peripheral lymph nodes was first made in 1983.[1,5] In about 90% of cases, MZBCL of

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Address for correspondence: Prof. Adam ELZagheid, Department of Genetic Engineering, Biotechnology Research Center, Tripoli, Libya. E-mail: elzagheid@yahoo.com

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MALT type is associated with *Helicobacter pylori* infection which was shown to play a causative role in the pathogenesis of gastric MALT lymphoma.[1,6] Primary gastric aggressive-type lymphomas are classified as diffuse large B-cell lymphomas (DLBCL). They contain an indolent MALT component in about one-third of the cases. This lesion likely represents a progression of disease from indolent to the aggressive stage.[1,7,8] The other two-thirds of the high-grade lymphomas (HGLs) have no detectable low-grade MALT stages. However, it is controversial whether these tumors arise from indolent lesions with subsequent obliteration of the low-grade component in any case or whether these tumors may be considered better as de novo extranodal DLBCL rather than transformed MALT lymphomas.[1] However, for both types, a causal association with *H. pylori* has been described as it induces acquired MALT in the gastric mucosa, promotes malignant transformation of reactive B-cells and induces genotoxic effects through neutrophil released reactive oxygen species, causing a wide range of genetic abnormalities.[1,9] Hence, newly described translocations such as *t* (11;18) (q21;q21) or *t* (1;14) (p22;q32) may play a key role in patient's stratification for the most effective therapeutic approach in the future.[1]

As simple as the initial diagnostics, staging and therapeutic approach to patients with gastric MALT lymphoma may seem, proper patient management is crucial. Furthermore, there is still a controversy regarding the most effective treatment strategy, especially in patients who do not respond to *H. pylori* eradication therapy.[1] Furthermore, regional and geographic differences in the various histologic subtypes of lymphomas exist. An understanding of the pattern of these differences may provide an insight into the pathogenesis of the disease.[10] We have, therefore, performed this study of the primary gastric lymphoma to examine the histological features and immunophenotyping of 30 cases from Tripoli, Libya and compare them to 24 cases from Antwerp, Belgium.

Materials and Methods

Patients with primary gastric lymphoma diagnosed at Tripoli Medical Center (TMC) during 2009–2016 and Universitair Ziekenhuis Antwerpen (UZA) during 2006–2016 years were retrospectively studied. Paraffin blocks were recovered from the pathology archives of TMC of 30 consecutive gastric lymphoma cases diagnosed by hematoxylin and eosin (H and E) stain. A series of 20 cases diagnosed with the same primary diagnosis at the UZA were identified. In this group, select stains such as periodic acid–Schiff (PAS)–diastase and Giemsa and an immunohistochemical lymphoma panel were applied for diagnosis of the lymphoma and to determine the specific entity according to the WHO/REAL classification.

Demographic characteristics such as age, sex, and the geographical location were documented. Histological parameters were identified such as the presence of atypical lymphoid infiltrate with or without lymphoid follicles, mucosal erosion, lymphoepithelial lesion (LEL), and determination of the cell size, i.e., low-grade lymphoma (LGL) and HGL. These are illustrated in Figure 1a and b. *H. pylori* infection status was recorded based on histology (H and E), using Giemsa stain and Warthin–Starry silver stain and *H. pylori* immunohistochemistry in selected cases. Furthermore, immunohistochemical antibodies (CD20 and CD79a, CD10, CD5, CD3, pan-cytokeratin, and CyclinD1) were employed on the TMC cases. These are illustrated in Figure 2 a and b.

Samples from the UZA archives were stained by the same immunohistochemical panel of antibodies used in TMC studied cases. Few selected cases were also stained by the following antibodies light chain (lambda and kappa), bcl2, bcl6, CD30, CD23, TdT, Ki67, and Epstein-Barr virus (EBV) latent membrane protein I. In certain cases, *in situ* hybridization (ISH-EBER) was applied when needed.

All TMC cases were reviewed again in UZA, special stains (PAS-diastase and Giemsa and Warthin-Starry) and an immunohistochemistry lymphoma panel was applied on all cases, and the diagnosis was confirmed with further immunophenotyping according to the WHO/Real classification.

Results

From the TMC group of cases, five cases were highly suspicious of primary gastric lymphoma with a differential diagnosis of undifferentiated carcinoma; undifferentiated carcinoma and severe chronic gastritis are important differential diagnoses. Their exclusion was based on the result of PAS-positive diastase-resistant stain, in addition to using immunohistochemical markers, such as pan-cytokeratin and a lymphoma immunostain panel such as CD45 and CD20. The remaining 24 confirmed lymphomas were included in the analysis.

The mean age of patients was 54.3 years (median 57 years), 45.8% of patients were between 20 and 50 years and two-thirds were male. Histologically, all cases revealed an atypical lymphoid infiltrate and showed LELs in 75% of the cases with presence of lymphoid follicles in one case, and the presence of neutrophilic infiltration (and erosions) in 85% of the cases [Table 1]. The 20 cases of primary gastric lymphoma from UZA, had a mean age of 60.3 years (median 63.5 years), fifth of the patients were between 20 and 50 years of age, and 85% were male. Histologically all cases revealed an atypical lymphoid infiltrate forming LELs in three quarters of the cases, but the presence of lymphoid follicles was seen in only two cases. Neutrophilic infiltration was seen (and erosions) in 85% of the cases.

TMC cases comprised 16 cases of HGL (66.7%) and eight cases of LGL (33.3%). UZA cases comprised nine cases of HGL (45%) and 11 cases of LGL (55%). Primary gastric lymphoma in UZA, occurs in a slightly older age with clear male predominance while in TMC group it occurs in a slightly younger age with slight male predominance [Table 1]. Two-thirds of the TMC cases were HGL and eight cases of

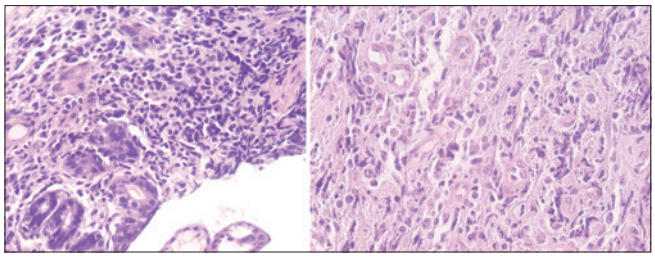

Figure 1: Gastric mucosa-associated lymphoid tissue lymphoma showing typical lymphoepithelial lesion in the upper panel (1a) and gastric diffuse large B-cell lymphomas showing proliferation of large cells (1b) using (H and E stain, ×40)

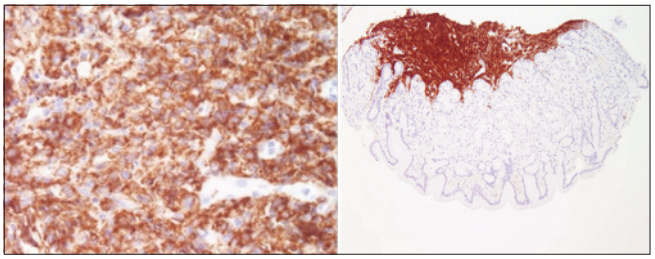

Figure 2: Immunostaining in diffuse large B-cell lymphomas in the upper panel (a) and in mucosa-associated lymphoid tissue lymphoma in the lower panel (b). The neoplastic cells show positive staining for CD20 (×40)

LGL [Tables 2 and 3]. UZA cases comprised nine HGL cases (45%) and eleven LGL cases (55%) [Tables 2 and 3]. Of the TMC primary gastric lymphoma cases, 12 were infected with *H. pylori* (50%), and eight cases were MALT-L (of which three cases had *H. pylori* infection) [Table 3]. In the UZA hospital cases, *H. pylori* infection was evident in seven of the total 20 primary gastric lymphoma (35%) and three of six MALT-L cases had *H. pylori* infection (50%) [Table 3].

Discussion

The number of diagnostic methods used in our study was probably insufficient, as all patients were only evaluated by histology and probably underestimated the *H. pylori* infection. A higher *H. pylori* prevalence has been described when serologic rather than histological methods were used.[11,12] This probably could be explained by a decrease in bacterial colonization due to gastric atrophy and hypochlorhydria; however, antibodies against bacteria can be detected even years after mucosal clearance.[11-18] On the other hand, *H. pylori* gastric mucosa colonization is not uniform; thus even in the presence of the infection, microorganisms may not be detected if the biopsy is taken from a noncolonized mucosal area. Therefore, *H. pylori* detection by histology is highly dependent on the number of gastric biopsies.[13,14,16]

TMC morphologically HGLs cases numbered 16 of 24 cases. They were phenotypically DLBCL (10 cases), DLBCL with MALT component (three cases), follicular lymphomas high-grade (FL-HG) (two cases), and one case is MCL pleomorphic variant (HG). *H. pylori* infection is expressed in eight cases of HGL (50%). Morphologically LGL was seen in eight cases, and all were phenotypically confirmed as being MALT-L. *H. pylori* infection was expressed in half of these cases.

UZA morphologically HGLs numbered nine cases, and phenotypically included DLBCL (seven cases), FL-HG (one case), and BL (Burkitt's lymphoma-one case). *H. pylori* infection was expressed in three of them (33.3%) and morphologically there are 11 cases of LGL which phenotypically were MALT-L (six cases), and five cases were MCL (classic variant). *H. pylori* infection was found only in four cases of LGL including three MALT-L and one MCL (36.3%), while among MALT-L cases of 30% were positive.

Table 1: Comparison of the demographic characteristics, histopathological parameters and *Helicobacter pylori* infection status of gastric lymphoma between cases from the Tripoli Medical Center and the Universitair Ziekenhuis Antwerpen

Characteristics	TMC, *n* (%)	UZA, *n* (%)
Age		
Mean age (years)	54.3	60.2
20-50	11 (45.8)	4 (20)
51-100	13 (54.2)	16 (80)
Sex		
Male	16 (66)	17 (85)
Females	8 (33)	3 (15)
Histopathological parameters		
LEL	18 (75)	15 (75)
Atypical lymphoid infiltrate	24 (100)	20 (100)
Lymphoid follicles	1 (4.1)	2 (10)
Neutrophils (erosion)	14 (58.3)	15 (75)
Lymphoma grade		
HGL (LC + IL) or (LC + SC)	16 (66.7)	9 (45)
LGL (SC + IC)	8 (33.3)	11 (55)
Helicobacter pylori positivity status	12 (50)	6 (30)

TMC: Tripoli Medical Center, UZA: Universitair Ziekenhuis Antwerpen, LEL: Lymphoepithelial lesion, HGL: High-grade lymphoma, LGL: Low-grade lymphoma

Table 2: Comparison between various gastric lymphoma entities in studied cases of Tripoli Medical Center and Universitair Ziekenhuis Antwerpen hospital based on the World Health Organization/real classification

Diagnosis	Histological grade	TMC	UZA
MALT-L	LG	8	6
DLBCL + MALT-L component	HG	3	
DLBCL	HG	10	7
FL-HG	HG	2	1
MCL	HG - LG	1 (pleomorphic)	5 (classic)
Burkitt's lymphoma	HG	0	1

TMC: Tripoli Medical Center, UZA: Universitair Ziekenhuis Antwerpen, MALT-L: Mucosa-associated lymphoid tissue lymphoma, DLBCL: Diffuse large B-cell lymphomas, MCL: Mantle cell lymphoma, FL-HG: Follicular lymphomas, HG: High-grade, LG: Low-grade

Table 3: Compares the proportions of high-grade lymphoma and low-grade lymphoma and proportions *Helicobacter pylori* infection in the whole group and in the mucosa-associated lymphoid tissue lymphoma subgroup

Characteristics	TMC, *n* (%)	UZA, *n* (%)
Grade of lymphoma		
HGL	16 (66.7)	9 (45.0)
LGL	8 (33.3)	11 (55.0)
Helicobacter pylori infection status		
All	12/24 (50)	7/20 (35)
MALT-L	4/8 (50)	3/6 (50)

Between Tripoli Medical Center and Universitair Ziekenhuis Antwerpen hospitals. HGL: High-grade lymphoma; LGL: Low-grade lymphoma, MALT-L: Mucosa-associated lymphoid tissue lymphoma, TMC: Tripoli Medical Center, UZA: Universitair Ziekenhuis Antwerpen

Gastric MALT-L lymphomas are subdivided into high and LGLs according to the presence of blast cells in the gastric biopsy sample. At the time of the diagnosis, 75%–80% of lymphomas are classified as low grade.[10,16-20] It is very common to find both types of lymphoma in the same lesion, and it has been reported that up to one-third of LGLs progress to HGLs[8,21,22] suggesting that both lesions represent the same tumor process, and in three cases of TMC both types of lymphoma in the same lesion were found. Lymphoproliferative disorders probably represent the only tumor type in which immunohistochemical evaluation is mandatory in all cases. In fact, the current WHO lymphoma classification has been largely formulated as a result if the integration of clinical and morphological features with immunophenotypical and molecular genetic data, thus defining clinicopathological entities.

Careful examination of the histological appearance of a pathological lesion is the main basis of pathological diagnosis. The distinction between lymphoma and reactive lymphoid infiltrate, between lymphoma and undifferentiated carcinoma or even sarcoma, and between different types of lymphoma can be extremely difficult using conventional histological techniques in many cases, but of great clinical importance. Hence, immunohistochemical demonstration of the antigenic determinant of cells helps the pathologist to distinguish more reliably between the most neoplastic and reactive proliferation of lymphocytes, nonlymphoid disorders, and different lymphoma types.[23]

The immunohistochemistry applied on the studied cases of gastric lymphoma revealed that there is diffuse membranous positivity of CD20 in all cases except in one case of MALT-L, which was CD20 negative, but CD79a strongly positive. MALT-L immunophenotype revealed positive for pan-B-cell markers CD20 and CD79a and negative for CD5 and CD10. MCL was positive for CD5 and showed nuclear staining for cyclin D1, and both MALT-L and MCL were negative for CD10. Follicular lymphoma showed CD10 positivity while CD5 was negative. DLBCL showed CD5, CD10, and Bcl6 negativity in the studied cases. Burkitt's lymphoma revealed positivity for pan-B-cell markers (CD20 and CD79a) and CD10. Furthermore, it was positive for EBV immunostain and ISH, while Ki 67 revealed nuclear positivity more than 95% of the tumor cells. Lambda and kappa immunoglobulin light chains immunostain was applied on one case of MCL, which confirmed the monoclonality of the atypical lymphoid infiltrate.

Conclusions

Primary gastric lymphoma mostly depends on the histological features and immunohistochemical phenotype added to the genotype and the clinical features. This study showed no differences between the two patient populations from Libya and Belgium.

Author's contribution

All authors contributed to the conception and conduct of the study, drafting and revision of the manuscript and approval of the final version thereof. Our coauthor Eric Van Marck has unfortunately passed away soon after completion of the study.

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Conflicts of interest

There are no conflicts of interest.

Compliance with ethical principles

This study was approved by the Institutional Review Board for retrospective analysis of archived samples. No consent was required.

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