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Original Article

HISTOLOGICAL ASPECTS AND FLOWCYTOMETRIC IMMUNOPHENOTYPING IN PATIENTS WITH CHRONIC GASTRITIS AND ANTIBODIES AGAINST HELICOBACTER PYLORI

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Abstract

***Background**. Helicobacter Pylori infection is frequently encountered in clinical practice in patients with upper digestive complaints. Chronic gastritis, diagnosed using endoscopy combined with histological examination of gastric mucosal fragments in Helicobacter Pylori positive patients, is a risk factor for gastric cancer development.*

***The aim** of the study is to determine the histological changes of gastric mucosa in patients with antibodies anti HP and endoscopic diagnosed gastritis, and to assess the immune status of these patients using flow cytometric immunphenotyping of peripheral lymphocytes.*

***Material and methods.** The cohort of patients was selected from the outpatients with dyspeptic symptoms, admitted to the Endoscopy Department of, , Sfâtul Ioan", Emergency Hospital. The group consisted of 50 patients, with ages between 40 and 70 years and serologically positive for Helicobacter Pylori antibodies. The patients were examined on one hand endoscopically and one the other hand, in those cases with gastritic lesions, the examination was completed with histology and immunophenotyping of peripheral lymphocytes using flow cytometry.*

***Results.** The group consisted of 33 (66%) male patients and 17 (34%) female patients, HP positive at serological testing. It was divided into three age subgroups: one between 40-50 years with 25 patients (50%), other one between 50-60 years - 21 (42%) patients and another between 60-70 years 4 patients (8%). Upper digestive endoscopy was performed in these patients and in 17 subjects (34%) were identified gastritic lesions, that were characterized into four different endoscopical types: eritematous gastritis, erosive gastritis, atrophic gastritis and biliary reflux gastritis. Histological examination of gastric samples from these patients confirmed gastritis only in 10*

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patients (58%) from the 17 subjects found with macroscopic mucosal changes. All 17 patients with endoscopical changes suggestive for gastritis were sent for performing immunophenotyping of peripheral lymphocytes using monoclonal antibodies.

Conclusions. *HP positive patients at serological testing who present chronic gastritis at upper digestive endoscopy, express an altered immune status as results from immunophenotyping of immune cells. The most common histological type of gastritis identified in the study group was eritematous gastritis.*

Keywords: *Helicobacter pylori - chronic gastritis – immunophenotyping – flow cytometry*

Rezumat

Infecția cu Helicobacter Pylori (HP) este frecvent întâlnită în practică la pacienți cu afecțiuni digestive superioare. Gastrita cronică diagnosticată histologic pe fragmentele de mucoasă cu Helicobacter Pylori pozitiv recoltate endoscopic, reprezintă un factor de risc pentru cancer gastric.

Obiectivul *acestui studiu este identificarea modificărilor histologice mucoase la pacienți cu anticorpi antiHP, diagnosticarea endoscopică a gastritei şi studiul statusului imunologic al pacienților prin imunofenotipizarea citometrică a limfocitelor periferice.*

Material şi metodă. *Lotul pacienților a fost selectat din cei cu tulburări dispeptice, internați in Departamentul de Endoscopie al Spitalului Clinic de Urgențe, , Sfântul Ioan", din Bucureşti. Vârsta celor 50 de bolnavi a fost de 40-70 ani, cu anticorpi antiHP pozitivi serologic. Pacienții au fost examinați endoscopic şi, la cei cu leziuni cronice de gastrită, s-a practicat flow-citometrie din limfocitele periferice.*

Rezultate. *Un grup de 33 (66%) bărbați şi 17 (34%) femei au fost depistate cu HP pozitiv serologic. Au fost grupați in 3 categorii de vărstă: 40-50 ani (25 pacienți - 50%), 50-60 ani: 21 pacienți (42%) şi 60-70 ani: 4 pacienți (8%). La 17 subiecți (34%) s-au depistat 4 tipuri de gastrită: eritematoasă, erozivă, atrofică şi gastrită de reflux biliar, s-a practicat endoscopie digestivă superioară. Examenul histopatologic a evidențiat gastrită cu modificări mucoase la 10 din cei 17 bolnavi. La toți 17, s-a practicat fenotipizarea citometrică a limfocitelor periferice cu anticorpi monoclonali.*

Concluzii*. Pacienții HP pozitivi testați serologic, cu leziuni endoscopice de gastrită cronică prezintă un status immunologic alterat, diagnosticat prin flow-citometrie.*

Gastrita eritematoasă este forma cea mai frecvent intâlnită.

Cuvinte-cheie: *Helicobacter pylori, gastrită cronică, imunofenotipizare, flow-citometrie*

Introduction

Helicobacter Pylori (HP) infection is largely encountered worldwide and the prevalence is correlated with socio-economical status (1). So, in developing countries the prevalence is around 80% in adult population, while in western countries the incidence is under 50% (2). It is now well recognized the role of HP in certain digestive conditions, like chronic gastritis, peptic ulcer disease, gastric cancer, MALT lymphoma (3, 4). HP infection has a pivotal role in gastric carcinogenesis as several studies demonstrated (5, 6), following the sequence of intestinal metaplasia-dysplasia-carcinoma. WHO classified this microorganism as a type I carcinogen, since 1994 (2). HP is responsible for more than 80% of cases with chronic gastritis. Inflammatory changes of gastric mucosa present a progressive course, evolving to atrophic lesions, intestinal metaplasia, dysplasia with subsequently malignization.

The main tool used in clinical practice to diagnose gastritis is upper digestive endoscopy, associated with histological examination to clearly define the mucosal lesions.

The issue of this study is to determine the immune status of patients with chronic gastritis Helicobacter Pylori positive and to establish if there is a link between an altered immunity and the initiation of the events responsible for gastric cancer development.

The examination of peripheral lymphocytes through flow citometry using a panel of monoclonal antibodies is able to provide a complete picture of the relationship between Helicobater Pylori infection, chronic gastritis and the risk of a future gastric cancer.

Flow cytometry represents a high resolution technique that measures multiple characteristics of a single particule flowing in a stream of fluid (8). The method represents a reliable tool for defining different cell populations and for identyfing phenotypic aberrancies suggestive for malignancy (9). Flow cytometry can be used for immunophenotyping both solid tissues and fluid samples (8). This technique is able to provide qualitative and also quantitative data (9).

The method allows to characterize different cell populations from peripheral blood, including lymphocytes, monocytes, granulocytes, from morphological and phenotypical point of view. The goal of immunphenotyping is to assess the proportion of lymphocytes sets from peripheral blood. Taking into account their biological function and surface antigen expression, the lymphocytes can be divide into three major groups: T lymphocytes (CD3+), B lymphocytes (CD 19+) and natural killer cells (NK-CD3-CD16+/CD56+). T lymphocytes are involved in cell mediated specific – antigen immunity and regulate immunoglobulin synthesis by B lymphocytes. On the other hand, T lymphocytes can be classified on functional basis in helper/inducer lymphocytes (CD4+) and suppressive /cytotoxic (CD8+). At the same time, this technique provides an evaluation of activated lymphocytes, using the expression of the membrane antigen HLA-DR, that belongs to the major histocompatibility complex, MHC class II.

NK cells mediate antibody-independent cell-mediated cytotoxicity, acting as a first defence line to neutralize tumoral cells and viral infected cells.

The analysis of B and T lymphocytes populations, NK cells, T lymphocytes subsets and also of the ratio CD4+/CD8+ has an important role in characterizing the immune status and in detecting a possible immunodeficiency responsible for tumor development.

Material and methods

The study group consisted of fifty patients irrespective of sex, with dyspeptic complaints and antibodies against Helicobacter Pylori, selected from the outpatients admitted to the Endoscopy Department of the, , Sfântul Ioan" Emergency Hospital. The age varied between 40 and 70 years. We designed a certain protocol for a uniform examination of the patients : superior digestive endoscopy for gastritis detection, followed, on one hand by biopsy collecting and histological examination in positive cases. On the other hand, in the cases with endoscopical diagnosed gastritis, immunophenotyping of peripheral lymphocytes using flow cytometry was performed. For the upper digestive endoscopy we used the System Olympus Exera CV 160. The histopathological examination of the gastric mucosal samples taken during endoscopy used the well-known method of coloring with hematoxilin-eozin. An important part of the study consisted of immunophenotyping technique of lymphocytes populations.The method determines the percentages of lymphocytes populations and subpopulations from peripheral blood and uses the kit Simultest IMK-Lymphocyte (Becton Dickinson, CA). This kit contains a mixture of monoclonal antibodies directed against targeted antigenes, antibodies that are conjugated to fluorochromes using direct immunoflorescence technique. The kit contains several types of antibodies. IgG1/IgG2a are used to appreciate the quantity of the antibodies that are not specifically binded to standard cells in human blood, through Fc receptor. The role of CD45/CD14 is to establish the lymphocyte gate in SimulSet programe. CD3/CD19 are used to recognize B lymphocytes and CD4/CD8 to recognize T lymphocytes. CD3/Anti-HLA-DR detect activated T lymphocytes, CD3/CD16+CD56+ identify NK cells and CD25 recognize IL 2. After the lymphocytes are labelled with specific antibodies, anucleate elements are removed using a lysis solution. The samples were rinsed with PBS and then fixed in paraformaldehyd. The data was collected using an aparatus FACScan (Becton-Dickinson) in FACScan programe, and the results were expressed in percentages of positive cells (IIF%) and in median values of fluorescence intensity (MFI sau mean). The analysis of the data and the graphic presentation was achieved using the programe WinMdi 2.8, on a PC.

Results

The majority of patients consisted of 33 (66%) male patients and 17 (34%) female patients (Figure no. 1 – see next page), who were serologically tested for HP and expressed positive antibodies.

The group was divided into three age subgroups: 25 (50%) patients were between 41 and 50 years, 21 (42%) patients between 51-60 years and 4 (8%) patients between 61-70 years (Figure no. 2).

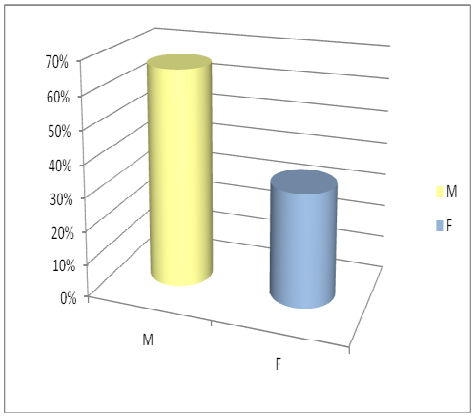

Figure 1 -
Sex distribution in study group

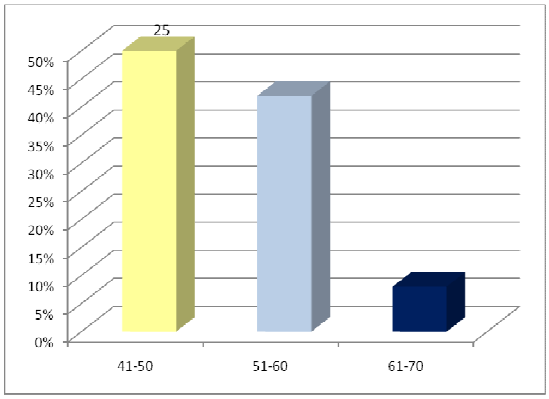

Figure 2 -
Age distribution in study group.

All patients with gastric complaints underwent upper digestive endoscopy, and 17 (34%) patients presented lesions suggestive for gastritis. Taking into account the macroscopic appearance (Figure no. 3a, 3b), we classified gastritis in four different endoscopical types: eritematous gastritis in 13 cases (76%), erosive gastritis in 2 cases (12%), atrophic gastritis and biliary reflux gastritis one case respectively (5%).

Figure 3a - Eritematous gastritis – endoscopic appearance

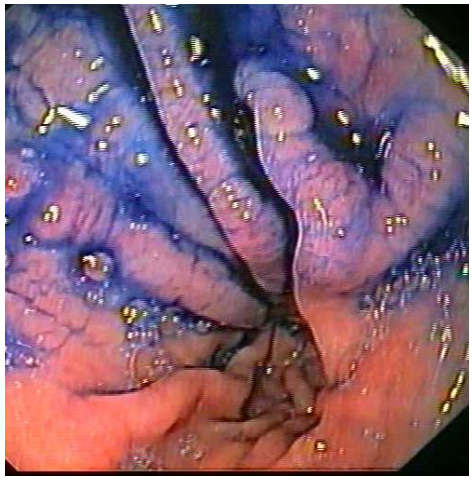

Figure 3b - Eritematous gastritis- chromoendoscopy with indigo carmin

Gastritis was histological diagnosed in 10 (58%) patients from the entire study group (Figure no. 4).

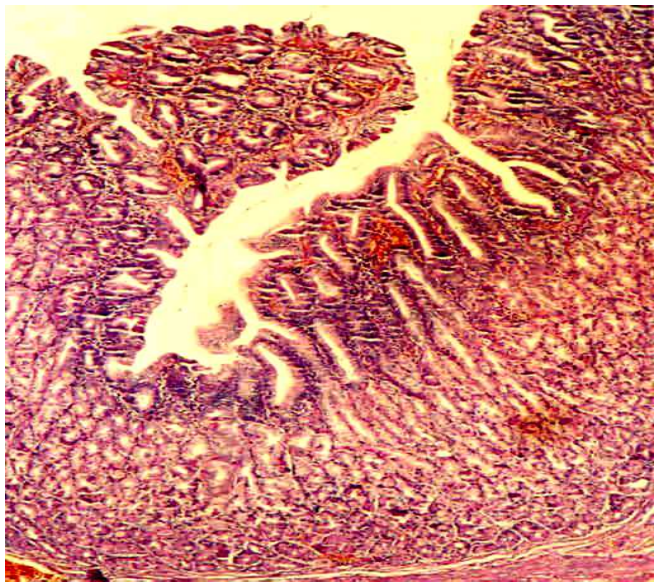

Figure 4 - Fundic atrophic gastritis (HE x 10)

All the patients with endoscopic gastritis were immunologically analysed through flow cytometry in order to establish their immune status. The results were presented like bidimensional graphics dot-plot. Each cell is represented through a point localized on the graphic taking into account the intensity of the parameters on the axis (FSC, SSC, FL-1 sau FL-2). In the first dial there are represented the cells that binded the antibody marked with PE but not with FITC (FL-1-/FL-2+), in the second dial there are the cells that binded both types of antibodies - duble staining, double positive, (FL-1+/FL-2+), in the third dial there are duble negative cells (FL-1-/FL-2-), and in the fourth dial, there are visualized the cells that binded the antibody marked with FITC, but not with PE (FL-1+/FL-2-). The programe calculates then, automatically the percentage of events in each dial. The results of the analysis regarding the percentages of lymphocytes populations and subpopulations in the cohort of patients are presented in the tabel **(Tabel no. 1).**

The mean values of the antigen expression associated with peripheral lymphocytes in HP positive patients were the following:

- CD3: 74.3 + 6.2%
- CD19: 12.36+3.0%
- CD4: 42.71+5.2%
- CD8: 28.43%+7.0%
- HLA-DR: 24.5+5.7%
- NK: 5.3+7.6%
- CD25: 3.9+1.2%

Tabel no. 1: Analysis of the percentages of lymphocytes populations and subpopulations in cohort patients

Nr. crt.	Pacient	CD45	CD3 (Lf.T total)	CD19 (Lf.B total)	CD4 (Lf.Th)	CD8 (Lf.Tc)	CD4/CD8	CD3/*HLA-DR*	CD3/*CD16+ CD56+* (Lf.T total/NK total)	CD 25
1.	BA (443)	96.22	79.55	12.61	44.82	29.62	1.51	84.14/ **21.84**	81/ **1.52**	3.99
2.	CR (444)	97.45	63.78	12.66	36.9	33.97	1.09	64.4/ **22.3**	62.4/ **14.95**	1.43
3.	LC (445)	97.2	60.71	9.44	34.75	33.52	1.04	59.29/ **18.88**	54.16/ **27.56**	2.27
4.	DA (446)	94.61	51.69	8.93	30.13	37.62		54.41/ **20.97**	53.22/ **29.98**	1.86
5.	PV (447)	94.66	61.27	9.05	34.49	35.99		60.83/ **26.92**	61.41/ **20.79**	1.47
6.	TO (448)	93.76	65.99	15.68	41.77	27.15	1.54	66.49/ **25.22**	65.16/ **12.35**	2.26
7.	DO (449)	92.39	64.06	16.08	25.57	40.01		64.73/ **30.56**	62.72/ **11.33**	1.49
8.	FT (450)	86.52	70.10	11.45	37.93	33.73	1.12	70.79/ **39.21**	69.55/ **9.30**	1.82
9.	BN (451)	92.41	63.77	13.04	39.09	24.65	1.59	64.99/ **21.84**	60.57/ **10.82**	1.93
10.	SM (452)	93.58	65.08	6.82	34.91	33.8	1.03	63.04/ **18.79**	60.96/ **11.42**	2.9
11.	MM (453)	97.45	59.33	6.32	29.52	37.82		61.05/ **18.22**	58.66/ **26.46**	4.75
12.	GZ (454)	89.24	67.10	9.53	41.14	25.86	1.59	61.92/ **26.74**	66.63/ **15.21**	1.07
13.	DA (455)	92.8	72.61	7.53	42.83	30.39	1.41	74.4/ **22.13**	74.58/ **9.06**	3.33
14.	CA (456)	98.35	65.27	6.72	31.53	42.15		66.38/ **15.96**	65.12/ **19.06**	1.65
15.	PE (457)	95.63	62.55	12.89	34.25	29.29	1.17	64.87/ **20.33**	61.66/ **10.91**	0.42
16.	BA (458)	96.57	57.70	10.15	35.78	12.81	2.8	60.5/ **18.6**	58.28/ **15.27**	0.75
17.	**AV (459)**	**96.57**	**69.04**	**12.11**	**40.60**	**27.24**	**1.49**	**66.61/ 27.13**	**68.2/ 9.05**	**3.76**

The data showed that some patients presented with values of NK cells lesser than the normal range (Figure 5a), while others presented increased levels of NK cells (Figure 5b):

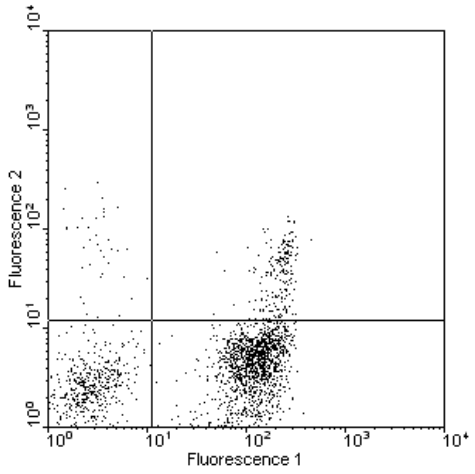

Figure no. 5a: NK=1.53%, up left dial

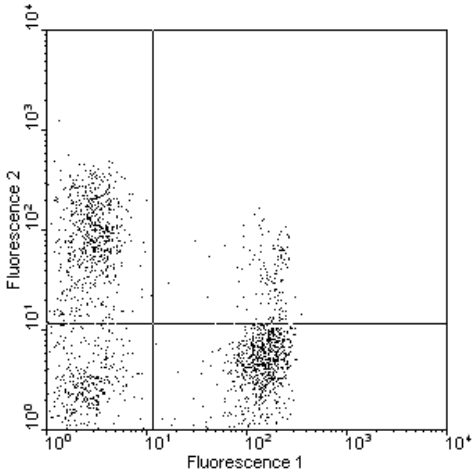

Figure no. 5b: NK=30.48%, up left dial

At the same time, we found out that some patients showed decreased value for the ratio CD4/CD8 (Figure 6).

The analysis of the results showed increased values for HLA-DR in all patients from the group (Figure 7). The data obtained from the studied patients suggested a possible depressed immune status in patients with chronic gastritis HP positive.

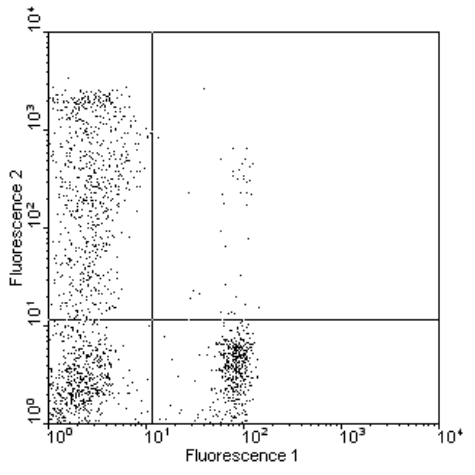

Figure 6 -
CD4/CD8 = 0.801

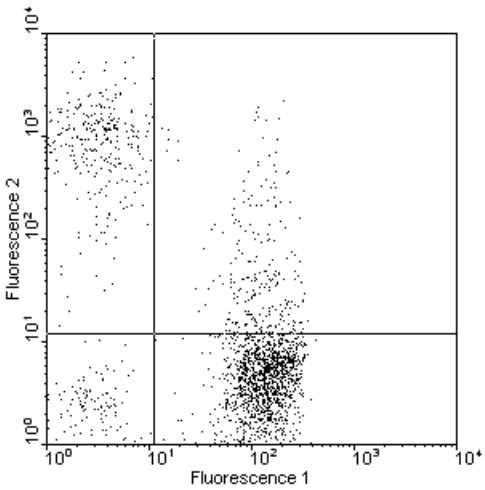

Figure 7 -
HLA-DR=21.84%, superior dials

Conclusions

The endoscopical and histological analysis, associated with immunophenotyping data stated that HP positive patients at serological testing who present with chronic gastritis diagnosed macroscopically, most frequently eritematous gastritis, may present with an altered immune status. This could be related to the development of gastric cancer, in cases that progress to atrophy, metaplasia and dysplasia. Assessing the immune status of patients HP positive with chronic gastritis using flow cytometry may represent a long-term method of follow-up for the risk of gastric cancer development. It can also be helpful for including the patients found to be at risk in endoscopic surveillance programe.

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