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ANALYSIS OF TISSUE EOSINOPHILS IN THE STRUCTURE OF GASTRIC POLYPS

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ABSTRACT — A study of the diagnostic value of tissue eosinophil infiltration in the structure of polyps of the human gastrointestinal tract and surrounding tissues was carried out. We investigated intestinal biopsies from 189 patients aged 40–90 years with polyps, cancer, metastases. This retrospective study allows us to note the possibility of using eosinophils in the development of criteria for early malignancy and a promising outcome of neoplasms in the mucous membrane. Eosinophils are important players in intercellular interactions within the polyp structure and the tissues around it. The complete absence of eosinophils in the tissue of malignant polyps indicates the malignancy of the neoplasm. We revealed relationship between apoptosis and eosinophilic infiltration; both of them affect the outcome and prognosis of polyp development. Apoptosis induction by eosinophils and programmed cambium necrosis in tumor progression need to be further researched.

KEYWORDS — human gastrointestinal tract, polyps, malignancy, mucous membrane, monocytes / macrophages, eosinophils, colon; gastrointestinal disease; neoplasms.

INTRODUCTION

The aggregate indicator of the prevalence of oncopathology in Russia reaches 2.399% with an annual increase of 1.7%. In the Primorsky Krai (Russian Far East. 1.9 million population), in 2016 only, gastric cancer was diagnosed in 493 people [1]. However, the level of early diagnosis of malignant polyps in stomach remains very low. Detected malignant neoplasms are confirmed morphologically in 91.3% of cases, which indicates a late diagnosis of malignancy [2]. One of the tasks of solving the problem of early diagnosis and prevention of cancer is the development of morphological criteria for the malignancy of benign formations

in the structure of the gastrointestinal tract and the strategy of pathogenetically substantiated treatment of neoplasms for the prevention of their malignancy and relapses.

According to Chetty R. (2016), at the present stage, the most discussed are the mechanisms of dysplasia, as well as issues related to the nomenclature, diagnostic criteria, histological variants, coexistence with other types of polyps, and those differential diagnosis [3]. The exceptional immunological properties of eosinophils and their ability to induce tissue remodeling, which is especially important in the destruction and disruption of cellular interactions in the system of epithelial-mesenchymal tissues during carcinogenesis, puts the study of eosinophilic infiltration during tissue malignancy at the forefront of relevance [4, 5]. Cansiz Ersöz C., Kiremitci S., Savas B., Ensari A. (2020) recognize eosinophilia against the background of polyps in 88% with high sensitivity and specificity in predicting carcinogenesis [6]. Clinical and morphological studies in the dynamics of treatment for patients with polyps in the gastrointestinal tract are necessary to elucidate the morphological criteria of malignancy and provide pathogenetically based recommendations for assessing the risk and predicting the outcome of benign polyps.

Aim of study:

To investigate eosinophils localization among the cellular phenotypes of myeloid cells in the structure of polyps in various parts of the human gastrointestinal tract.

MATERIAL AND METHODS

Analysis of the clinical material based on the study of 189 patients aged 40 to 90 years treated at surgical departments of hospitals in the Primorsky Krai (Russia) 2018 to 2020 were carried out. Clinical material was obtained with informed consent of the patients and in accordance to the Helsinki Declaration (Revision 2013). The study was approved by Ethic Committee of Far Eastern Federal University (Vladivostok, Russia).

Immunohistochemical methods for processing biopsies of polyps from various parts of the gastrointestinal tract using monoclonal antibodies (MCA) (clone KP1, code No. M 0814, lot 119) were used. Macrophages were also identified by the CD68 marker

(a highly glycosylated transmembrane glycoprotein that is localized in lysosomes). Molecular clone CD68 showed that LGP (a family of lysosomal glycoproteins) with plasma membrane proteins play a role in lysosomal traffic and endocytosis, including lysosomal membrane protein-1 and 2 associations (LAMP-1 and LAMP-2). For labeling CD163 used clone 10 D6, class of immunoglobulins IgG1. The antibodies were labeled with horseradish peroxidase and diaminobenzidine was used to visualize the binding. Cleansing and detection of antigenic determinants was carried out in a glass container filled with a reducing solution and creating a water bath for 1 hour. Some of the preparations were processed using microwave radiation, which gives the best unmasking effect, within half an hour. Used to unmask antigens 10 mmol/L citrate buffer, pH 6.0 or DAKO TRS (Target retrieval solution, code No. S 1700) The cooled preparations were washed in distilled water. Antibodies were used at a dilution of 1:50 and 1: 100. Brown staining indicated a positive reaction.

RESULTS AND DISCUSSION.

Analysis of clinical material and case histories in patients showed that against the background of cancer in various localization of the gastrointestinal tract, the stomach is in the lead, cancer of which in 2016 amounted to 199 cases per 100,000 population, which was 2 times higher than the intermediate data for Russia (19.83 versus 9.73). The share of the colon accounts for 451 cases, which amounted to 25.71 cases per 100 thousand of the population; rectal cancer was 294 cases (146 per 100 thousand population) [1]. In men, the number of patients with polyps in the group of 31–40 years old is 57%, 41–50 years old — 40%, 51–65 years old — 61%, 66–70 years old — 22%, 72–74 years old — 71%. In the examined women with clinical manifestations at the age of 40–50, polyps were found in 100%; in the 51–65 age group — polyps are identified in 40%; at 66–70 years old — at 25%; there are no women with polyps in the group over 70 years old among the examined patients (Fig. 1)

Polyps were more often detected by endoscopy, mainly in the large intestine, in both men and women. Cases of malignancy are established both by clinical diagnosis and histologically confirmed.

Surgical treatment was performed in 95% of patients, conservative treatment — in 2% of patients, the outcome was favorable, the prognosis was positive in 93% of patients. The use of the fast track surgery (FTS) protocol — *accelerated recovery after surgical operations* in combination with the laparoscopic approach made it easier for patients to tolerate surgical treatment and leave the hospital 5–6 days after the

operation. Processed and analyzed biopsies of the gastrointestinal mucosa were obtained from 189 patients, 112 men and 77 women) aged 40 to 90 years, who had polyps, cancer, metastases. (Table 1).

A higher relative number of male patients among patients with pathology of gastrointestinal mucosa indicate a higher incidence of morbidity and mortality in the male population. The distribution of patients in Primorsky Krai with clinical manifestations and identified pathology of the gastrointestinal tract is shown in figure 2.

Age-related changes in the structure of the gastrointestinal mucosa are also characterized by the fact that almost all sections from biopsies obtained for the purpose of diagnostic measures revealed the presence of leukocytes infiltration around the vessels in the connective tissue of the lamina propria and submucosa. At the same time, in the composition of the infiltrate, eosinophils were identified in large quantities. In the area around the polyp, various types of eosinophils are present. In the first group, eosinophils have a clearly identifiable cytolemma, a nucleus of a round, oval or irregular shape. Another type is represented by degranulating cells with a nucleus of two or one fragments. Eosinophils were identified not only in the lamina propria, but also penetrated into the basal epithelial layers (Fig. 3).

Within the changes of epithelium of the mucous membrane in the structure of the polyp and its metaplasia, eosinophils were absent in our observations. The morphological findings were process eosinophils with signs of degranulation identified in the epithelial lamina with abnormally wide space involved in to apoptosis. As part of the infiltrate, eosinophils are grouped near the stem migrants, near the vessels and inside the preserved blood vessels. In the area surrounding degranulating eosinophils, cells with signs of apoptosis are observed (Fig. 4).

In addition, immunohistochemical study showed immunoreactivity to CD4, CD8, CD68, CD163, which were observed in all studied biopsies of polyps and their surrounding tissue (Fig. 5).

These results suggest that CD4, CD8, CD68, CD163 are key markers for clinical diagnosis and prediction of polyps malignancy in the structure of the gastrointestinal tract. As well as eosinophils has unlimited importance in the analysis and prognosis of polyps malignancy based on a thorough analysis of preparations stained with hematoxylin and eosin with immunostained sections.

We noted that with the formation of a polyp on the surface of the mucous membrane, the morphological picture of the altered tissue is characterized by deformation of the basement membrane, apoptosis

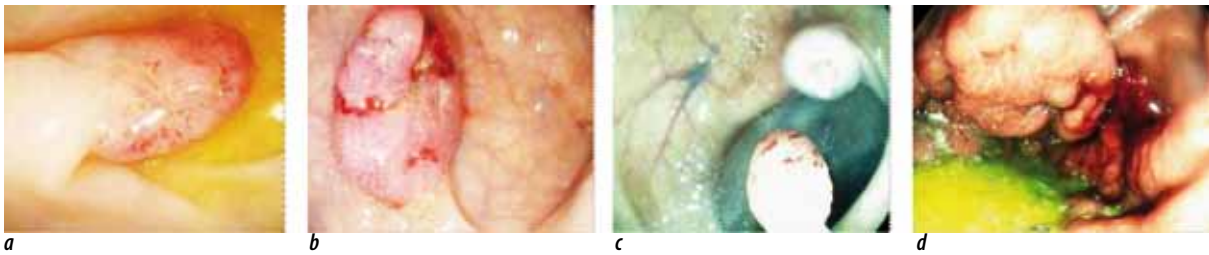


Fig. 1. Endoscopic examination of patients with gastric mucosa. a) 65-year-old man patient. Gastric polyp. b) 67-year-old man patient. Gastric polyp a; c) 72-year-old man patient. Gastric cancer. d) 75-year-old man patient. Gastric cancer.

Table 1. Distribution of material according to the revealed pathology

Age (years old)	Patients (number)	Disease		
		Polyps	Cancer	Metastasis
40–49	6	4	1	1
50–59	9	7	2	0
60–74	85	64	21	1
75	81	66	15	2
90	8	4	4	2
Total:	189	140	43	6
Total: %	100%	71%	26%	3%

Neoplastic changes in patients involved in study

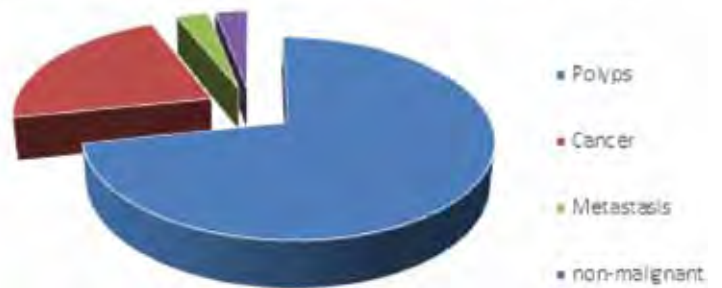


Fig. 2. Distribution of older age groups of patients with complaints by the presence of pathology

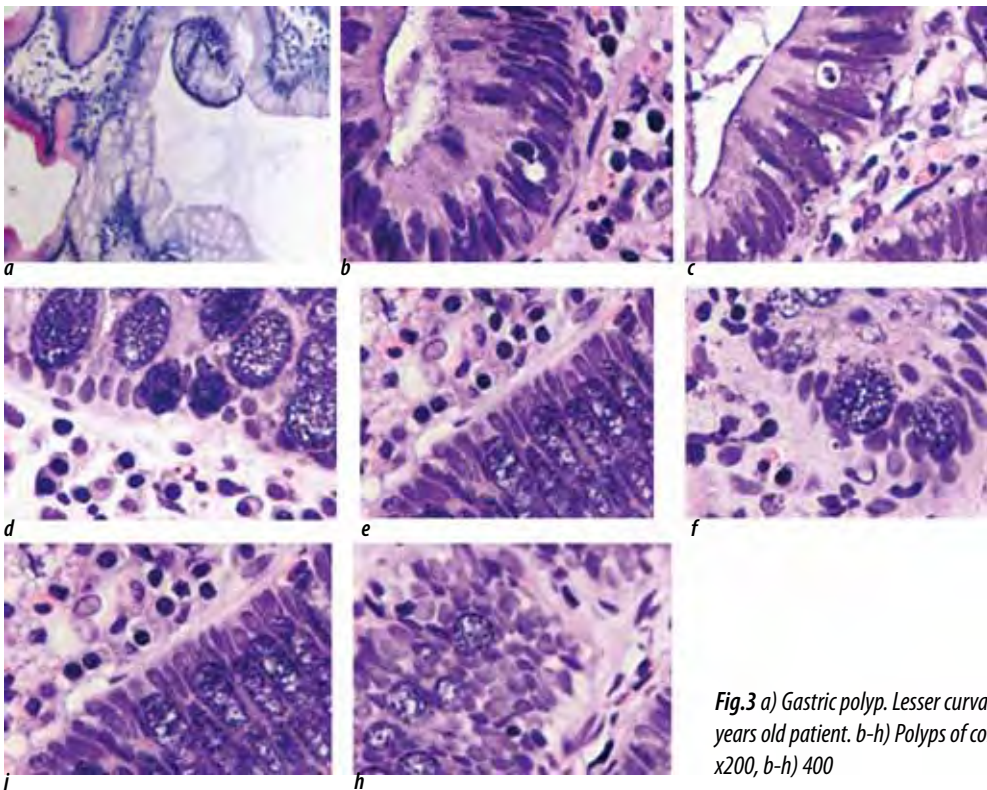


Fig.3 a) Gastric polyp. Lesser curvature of the stomach. 64 years old patient. b-h) Polyps of colon. H/e staining. Zoom a) x200, b-h) 400

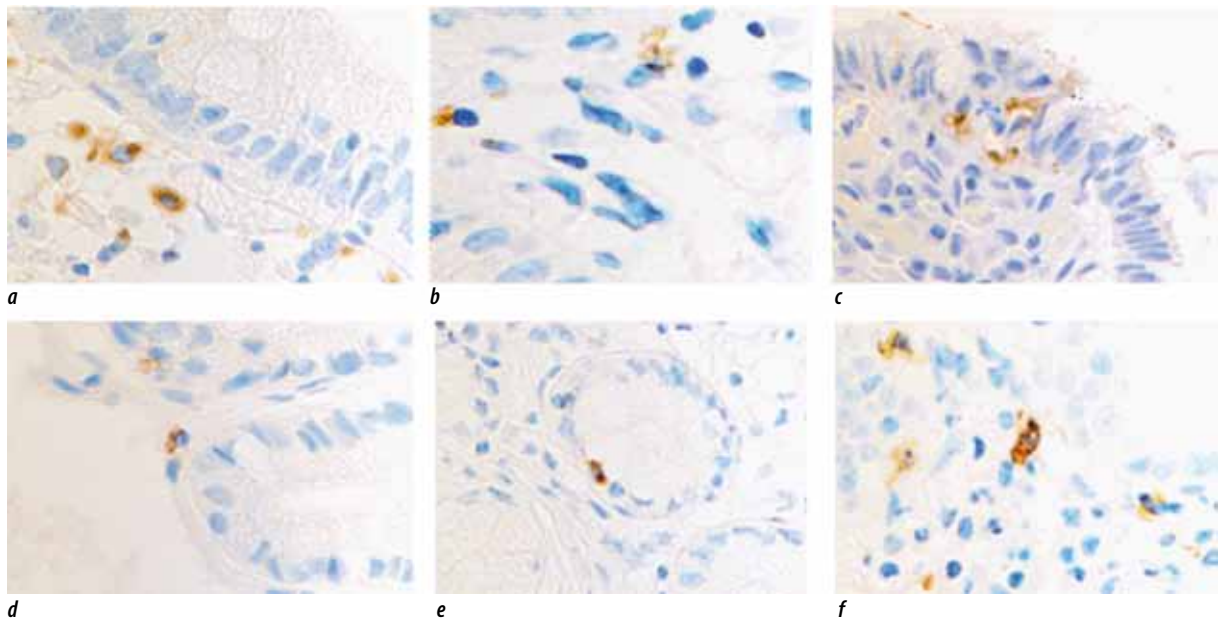


Fig. 4. Colon polyps. Immune staining. a) CD4; b) CD68; c) CD68; d,e,f) CD163. Zoom $\times 400$

of epithelial cells and infiltration of migrant cells from the blood into the area of altered tissue. Identified cells of the infiltrate proliferate by amitosis, can be identified both near the epithelial plate, and at the site of destroyed epithelial cells, as well as outside the epithelium on the surface. The structure of the lamina propria of the mucous membrane is not identified, which may be associated with both the death of connective tissue cells responsible for the production of intercellular substance and their veiling with elements of a blood infiltrate containing migrant cells.

The long-term process of polyp formation is accompanied by apoptosis, folding of the basement membrane, and complete disruption of epithelial restitution. This indicates the peculiarities of reparative processes in the structures of the mucous membrane against the background of the formation of and their malignancy. We found that eosinophils in the surrounding polyps tissue are identified in 100%, and under conditions of malignancy, their number decreases by 20%. The number of cells in the monocyte pool also decreasing. But it is worth paying attention to the pathological forms of eosinophils, which do not normally occur in peripheral blood or tissues. A common eosinophil has a nucleus consisting of 2 segments; in our studies, the eosinophilic population is represented by cells with spherical, sometimes eccentric nuclei.

The second phenotype of eosinophils had nuclei of two segments, but also spherical in shape, while normally the segments of the nuclei of eosinophilic granulocytes have a small width of 12–20 μm and a

length of up to 7 μm . In our observations, the nuclei were dumbbell-shaped, but the venom chromatin was unevenly distributed. This may reflect not only quantitative, but also qualitative changes in protein synthesis and function in general. Caruso R.A., Branca G., Fedele F. et al. (2014), consider eosinophilic infiltration in the stroma of a gastric tumor associated with carcinoma [8]. But these data about the participation of eosinophils in the destruction of normal tissue structures and the synthesis of stromal elements of the tumor require additional research.

We noted that the eosinophilic population spreads from the lamina propria to the cambial layer of the epithelium, with corresponding co-localization with CD4, CD8, CD68 and CD163 positive cells, which indirectly indicates that in the case of polyps formation, eosinophils are able to acquire the properties of antigen-presenting cells, as well as macrophages. Beltran M, Khurana S, Gil Y, Lewis JT, Kumar R, Foran JM. (2020) described that tubular adenomas with dense transmural inflammatory infiltrates, which mainly consist of eosinophils and store Charcot-Leiden crystals of histiocytes, in some cases are preceded by parasitic infection, which leads to a high content of eosinophils in the leukocyte infiltrate of the forming polyps and their environment [9].

Cai L, Chen Y, Xiao SY. (2020), on the contrary, consider that eosinophilia in the tissue does not always occur against the background of parasite invasion [10]. According to our analysis of the anamnestic data, there were no parasitic infections in the patients. We believe

that the migration activity of eosinophils to the focus of polyp formation may be associated exclusively with a violation of signal interactions in the system of local immune homeostasis of the gastrointestinal mucosa.

Agaimy A., Mudter J., Märkl B., Chetty R. (2011) in two cases of polyps of cytomegalovirus etiology, tissue eosinophils were observed in the mucous membrane of the gastrointestinal tract [11]. Therefore, it can be assumed that the threshold values of the number of tissue eosinophils in the gastrointestinal mucosa against the background of neoplasms require further research, since they may be important in the differential diagnosis of the etiology of polyps.

Moezzi J, Gopalswamy N, Haas RJ Jr, (2000) observed in 75% of all adenomas from mild to moderate eosinophilia, especially pronounced in the tissue surrounding the polyp and their absence in the stroma of invasive adenocarcinomas [12]. Similar data obtained in our study indicate that the quantitative dynamics of tissue eosinophils can be taken into account in the development of a treatment strategy and influence the prognosis of polyp outcome in clinical assessment.

It should be noted that over the 20th and 21st centuries, many microorganisms had laurels of a starting source in the pathogenesis of cancer, but the absence of 100% oncogenesis in all carriers of these microorganisms [13], as well as spontaneous recovery in 98% in the presence, for example, in polyps of papilloma virus, which is currently recognized as a trigger in the development of cancer with localization in the mucous membranes of the gastrointestinal tract, indicate that the key pathogenetically significant process in the oncogenesis of the gastrointestinal tract is a process unknown today [14, 15].

The almost complete lack of data on intercellular signaling interactions in the stem niches of the gastrointestinal mucosa of patients with confirmed polyp etiology is an obstacle to the introduction of cellular technologies in gastroenterology and oncology [16, 17]. Although a routine biopsy is recommended in accordance with the Sydney system, this is not enough for an adequate assessment of the pathological process, since the sampling zones are actually endoscopically distinguishable areas, excluding the surrounding tissues from the complete analysis. Therefore, histological studies in areas at the border of the polyp zones and unaltered macroscopically tissue are of high importance. The persistence of dyspeptic symptoms in patients after therapy or the absence of symptoms after extirpation of polyps in patients dictate the search for more evidence-based criteria for the risk of developing gastrointestinal cancer. In cancer, the presence and degree of penetration of eosinophilic granulocytes into malignant tissue can provide important prognostic information [18].

We have found that the formation of polyps is associated, first of all, with damage of epithelial restitution, increased proliferative activity, accompanied by depletion of the cambial stem elements of the integumentary epithelium. Migration of tissue eosinophils is required for adaptive tissue remodeling in the structure of the polyp and its environment [19]. We noted that with the formation of a polyp on the surface of the mucous membrane, the morphological picture of the altered tissue is characterized by the disappearance of the basement membrane, apoptosis of epithelial cells and migration of immunocompetent cells from the blood to the zone of altered tissue.

Ieni A, Branca G, Parisi A, (2015) in patients over 72 years of age, a distinctive morphological sign of malignancy is associated with an increase in neutrophil infiltration, although the true mechanism of this process is also unclear [20].

CONCLUSIONS

The mechanism of HPV-induced carcinogenesis, despite the widespread nature of this infection, has not yet been elucidated and substantiated. Based on the integral assessment and comparative analysis of pathological changes in the gastrointestinal mucosa, there are still no definite definitive morphological markers of epithelial metaplasia of polyps and malignancy, as well as no explanation for malignancy of only 1% of polyps, while tumors are often found near polyps. These facts indicate that local immune homeostasis plays a role in malignancy, which must be guided by when making a diagnosis, predicting the outcome of polyps, determining the scope of surgical intervention, and postoperative treatment strategies.

An increase in the number of eosinophils in the general inflammatory cellular spectrum in the area of malignancy is an autonomous, antitumor mechanism, the decoding of which can contribute to the development of conservative methods for the treatment of gastric polyps. The obtained data open up prospects for the development of a new strategy for the correction of malignancy using the secretory activity of eosinophils by inducing an increase in the number of required phenotypes. One of the focuses of targeted treatment and prevention of malignancy is the eosinophilic pool of leukocyte infiltrate.

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