The study of tissue IgG4 in the mucous membrane of the colon in patients with inflammatory bowel disease

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Abstract. **Background.** Inflammatory bowel disease (IBD) is a global problem today, with a growing prevalence in the world. It significantly increases the economic burden on the health care system. Recently, many studies indicate the important role of immunoglobulin G4 (IgG4) in the formation of chronic inflammation in IBD and the possibility of using it as a biomarker of the inflammatory process. The purpose was to improve the diagnosis of chronic inflammatory bowel diseases by studying the status of IgG4-positive plasma cells in the mucous membrane of the colon in patients with ulcerative colitis (UC) and Crohn’s disease (CD). **Materials and methods.** We have examined 34 patients with IBD, 25 with UC and 9 with CD, of them 20 women and 14 men, with an average age of (38.8 ± 3.0) and (38.2 ± 3.7) years, respectively. Patients were divided into groups depending on the nosology and severity of the disease. All patients underwent endoscopic examination of the colon to establish or clarify the diagnosis, and biopsy specimens were taken for histological and immunohistochemical examination. **Results.** In 13 (38.3 %) of 34 examined patients, a positive result for the presence of tissue IgG4 (≥ 10 cells in the field of view) was found. Among patients with UC, 48 % have a positive result of immunohistochemical examination of tissue IgG4, in people with CD, this figure is 11.1 %. This gives us reason to say that in UC, elevation of tissue IgG4 levels occurs 4.4 times more often. Positive tissue IgG4 in patients with moderate UC was found 1.1 times more often than in severe UC. Among patients with mildly active disease, tissue IgG4 was not detected. **Conclusions.** In UC, IgG4-positive cells in the mucous layer of the colon are more common than in CD, which makes it possible to use this indicator for the differential diagnosis of ulcerative colitis and Crohn’s disease. Positive tissue IgG4 is more common in moderate form than in severe one. **Keywords:** inflammatory bowel diseases; ulcerative colitis; Crohn’s disease; IgG4

Introduction

Today, inflammatory bowel disease (IBD) has become a global problem with increasing prevalence on all continents. It is estimated that more than 1 million people in the United States and 2.5 million in Europe have IBD, which significantly increases health care expenses. The main factors that influence the financial component are the cost of hospitalization and surgery. At the same time, the cost of reduced quality of life is difficult to measure but this component invariably affects patients and their families [1–3].

The term IBD was introduced to denote two conditions: ulcerative colitis (UC) and Crohn’s disease (CD). These nosologies are divided into categories depending on the severity of the disease, the location of the lesion and the duration of the disease [4, 5]. Diagnosis of inflammatory bowel disease is usually based on clinical, endoscopic, radiological, histological examinations. This is a standard that remains unchanged, despite the new understanding of the immunological and genetic component of IBD [1, 6, 7].

Inflammatory bowel diseases are chronic disorders that occur as a result of the interaction between genetic and environmental factors [8]. In most patients, IBD is diagnosed at a young age. The accurate etiology of IBD is unknown, and therefore causal therapy for this disease is not yet available [9].

The importance of the role of humoral immunity in the formation of chronic inflammation in IBD has been overlooked for many years, and only in the last 5 years, there have been studies that indicate the importance of B-lym-
phocytes in the pathogenesis of inflammation. B-lymphocytes are finally differentiated into plasma cells, which are characterized by the production of various antibodies. These antibodies are actively involved in the regulation of humoral immunity and can cause allergic reactions and multiple autoimmune disorders [10, 11]. Excessive plasmacytic infiltration in the lamina propria is a classic pathological characteristic of IBD, which indicates the potential involvement of B-cells in the pathogenesis of this condition [12, 13].

The study of the role of humoral immunity in the pathogenesis of IBD and the possibility of using immunoglobulins as inflammatory biomarkers is one of the promising directions of diagnosis. And the use of tissue biomarkers for the differential diagnosis of UC and CD will help expand and deepen the research area for IBD [12, 14, 15].

The main indicator of the status of humoral immunity is the content of immunoglobulins (IgA, IgM, IgG) [12]. IgG4, which is the smallest of all IgG isotypes, usually was ignored because it was considered a non-inflammatory antibody. However, its role in various autoimmune disorders and even in the occurrence of cancer has been the subject of research in recent years, along with the detection and prevalence of IgG4-related diseases [5, 11, 16].

IgG4 is a heterogeneous antibody, which may be directly pathogenic, has a protective role, or may be a random marker of an aberrant inflammatory response. IgG4 antibodies have exceptional structural and functional characteristics, indicating anti-inflammatory and resistance-promoting effects [17, 18]. This emphasizes the need for research in this area, which aims at improving the diagnosis, the ability to predict these diseases that will allow improving the quality of treatment and life of patients with IBD.

The purpose was to improve the diagnosis of chronic inflammatory bowel diseases based on the study of IgG4-positive plasma cells in the mucous membrane of the colon in patients with ulcerative colitis and Crohn’s disease.

Materials and methods

We have examined 34 patients with IBD. There were 25 individuals with UC and 9 with CD, including 20 women and 14 men, with an average age of (38.8 ± 3.0) and (38.2 ± 3.7) years, respectively. Patients were divided into groups depending on the nosology and severity of the disease determined by the Mayo score (Table 1). Among people with UC, mild form (3–5 points) has been detected in 3 patients (group I), moderate (6–10 points) — in 18 (group II), and severe (11–12 points) — in 4 (group III). In patients with CD (group IV), the severity was evaluated by the Crohn’s disease activity index developed by W.R. Best (Table 2).

Patients were enrolled in the study regardless of gender and age.

Endoscopic examinations of the colon were performed according to conventional methods using a Pentax EC-380LKp video colonoscope (Japan) to establish or clarify the diagnosis, analyzing changes in the state of the colon mucosa, taking biopsy specimens for histological and immunohistochemical examinations.

To determine the endoscopic activity of UC, the Mayo score was used: remission (degree 0), mildly active (degree 1), moderately active (degree 2), severely active (degree 3).

### Table 1 — Disease severity according to the Mayo score

| Parameter | Score |
|-----------|-------|
| Stool frequency | Normal number of stool for patient — 0 points; 1 to 2 stools per day more than normal — 1; 3 to 4 stools more than normal — 2; ≥ 5 stools more than normal — 3. Total score — from 0 to 3 points |
| Rectal bleeding | No blood seen — 0 points; streaks of blood with stool less than half the time — 1; obvious blood with stool most time — 2; blood alone passes — 3. Total score — from 0 to 3 points |
| Endoscopic finding | Normal or inactive disease — 0 points; mild disease — 1; moderate disease — 2; severe disease — 3. Total score — from 0 to 3 points |
| Physician’s global assessment | Normal — 0 points; mild disease — 1; moderate disease — 2; severe disease — 3. Total score — from 0 to 3 points |

Score: ≤ 2 points — clinical remission; 3–5 points — mild activity; 6–10 active — moderate activity; 11–12 score — severe activity

### Table 2 — Crohn’s disease activity index

| Clinical or laboratory variable | Weighting factor |
|-------------------------------|-----------------|
| Number of liquid of soft stools each day for 7 days | ×2 |
| Abdominal pain (each day for 7 days): 0 — none; 1 — mild; 2 — moderate; 3 — severe | ×5 |
| General well-being (each day for 7 days): 0 — well; 1 — slightly under par; 2 — poor; 3 — very poor; 4 — terrible | ×7 |
| Complications: arthritis/arthralgia; mucocutaneous lesions (erythema nodosum, aphthous ulcers); anal disease (fissure, fistula, etc.); external fistula; fever > 37.5 °C each day for 7 days | ×20 each item |
| Use of diphenoxylate for diarrhea | ×30 |
| Abdominal mass: 0 — none; 2 — questionable; 5 — definite | ×10 |
| Absolute deviation of hematocrit: from 47 % in men; from 42 % in women | ×6 |

Deviation from standard weight, % | ×1

Index of severity | Total score

Score: < 150 points: remission; 150–300 points: mild disease; 301–450 points: moderate course; > 450 points: severe CD
gree 3) [19, 20]. This classification is based on the presence of changes and on the severity of macroscopic signs characteristic of UC: fade (loss) of vascular pattern, bleedings and defects of mucous membrane of the colon. It is proved that there is a strong relationship between the clinical activity of UC and the severity of endoscopic signs, as well as the prevalence of the colon lesions [21–23].

Typical signs of Crohn’s disease during endoscopic examination were the presence and severity of aphthae, deep longitudinal ulcers, cobblestone appearance, pseudopolypsis, strictures, and changes in the perianal area.

Immunohistochemical studies of biopsy material were performed on deparaffinized sections of the intestine, 3–5 μm thick. On the prepared sections, we applied 50–100 μm of rabbit serum diluted 1:200 (Abcam, USA) at +5 °C in a wet chamber for 8 hours. After that sections were washed in phosphate-buffered saline, pH 7.4, three times for 5 minutes. Avidin-streptavidin complex labeled with horseradish peroxidase was applied for 60 minutes. Then horseradish peroxidase was visualized with 0.005% solution of 3,3-tetrahydrochloride benzidine (Sigma, USA) in phosphate-buffered saline, pH 7.4. The number of IgG4-positive plasma cells was counted in 5 consecutive fields of view. A case was considered positive when ≥ 10 IgG4-positive cells were detected in one field of view in sections.

To optimize the results and automate data processing, the obtained indicators were entered into the database management system, which is created using the integrated application package Statistica for Windows 6.0.

Descriptive and inductive statistics were used for statistical data analysis. The average values of variables were compared by means of parametric methods (Student’s t-test) with a normal distribution of these features expressed in the interval scale. The χ² goodness of fit test was used to compare qualitative indicators. Differences between the two indicators were considered probable at p < 0.05 [24–26].

Results and discussions

Chronic relapsing course was observed in most patients with IBD. In 7 people, 5 with UC and 2 with CD, the diagnosis was established for the first time. The mean duration of IBD was (4.4 ± 0.7) years with no significant difference between groups.
As for the extent of intestinal lesions in patients of groups I and II, left-sided lesions of the colon are more typical (66.7 %). In group III, more extensive lesions of the colon were detected (50.0 % — left-sided and 50.0 % — total) (Fig. 1). In patients with CD (group IV), terminal ileitis prevailed (55.6 %).

The analysis revealed that in group I the remission stage prevailed (66.7 %) and a third of patients had a minimal stage of activity; in the group with moderate disease, the pronounced activity of ulcerative colitis prevailed in 55.6 % (p < 0.05) of cases, and in group III, all patients had severely active disease (p < 0.05).

In patients with Crohn’s disease, segmental lesions of the colon dominated (77.8 %), when foci of inflammation with defects alternate with areas of unchanged mucous layer. Left-sided colon lesions (44.4 %) were more frequent, in a third of patients, the terminal ileum was involved in the inflammatory process. Total colitis and rectal lesions were detected with a frequency of 11.1 %, in 2 cases (22.2 %), there was a lesion of the perianal area in the form of scarring, which indicated previous fistulas (Fig. 2).

In 55.5 % of patients, fibrotic strictures of the colon were determined, most of them were localized in the sigmoid section of the colon that is typical for CD. Two people (22.2 %) had fistulas.

To detect tissue IgG4, immunohistochemical staining of biopsy material was performed. The accumulation of IgG4-positive cells in UC and CD is shown in Fig. 3, 4.

In 13 (38.3 %) of 34 examined patients, a positive result for the presence of tissue IgG4 (≥ 10 cells in the field of view) was found. In 7 (20.5 %) people, the amount of IgG4 cells was 3–5 in the field of view, in 14 (41.2 %) cases, IgG4-positive cells were not detected (Fig. 5, 6).

In 48 % of patients with UC, the result of immunohistochemical examination for tissue IgG4 was positive. Among patients with CD, this figure was 11.1 % that allows us to say that in UC, tissue IgG4 elevation occurred 4.4 times more often.

Among 18 patients with moderate UC according to the Mayo score, a positive result for tissue IgG4 was found in 10 (55.5 %) cases, and in those with severely active disease — in 2 of 4 (50.0 %) (Fig. 7).

From the above data, we can conclude that with moderate severity of UC, positive tissue IgG4 is found 1.1 times more often than in severe UC. Among patients with mild severity, positive tissue IgG4 was not detected. Given the small sample of patients with CD, these data require further study.

Conclusions

1. More extensive lesions of the colon have been found in patients with severe UC than in those with mildly and moderately active disease.

2. In ulcerative colitis, IgG4-positive cells in the colonic mucosa are found 4.4 times more often than in Crohn’s disease, which may allow using this indicator for the differential diagnosis of UC and CD.

3. There was a tendency to more frequent detection of positive tissue IgG4 in moderate IBD than in severe.

4. It is planned to further study the dependence of tissue IgG4 levels in patients with IBD on the duration of the disease and the localization of lesions in the mucous membrane of the colon, which will allow developing methods to predict the severity of inflammatory bowel diseases, and in the future will help optimize therapy and improve the quality of life of patients.

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Вивчення тканинного IgG4 у слизовій оболонці товстої кишки в пацієнтів із запальними захворюваннями кишені

Резюме. Актуальність. На сьогодні запальні захворювання кишені є глобальною проблемою, поширеність якої зростає в усьому світі. Це значно підвищує економічне навантаження на систему охорони здоров’я. Останнім часом у багатьох дослідженнях вказують на важливу роль імуноологобуліну G4 (IgG4) у формуванні хронічного запалення захворювання, включно з біомаркером запального процесу. Метою дослідження було дослідження тканинного IgG4 у пацієнтів із запальними захворюваннями кишені відомих у високій частоті тяжких захворювань, з метою виявлення позитивних показників IgG4 у слизовій оболонці товстої кишки.

Матеріали та методи. Обстежено 34 пацієнтів з запальними захворюваннями кишені, які були розділені на дві групи: 1) пацієнти з виразковим колітом (VK) та ХК. Матеріали включали уретероскопічне та коліоскопічне дослідження товстої кишки, а також біопсії для гістологічного та імуногістохімічного аналізу.

Результати. У 13 (38,3%) пацієнтів виявлено позитивний результат на наявність тканинного IgG4 (10 клітин у полі зору). Серед пацієнтів з VK цей показник становить 48,0%, замикаючи 11,1% у пацієнтів з ХК. При середньому ступені тяжкості порівняно з тяжким течією, частота позитивних випадків IgG4 збільшується в 1,1 раза. Позитивний тканинний IgG4 виявляється частіше при тяжкому ступені тяжкості, ніж при середньому.

Висновки. Позитивний тканинний IgG4 у пацієнтів з VK змінюється більшістю, ніж при ХК. Це дає можливість використовувати цей показник для диференційної діагностики тяжких захворювань кишені.

Ключові слова: запальні захворювання кишені; виразковий коліт; ХК; IgG4