Endoscopic semiotics with immunohistochemical analysis of gastric non-Hodgkin's lymphoma

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Summary. Comparative study of endoscopic view of different forms of gastric non-Hodgkin's lymphoma and their immunohistochemical type was made for 56 patients. It was shown that among all studied lymphoma cases at gastroscopy the most common were ulcerative infiltrative and ulcerative forms of gastric NHL. At immunohistochemical study of biopsy specimens MALT lymphoma and diffuse large B-cell lymphoma were determined in the majority of cases. Immunohistochemical verification of lymphoma determines approaches to treatment policy selection and subsequent endoscopic monitoring of patients.

Key words: gastric non-Hodgkin's lymphoma, immunohistochemical types, endoscopic diagnostics.

Non-Hodgkin's lymphomas (NHLs) – are lymphoid tissue clonal disorders that originate from lymphoid cells of various differentiation levels that

have different malignancy grades and localization. NHLs account for about 20% of all tumors of human lymphoid system. According to different authors data gastrointestinal tract is the most commonly affected (15-24%) that comprises 30-45% of all extranodal NHLs [1-3]. In many countries including Ukraine, NHL incidence during recent two decades increased by more than 50% that is possible to associate with both diagnostics improvement and adverse environmental factors [4]. In recent years gastric cancer morbidity has declined and for NHL constant upward trend has been registered [5].

Because of absence of clear pathognomonic endoscopic gastric NHL characteristics, the diagnostics based on endoscopic semiotics only in the majority of cases is complicated, and it necessitates multiple biopsy material sampling.

In recent years immunohistochemical examinations were widely applied for diagnostics of malignant tumours, including lymphomas [6-8]. Immunohistochemical examinations (IHCE) – is a method of tissues microscopic examinations that provides detection in tissues of the most specific substances of interest, based on microscopic sections treatment with labeled specific antibodies to detectable substance, which in this situation acts as antigen. The method allows identifying cells of different types by their unique marker characteristics, and studying synthetic and secretory processes in cell.

The following markers are indicative for lymphoid cells: CD1a, CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD19, CD20, CD22, CD79a, CD23, Bcl2, PAX-5, TdT, and others [9]. These markers are applied for lymphoma diagnosis

verification, lymphoma differential diagnostics with cancer, and lymphoma type determination.

Based on this method in 2008 WHO proposed lymphomas classification [10]. In stomach the following NHL types are found:

- diffuse large B-cell lymphoma
- mantle cell lymphoma
- Burkitt lymphoma
- MALT lymphoma
- follicular lymphoma

First three lymphoma types (diffuse large B-cell lymphoma, mantle cell lymphoma, Burkitt lymphoma) are more often seen at young and middle age, and their course has aggressive character that requires polychemotherapy (PCT) delivery. In that case the permanent endoscopic monitoring during patients' treatment is necessary to alter timely PCT scheme if therapeutic effect either absent or minor.

Indolent MALT lymphomas and follicular lymphomas are seen more often in elder patients. According to the number of authors' opinion, their formation is associated with Helicobacter pylori presence [11-13]. Clinical manifestations of these gastric NHL forms are insignificant and of torpid character.

Aim of the work – to determine interconnection between endoscopic view of gastric NHL and immunohistochemical disease variant.

Materials and methods of the study

In the Division of Endoscopic Diagnostics of the National Cancer Institute of the MOHC of Ukraine in the period from 2000 till 2011 NHL was diagnosed in 267 patients. From this patients' number IHCE were implemented in 56 (21%) patients. Endoscopic examinations were provided with videogastroscopes «Fujinon» and fibrogastroscopes «Olympus» (Japan). Endoscopic examination was accompanied with sampling of tissue fragment from affected zone for morphological examination and IHCE. Biopsied tissues fragments were fixed in 10% formalin or zinc-formalin, dehydrated and embedded into paraffin. Sections of 4 mcm thickness were prepared from paraffin blocks, stained with hematoxylin-eosin and azure II-eosin. Diagnose was established on the basis of histological preparations study results, and the panel of immunohistochemical markers that were necessary for differential diagnostics of the detected pathological process and morphologically similar lymphoid tissue tumors was prepared. For IHCE the sections of 4 mcm thickness were stuck on slides with polylysine, they were deparaffinated, and treated for antigen determinants restoration. In the study the antibodies to CD5, CD10, CD19, CD20, CD22, CD79a, CD23, and bcl-2 were used.

The antibodies were applied onto sections after endogenous peroxidase blocking with hydrogen peroxide solution in methanol. For the reaction results visualization the method ABS or EnVision was applied, as chromogen 3',3'-diaminobenzidine was used. In every case, negative control studies were conducted.

In the study group there were 31 men (55%) and 25 women (45 %), their age varied from 30 to 75 years. Average age was 58 years.

Results and their discussion

Endoscopic diagnostics in patients demonstrated that in the majority of cases NHL localized in distal department and in body of stomach.

From the data presented in the Table 1 it is seen that the most common gastric NHL forms were ulcerative infiltrative and ulcerative. In all the patients NHL diagnosis was histologically verified based on biopsy material examination. However, only in 25% of observations morphological verification was obtained at study of biopsy material that was sampled at first endoscopic examination. It is important to emphasize that in 75% of patients NHL was confirmed morphologically based on study of biopsy material obtained at repeated, and in many cases multiple endoscopies. IHCE application even at small fragmentary bioptate offers the opportunity to establish directly correct diagnosis and to discover immunohistochemical type of lymphoma (Table 2).

Table 1

Endoscopic forms of gastric NHL

N	Endoscopic form of gastric NHL	Patients' number	
		n	%
1	Exophytic	2	3.4
2	Ulcerative	19	34
3	Infiltrative	8	14.2
4	Ulcerative infiltrative	25	45
5	Mixed	2	3.4
	Total	56	100

Table 2

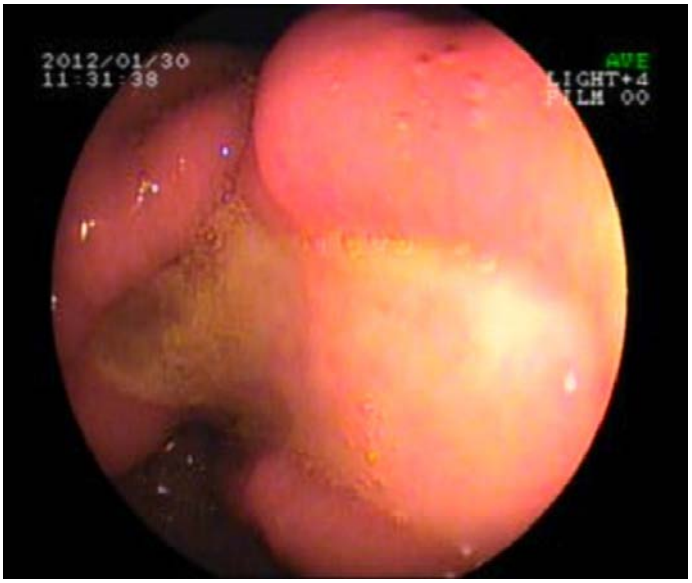
Immunohistochemicl types of gastric NHL

N	Diagnosis based on immunohistochemical study results	Patients' number	
		n	%
1	Diffuse large B-cell lymphoma	8	14.3
2	Mantle cell lymphoma	1	1.8
3	Burkitt lymphoma	1	1.8
4	MALT lymphoma	44	78.5
5	Follicular lymphoma	2	3.6
	Total	56	100

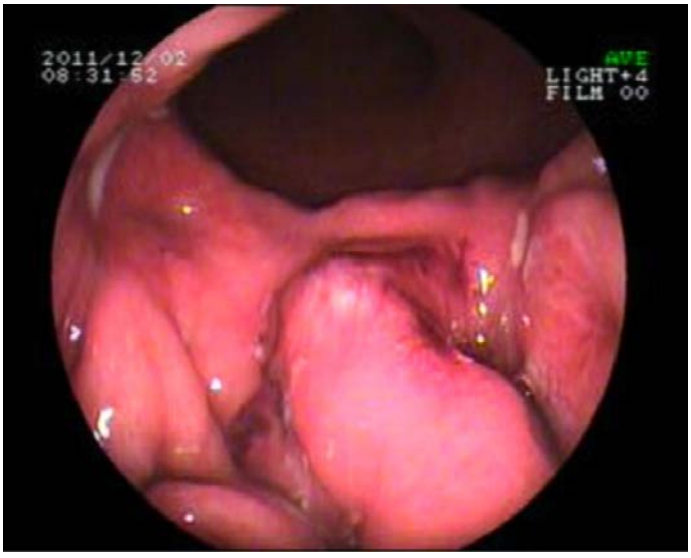
It was determined that at diffuse large B-cell lymphoma ulcerative infiltrative and ulcerative forms were revealed by endoscopy in 50% of patients (Picture 1). This lymphoma type was characterized by CD19, CD20, CD79a, and PAX-5 expression. In mantle cell lymphoma and Burkitt lymphoma endoscopic view in patients appeared in ulcerative form (multiple deep ulcerations). At that substantial CD5, CD20, CD79a, PAX-5, and bcl-1 expression was observed. At Burkitt lymphoma, expression of pan-B cell antigens CD20 and CD79a in association with CD10 and bcl-6. was observed. At MALT lymphoma (Picture 2) ulcerative infiltrative affection was seen in 56% of patients, and ulcerative form – in 30% of patients (in a form of surface multiple ulcerations), infiltrative – in 9%, and mixed form – in 5% of cases. Immunohistochemical analysis demonstrated that for this lymphoma type was indicative expression of CD19, CD20, CD35, CD79a, PAX-5, and bcl-2. Our obtained results agree with other authors results [14]. In 3.6% of examined patients,

follicular NHL form was observed (Picture 3). By endoscopy in these patients exophytic gastric affection was observed. Follicular lymphoma phenotype was characterized by CD19, CD20, CD10, CD79a, bcl-2, and bcl-6 expression.

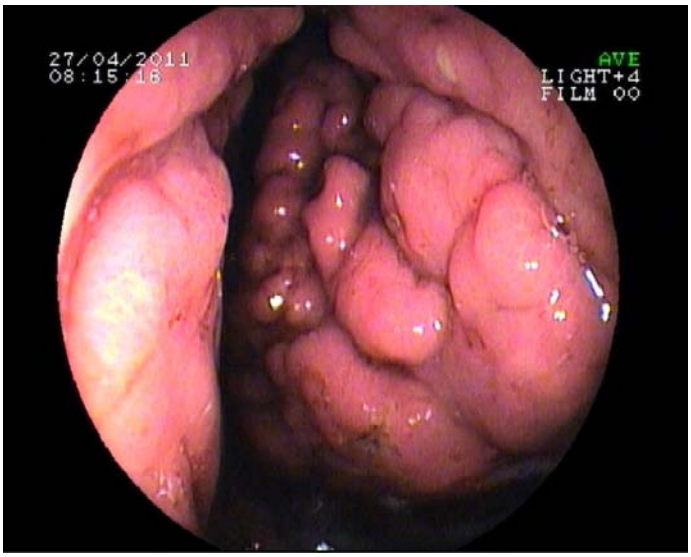
Stomach affection at indolent lymphomas was presented by ulcerative infiltrative, infiltrative, exophytic and mixed affections.



Picture 1. Ulcerative NHL form of gastric antral department (ulcerative defect of irregular shape, located on greater gastric curvature, at pinch palpation the tissue is tight, at biopsy sampling the forceps slide along neoplasia surface), at IHCE – difuse large B-cell lymphoma



Picture 2. Ulcerative infiltrative NHL form of lower third of the stomach body (two ulcerative defects along anterior and posterior walls, along large curvature – mucous membrane infiltration, at pinch palpation and biopsy sampling – the tissue in ulcers site is tight, in infiltration site – the tissue is loose, it is easily detached), at IHCE – MALT lymphoma



Picture 3. Infiltrative NHL form of gastric body (at pinch palpation gastric wall is confining, at biopsy sampling mucous membrane is poorly dragged after forceps, it is easily detached), at IHCE – MALT lymphoma. At follicular lymphoma exophytic gastric affection is revealed.

From obtained data it is possible to conclude that aggressive lymphomas forms (diffuse large B-cell lymphoma, mantle cell lymphoma, and Burkitt lymphoma) endoscopically manifest more commonly in the form of ulcerative and ulcerative infiltrative gastric affection comparing with MALT lymphomas and follicular lymphomas.

Conclusions

1. Endoscopic examinations offer the opportunity to determine gastric NHL affections that enable to predict disease course and patient's treatment policy.

2. Based on the material studied it was determined that from all examined lymphomas the most common are ulcerative infiltrative and ulcerative gastric NHL forms.
3. At biopsy material and IHCE more commonly MALT lymphomas and diffuse large B-cell lymphomas were determined.
4. Immunohistochemical lymphomas verification determines approaches to treatment policy choice and subsequent patients' endoscopic monitoring. Such approach allows individualizing therapy in patients with gastric NHL and correcting it timely.

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