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The Role of Immunopathology: As Predictor Factors in Patients with Inflammatory Bowel Diseases

*Kemelbekov Kanatzhan[1], Kozhakhmetova Ulzhan[2], Kuzenbayeva Aisulu[3], Tazhedinova Aigerim[4], Nurkeshova Elmira[5]
[1*]PhD doctoral Department of Pediatrics-2, South Kazakhstan medical Academy, Shymkent, Avenue Al-Farabi-1, Kazakhstan, E-mail: kanat-270184@mail.ru
[2]Assistant, Department of general practice with EBM course, NCJSC «Astana Medical University», Nur-Sultan, Street Beibitshilik 49A, Kazakhstan
[3,4]Senior Teacher, Department of molecular biology, histology, cytology and genetics, NCJSC «Astana Medical University», Nur-Sultan, Street Beibitshilik 49A, Kazakhstan
[5]Teacher, Department of molecular biology, histology, cytology and genetics, NCJSC «Astana Medical University», Nur-Sultan, Street Beibitshilik 49A, Kazakhstan

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ABSTRACT
Recently, significant progress has been made in understanding the pathogenesis of Inflammatory Bowel Diseases (IBD), but to date, their etiology has not been established. As a result, treatment is not always successful, resistance to basic therapies is formed, and severe, life-threatening complications are added.
Identify risk factors for cellular and humoral immunity in patients with inflammatory bowel diseases.The study included the actual material of an open prospective cohort study in parallel groups of 40 patients with UC and CD. The state of cellular immunity was assessed by determining the number of subpopulations of T-lymphocytes (CD4+, CD8+), and NK cells (CD16+). The results obtained allow us to present a possible scenario for the development of pathogenetic relationships as follows: expressing TLR 2,4 initiation or intensification of the inflammatory process in the colon mucosa, and, as a result, the appearance of the main clinical symptoms of the disease.
Keywords: inflammatory diseases of the intestines, immunopathology, predictors, forecasts
Correspondence:
Kemelbekov Kanatzhan
PhD Doctoral Department of Pediatrics – 2
South Kazakhstan Medical Academy, Kazakhstan
E-mail: kanat-270184@mail.ru
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INTRODUCTION

Recently, significant progress has been made in understanding the pathogenesis of IBD, but to date, their etiology has not been established. As a result, treatment is not always successful, resistance to basic therapies is formed, and severe, life-threatening complications are added [1,2]. The social significance of the problem of IBD is determined by a significant decrease in the quality of life of patients, frequent disability and disability of young people, and high economic costs [3,4].

It is difficult to establish an epidemiological indicator such as prevalence in IBD. The organization of screening studies is complex, time-consuming and requires large financial investments. Most authors assume that almost all patients have clinical symptoms of the disease, forcing them to seek medical help [5,6].

Detection of high concentrations of proinflammatory cytokines, mainly tumor necrosis factor alpha (TNF -), in intestinal biopsies of patients with IBD allowed us to develop a pathogenetic strategy for the treatment of these diseases – biological therapy, including the use of monoclonal antibodies that selectively block TNF - [Vermeire S. 2009,. et al., 2008].

The introduction of biologics into the IBD treatment regimen has significantly increased the proportion of patients who achieve stable remission in a short time. However, 30% of patients do not manage to reduce the activity of the disease against the background of TNF-blockers, more than 50% of patients experience a loss of therapy effectiveness during the first year of treatment [Sanchez-Muoz F. et al., 2008]. The reasons for the development of tolerance to TNF-blockers include the production of their own antibodies to the drug, as well as the involvement of TNF-independent pathways of IBD pathogenesis [7-11].

A personalized approach to the choice of a biological drug and an early prognosis of insufficient therapy effectiveness allows for timely correction of the treatment regimen, thereby significantly increasing the clinical and economic efficiency of treatment of these diseases [Svitich O. A., 2015].

Currently, a number of indicators have been identified that have predictive significance in evaluating the effectiveness of biological therapy. Clinical and anamnestic predictors include the duration of the disease, the form of pathology [Vermeire S. et al., 2012], the patient's history of surgery for complications of IBD [Orlando A. et al., 2015]. Predictive capabilities of laboratory tests were shown by the example of determining the serum content of anti-neutrophil antibodies [Taylor K. et al., 2011]. Currently, methods for predicting the effectiveness of infliximab are widely studied, based on the assessment of pharmacokinetic parameters, such as the residual serum concentration of infliximab [Maser E. et al., 2006].

These prognostic parameters have a number of limitations, since they do not allow us to assess the prognosis of biological therapy before it begins, exclude the possibility of making a prognosis in the dynamics of treatment, and were developed for adult patients with IBD [12-17].

There is data on the prognostic significance of determining the content of a number of cytokines before starting infliximab treatment in adult patients with IBD. So in the works. et al., 2012, Di Sabatino A. et al., 2010 serum concentrations of IL-23, IL-17A, IFN -, IL-6, and TGF-were proposed as predictors of the effectiveness of biological therapy [18-21].

Currently, the diagnostic and prognostic significance of determining markers of autoimmune inflammation in patients with IBD is being actively studied. The information content of determining antibodies to

Saccharomyces cerevisiae, to the exocrine part of the pancreas, and antibodies to glycoprotein 2 (anti-GP2) in assessing the inflammatory activity of IBD was revealed [Bogdanos D. et al., 2012, D. Roggenbuck et al., 2012].

The study of the content and functional activity of populations of lymphocytes that play an important role in the development of IBD, primarily Th17 lymphocytes, regulatory T-lymphocytes (Thed) and activated T-helpers, revealed the diagnostic significance of these indicators in assessing the inflammatory activity of the disease [Saruta M. et al., 2017, Di Sabatino A. et al., 2010]. The role of b-lymphocyte populations and the informativeness of their determination in the diagnosis of IBD is intensively studied [El-Hodhod M. et al., 2013, Mishima Y. et al., 2019]. Individual studies are devoted to the analysis of the prognostic significance of cellular immunity indicators in IBD. the information content of determining the number of circulating Treg lymphocytes in the forecast of infliximab effectiveness in adult patients with CD is shown [Di Sabatino A. et al., 2015].

Currently, there is strong evidence for the role of mitochondrial dysfunction in the pathogenesis of IBD [Alzoghaibi M., 2013, Santhanam S. et al., 2012]. Detection of direct and indirect signs of oxidative stress in cells of the intestinal mucosa and leukocyte infiltrates directly indicates a violation of the normal functioning of the mitochondrial apparatus in patients with IBD [Sifroni K. et al., 2010, Roessner A. et al., 2008, Rezaie A. et al., 2007]. The key enzyme that reflects the activity of mitochondria is succinate dehydrogenase (SDH). The diagnostic significance of determining SDH in various pathological conditions is proved [Sukhorukov V. S., 2011, Izmailova T. D., et al., 2012, Rutter J. et al., 2010]. The information content of determining the activity of SDH in peripheral lymphocytes in IBD and other gastrointestinal pathologies in children was revealed. [Semenova G. F. et al., 2007, Klimova S. V. et al., 2010].

Thus, the determination of the cytokine profile, the study of the state of lymphocyte populations and their functional activity is promising due to the potential for identifying new laboratory markers for predicting the effectiveness of biological therapy in patients with IBD [22-28].

THE PURPOSE OF THE STUDY

Identify risk factors for cellular and humoral immunity in patients with inflammatory bowel diseases.

RESEARCH MATERIALS AND METHODS

The study included the actual material of an open prospective cohort study in parallel groups of 40 patients with UC and CD (table. 1).

Table 1: Distribution of patients with inflammatory bowel diseases

Diagnosis	Ulcerative colitis		Crohn's disease	
Research group	I n=30		II n=30	
Subgroups	Ia	Ib	IIa	IIb
	12 (40%)	18 (60%)	14 (46,6%)	16 (53,4%)
Total	30 (100%)		30 (100%)	

After a comprehensive examination of patients and verification of the diagnosis of IBD, in accordance with the inclusion and exclusion criteria, two research cohorts were formed: I cohort - 30 patients with recurrent UC (17 - women and 13 - men), aged 20-65 years (average age 37.0±1.3 years) and II research cohort - 30 patients with recurrent UC (11 - women and 19 - men), average age 41.2±2.5 years. Patients of the I cohort, depending on the location of the inflammatory process, were ranked into 2 groups: Ia - 12 (40%) patients with distal UC, and Ib - 18 (60%) patients with total form of the disease. Patients in the II research cohort were divided into 2 subgroups depending on the segmental localization of intestinal lesions: IIa subgroup - 14 (46.4%) patients with colonic localization, IIb subgroup - 16 (53.4%) patients with combined lesions of the large and small intestines. The control group consisted of 20 healthy volunteers (10 women, 10 men), with an average age of 29.1±3.2 years.

After isolation of the research groups, all patients underwent a special laboratory examination, including immunological blood tests with determination of CD4+, CD8+, CD16+ HST test, CEC, Ig G, M, A, and determination of TLR 2, 4 expression on the surface of the patients ' blood monocytes.

In the second phase of the study, the patients were re-ranked into groups depending on the activity and severity of relapses from assigned course 8-week therapy, during which used standards of medical care to patients with IBD and previous experience with medication therapy.

At the end of the clinical trial, 8 UC patients (5 women and 3 men) and 5 BC patients (4 women and 1 man) achieved clinical and endoscopic remission, which was 25% of the patients included in the study. Clinical and endoscopic remission was understood as the absence or very slight severity of the main clinical symptoms and complete healing of the mucous membrane.

Clinical, endoscopic, morphological, and special (immunological) research methods were used to implement scientific tasks. The results of the complex study were included in the individual patient registration card (IRC).

Endoscopic examination was performed using a floor-by-floor segmental examination of the large and terminal small intestine using the standard method of video paleocolonoscopy using a video system and video colonoscopes. In the course of diagnostic video colonoscopy, a semi-quantitative score was performed for inflammatory erosive-ulcerative changes in the small and large intestine, based on macroscopic changes in CO, complementing the indices of IBD activity (Mayo D. Rachmilewitz, CDAI). Morphological study of ileo-and colonobioptates allowed us to dynamically assess the

degree of activity of inflammatory changes IN the small and large intestines during course of drug therapy, and exclude dysplastic changes. A special immunological laboratory examination included the determination of TLR 2, 4 expression on blood monocytes as well as an extended immunological study of blood with the determination of CD4+, CD8+, CD16+, nst test, CEC, Ig G, M, A. TLR Expression on peripheral blood monocytes was determined using an immunofluorescence test.

RESULTS AND DISCUSSION

During the examination, cellular and humoral immunity, indicators of the neutrophilic link of the immune system (nst) were evaluated, Nst art., To art.). The state of cellular immunity was assessed by determining the number of subpopulations of T-lymphocytes (CD4+, CD8+), and NK cells (CD16+). The number of cells expressing the membrane marker (CD16+) and cells with helper-inductive properties (CD4+) remained unchanged in comparison with the control group ($p>0.05$). Indicators of the humoral link presented in the study by lymphocytes with CD19+ on their surface increased by 1.3 times, which was 37% higher than the values of the control group ($p<0.05$). In patients with recurrent UC, the IgG and IgA levels exceeded the values of the group of healthy volunteers by 1.4 and 1.2 times, respectively, while the IDM content did not differ significantly. The number of CEC in the blood serum of patients with recurrent UC reached 78.7±3.1 cu, which was 1.4 times higher than the level of the control group ($p<0.05$). The study of the morbicidal activity of neutrophils showed a statistically significant 1.8-fold decrease in the stimulated activity of neutrophils and a similar decrease in the stimulation coefficient compared to the control group ($p<0.05$). Thus, the analysis of the parameters of the immune status in patients with recurrent UC indicates activation of the cellular link of the immune system, violation of the processes of intercellular cooperation, manifested in an imbalance in the ratio of subpopulations of T-lymphocytes towards an increase in the number of cells with cytotoxic activity, increased activity of the humoral link of the immune system, accompanied by an increase in the concentration of Ig A and G, as well as depletion of the neutrophil-phagocytic link of the immune system. During remission, normalization of cellular immune status parameters and insignificant activation of the humoral link of the immune system, as well as moderate depletion of phagocytic reserves, were observed.

Modern scientific sources distinguish two main components of the innate immune system: endogenous antimicrobial peptides and image-identifying receptors, in particular, TLR (Podolsky D. K., 2002; Cario E., 2010). In our study, we evaluated the expression of TLR 2, 4 in patients in different phases of the course of UC (table. 2).

Table 2: Expression of TLR 2, 4 on monocytes in patients with ulcerative colitis during relapse and remission of the disease

Phase of Ulcerative colitis	Recurrence n=30	Remission n=30	Control n=20
CD4⁺, %	78,1±0,3*, ***	64,0±0,1**	67,4±0,7
CD8⁺, %	14,2±0,4*, ***	5,1±0,2	2,3±0,5
CD16⁺, %	11,4±0,2*, ***	5,5±0,1	3,7±0,4

*Note: * - p<0.05 in comparison with the control group, * * - p>0.05 in comparison with the control group, * * * - p<0.05 in comparison with indicators during remission.*

The average values of CD4+ cells during UC relapse were 79.1±0.3%, which was 1.2 times higher than the values during remission ($p<0.05$). The expression level of CD4+ was 1.5 times higher in the remission phase and 3 times higher in comparison with the control group. The increase in CD4+ was 10.2±0.8%, which was 2.4 and 3.1 times higher, respectively, in the period of remission of UC and in the group of healthy volunteers ($p<0.05$).

As a result of the analysis of the distribution of TLR expression in patients with recurrent UC, depending on the prevalence of inflammation in the colon, it was found that in the distal form of UC, TLR expression increased by 7.7% ($p>0.05$), in the left - sided form by 16.3%, and in the total form of UC, the increase in indicators reached the maximum values-85.2±1.2, which was 18.5% higher than the level in the control group ($p<0.05$). No statistically significant changes in the number of monocytes expressing TLR 2 were detected during intragroup analysis. The dynamics of TLR 4 expression on the monocyte surface was more significant. Thus, the number of CD14+CD284+ cells increased by 2.4 times ($p<0.05$) in comparison with the control group when the distal colon was affected. In the group with left-sided localization of the inflammatory process, the expression increased by 3 times, in the total form of UC, the studied indicator increased by 3.7 times ($p<0.05$). Growth in the number of monocytes expressing TLR 6, is also dependent on the localization of the inflammatory process: while the distal form noted a twofold increase in the indicators, while left and total form of the YAK, the increase was 9.8±0,4 10,7±0,3 that 2.9 and 3.1 times higher than the values of a group of healthy volunteers ($p<0.05$) (table. 3).

Table 3: Expression of TLR 2, 4 on monocytes depending on the length of the inflammatory process during the exacerbation of ulcerative colitis

Localization inflammatory process's	Ia n=12	Ib n=18	Control group n=20
CD4⁺,%	71,9±1,8	82,4±0,2*	64,5±0,2

$CD8^+$,%	9,4±0,1*	10,8±0,9*	2,8±0,7
$CD16^+$,%	8,3±0,7*	7,5±0,5*	3,1±0,5

*Note: * - p<0.05 in comparison with the control group*

When comparing the expression of TLR 2,4 in the period of remission of UC with the indicators of the control group, the results were similar, but there were no statistical differences. Analysis of the detected changes in TLR 2,4 expression indicates a decrease in the activity of the innate immune response system in patients with UC (table. 4). When comparing the results presented in tables 3 and 4, it was found that differences in the expression of CD4+ in patients with left-sided and total UC significantly differ from the studied values during remission (p<0.05). The difference in expression increases with an increase in the prevalence of the inflammatory process: in the left-sided and total forms, 14.1% and 14.8%, respectively, exceeding the initial indicator by 1.1 times, while in the distal form of UC, the differences in the expression of CD4+ were only 5.2% (p>0.05). Analysis of the dynamics of the increase in the relative number of monocytes expressing TLR 4 revealed statistically significant differences in indicators during relapse and remission of UC in all groups of patients: in the distal form, the expression indicators of CD8+ increased by 1.3 times. for left-sided and total forms-2.1 times (p<0.05). The dynamics of increasing CD4+ indicators in different phases of the disease showed statistically significant differences in the distal, left-sided and total forms: 1.4, 2.2 and 2.6 times, respectively (p<0.05)

Table 4: Expression of TLR 2, 4 on monocytes depending on the length of the inflammatory process in patients with ulcerative colitis during remission

Research group	Ia n=14	Ib n=16	control group n=30
$CD4^+$	62,5±4,1**	63,2±0,9**	64,7±0,2
$CD8^+$	5,1±0,4**	5,2±0,1**	2,9±0,5
$CD16^+$	5,2±0,8**	4,9±0,5**	3,1±0,6

*Note: ** - p>0.05 compared to the control group*

Results of intragroup analysis of TLR 2,4 expression on monocytes depending on the severity of the disease are presented in table 5. In mild UC, a slight difference of 8.1% (p>0.05) was found between the expression of CD4+ during exacerbation and the control group. In severe cases, there was a 1.1-fold increase in the studied indicator (p<0.05). Expression of CD8+ was increased: 2.1 and 3.2 times in mild and moderate cases; in severe cases, the difference was 13.4±0.1, which was 3.2 times higher than the values of the control group (p<0.05). The mild course of UC was accompanied by an increase in the number of CD16 + cells by 2.4 times, the average severity by 2.6 times, and the severe course by 3.2 times, respectively (p<0.05).

CONCLUSION

The results obtained allow us to present a possible scenario for the development of pathogenetic relationships as follows: expressing TLR, further initiation or intensification of the inflammatory process in the colon mucosa, and, as a result, the appearance of the main clinical symptoms of the disease. As well as the obtained data can serve as a theoretical justification for the search and implementation of additional methods of pharmacological correction of detected disorders. The results of the study allow us to expand the diagnostic algorithm, optimize the management tactics of patients with IBD, and predict early recurrence of the disease.

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