Syrota B. V., Sydorchuk L. P. Are genes of interleukins' 10 (RS1800872) and heat shock protein 70-2 (RS1061581) predictive markers of cytokines production in patients with enterocolitis. Journal of Education, Health and Sport. 2018;8(7):615-622. eISNN 2391-8306. DOI http://dx.doi.org/10.5281/zenodo.1406189
http://ojs.ukw.edu.pl/index.php/johs/article/view/5888

The journal has had 7 points in Ministry of Science and Higher Education parametric evaluation. Part b item 1223 (26/01/2017).
1223 Journal of Education, Health and Sport eissn 2391-8306 7 ©

The authors declare that there is no conflict of interests regarding the publication of this paper.

Received: 25.06.2018. Revised: 30.06.2018. Accepted: 31.07.2018.

Are genes of interleukins' 10 (RS1800872) and heat shock protein 70-2 (RS1061581) predictive markers of cytokines production in patients with enterocolitis

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Abstract

Objective: to investigate the effect of 1267 A→G gene polymorphism of heat shock proteins (HSP) family 70-2 (HSP70-2) and *C-592A* gene polymorphism of interleukin 10 (IL-10) on the level *IL*-1*β*, *TNF*-*α* and *IL*-10 in the blood of patients with colienteritis.

Materials and methods: 95 patients suffering from Colienteritis participated in the study including 34.74% (33) men, and 65.26% (62) women, their average age was 38.66±3.11 years. Concentration of *IL*-1*β*, *TNF*-*α* та *IL*-10 in the blood plasma was examined by means of immune-enzyme method. Polymorphism of HSP70-2 (rs1061581) and IL-10 (rs1800872) genes was studied by means of polymerase chain reaction (PCR) method. The control group included 30 practically healthy individuals correlated by their age and sex who were not infected by diseases of any localization during the last 6 months.

Results. Changes of immune-inflammatory body response in patients with colienteritis are characterized by an increased synthesis of the first generation pro-inflammatory IL-1β and TNFα on 40.93% and 44.44% ($p<0.001$) respectively against the ground of low production of anti-inflammatory IL-10 – on 37.30% ($p<0.001$) and are indicative of certain mechanisms of specific immune response formation: imbalance between a high activity of the immune

cellular link at the expense of Th1 lymphocytes and macrophages/monocytes, and insufficient activity of the humoral link due toTh2 system and B-lymphocytes activity.

Single-factor dispersive analysis confirmed the association between the gene promoter of heat shock protein HSP70-2 (1267A→G) and increased level of IL-1β in the blood, more considerable in the carriers of GG-genotype on 45,05% ($F=11.66$; $p<0.001$), and decreased concentration of IL-10 ($F=4.56$; $p=0.035$), more considerable in the carriers of GG-genotype on 23.38% ($p=0.031$). On the contrary, promoter of IL-10 (C-592A) gene is associated only with decreased content of IL-10 ($F=3.14$, $p=0.048$), the most substantial in the carriers of mutation A-allele on 26.95% and 20.64% ($p<0.05$) respectively.

Conclusions: Availability of GG-genotype of HSP70-2 gene in patients with Colienteritis is associated with a higher content of IL-1β increase in the blood plasma and a low concentration of IL-10. Occurrence of A-allele of IL-10 gene in the genotype of patients with Colienteritis is characterized by the lowest IL-10 content.

Key words: Colienteritis, Coliescherichiosis, Gastroenterocolitis, genes HSP70-2, IL-10, markers, cytokines IL-1β, TNFα, IL-10.

Introduction. Coliescherichiosis (Colienteritis, Acute Gastroenterocolitis (AGEC) – ICD-10) as an infectious disease is registered in all the regions of the world, possessing mainly sporadic character in the form of outbreaks of acute intestinal diseases and food poisonings [1]. Unfortunately, the mechanisms of development of Coliescherichial endotoxicosis considering immunological, concomitant microbiological factors are studied insufficiently, and detection of the role of genetic predispositions till occurrence of Colienteritis and risks of severity of immunological disorders, or severity of dysbiosis in case of Coliescherichiosis has not been performed on the level of Ukrainian science at all.

An important role in this process belongs to immunological response to pathogen penetration. Neutrophils are one of the first reacting to chemoattractants – mediators of inflammation released from bacteria, dead cells, or produced by the endothelial or stromal cells in the places of inflammation. Neutrophils possess these properties due to availability/expression of appropriate receptors on their surface: adhesion (до *LFA*-1, *Mac*-1 integrins, E-, P-, L-selectins and their ligands), serpentine receptors (to fMLP, chemoattractants – LBT_4-rec, *PAFR, C5aR*, chemokinetic – *CXCR*1), Fc-receptors (F*cγR1, FcγRIIA*, or CD32, *FcγRIIIB*, or CD16, *cαRI, Fcε*RI, *Fcε*RIII), cytokine receptors (type 1 – *IL-4, -6, -12, -15, G-CSF, GM-CSF*; type 2 – *IFN*-α/β-rec, *IFN*-G, *IL*-10, *IL*-1family, *IL*-1RI,

IL-1RII(decoy), *IL*-18; family *TNF (TNF1, 2; FAS, TRAIL-R*2,3), receptors of natural immunity (*TLR* 1-9; lectins; *NOD*- and *RIG*-like receptors), etc. [2]. It should be noted that neutrophils' own production of cytokines influences upon the activation of the cellular immune response of Th1 and Th2 system, providing interaction of the cellular and humoral links of immunity [3]. Therefore, the change of neutrophil activity can be one of the factors determining the development and progress of pathological processes including Colienteritis. Although, genetic predictors promoting changes of cytokine production in case of Acute Colieschericchiosis are not studied sufficiently and require further investigations today.

Therefore, **objective** of our study was to investigate the effect of 1267 A→G gene polymorphism of heat shock proteins (HSP) family 70-2 (HSP70-2) and *C-592A* gene polymorphism of interleukin 10 (IL-10) on the level *IL*-1β, *TNF*-α and *IL*-10 in the blood of patients with AGEC.

Material and methods

The prospective study was conducted in compliance with the main provisions of the GCP (1996), the Council of Europe Convention on Human Rights and Biomedicine, the Helsinki Declaration of the World Medical Association on the ethical principles for the implementation of human medical research, with the informed consent of the patient to participate in the research. 95 patients participated in the study. By means of bacteriological method Enteropathogenic, Enterotoxigenic, Enteroinvasive, Enterohemorrhagic, or/and Enteroadhesive Escherichia coli and Hemolytic intestinal bacilli (E. coli Hly+) and lactose-negative E. coli were isolated and identified from the cavity of the colon. Availability of pathogenic causative agents such as Salmonellae, Shigellae, campylobacter, etc. was a reason to include patients into the study. The age of patients was from 25 to 52 (in an average 38.66±3.11 years). There were 62 women (65.26%) and 33 men (34.74%) among the examined patients. The control group included 30 practically healthy individuals without reliable differences by age and sexual distribution, who during the last 6 months and on the moment of examination did not have acute inflammatory or exacerbation of chronic inflammatory diseases of any localization, did not take antibacterial or antiseptic agents orally or rectally. Individuals who underwent screening signed an informed consent to participate in the study. A complex of clinical and laboratory examinations including genetic ones were performed.

Gene polymorphism of heat shock protein HSP70-2 (A1267G, rs1061581) and IL-10 (C-592A, rs1800872) was examined by means of polymerase chain reaction (PCR). DNA was isolated from the lymphocytes of the peripheral venous blood using the set of reagents "DNA-

sorb-B" (RU). PCR was performed using Taq-DNA-polymerase and specific primers for gene HSP70-2 (5′-CATCGACTTCTACACGTCCA-3′ – *forward* and 5′-CAAAGTCCTTGAGTCCCAAC-3′ – *reverse*) [4] and *IL-10* (5′-CCTAGGTCACAGTGACGTGG-3′ – *forward* and 5′-GGTGAGCACTACCTGACTAGC-3′ – *reverse*) [5]. Allele discrimination of HSP70-2 gene was performed by means of endonuclease restriction PstI ("Fermentas®", Lithuania). The products of amplification of DNA fragments (amplicons) of IL-10 gene experienced hydrolytic breaking up by means of endonuclease restriction *RsaI* ("Thermo Scientific", USA).

The concentration of anti-inflammatory cytokine of interleukin 10 (IL-10) and pro-inflammatory cytokines – Tumor Necrosis Factor alpha (TNF-α) and IL-1β was determined in the blood plasma by means of immune enzyme method according to the instruction of the reagents' producer: for IL-10 – Platinum ELISA, eBioscience® (Austria), for IL-1β, TNF-α – "ProCon TNF-α" and "ProCon IL-1β" (RU, ISO certificates 9001, 13485). Cytokine production was considered increased when the upper quartile of the control group was higher for pro-inflammatory IL-1β – >38.1 pg/ml, TNFα – >39.0 pg/ml, and decreased for anti-inflammatory IL-10 – lower than that of the lower quartile of the control group – <11.8 pg/ml, respectively.

Statistical analysis was performed using Statistica 7.0 (StatSoft Inc, USA) software. The reliability of the data for independent quantitative samples were calculated using Student's t-test (if distribution by Kolmogorov-Smirnov and W-Shapiro-Wilk test was close to the normal), or U-test Wilcoxon-Mann-Whitney (in case of uneven distribution), analysis of qualitative data (categorical variables) – by odds ratio (OR), with 95% confidence interval (CI) using a chi-square test (χ^2) (df=1). P values <0.05 were considered statistically significant.

Results and discussion

Patients with AGEC, high and average production of pro-inflammatory cytokines (IL-1β and TNFα) were registered 2.96 times as often than those with a normal one (χ^2=46.51, p<0.001), which is indicative of an increased activity of macrophages and T-lymphocytes helpers of the 1st type (Th1) (Table 1). On the contrary, individuals with normal synthesis of anti-inflammatory *IL-10* were 1,64 times less (on 24.22%), than the patients with a reduced production of *IL-10* respectively (χ^2=11.14, p=0.001). In patients the content of IL-1β and TNFα in the blood prevailed over that one in the control group on 40.93% (p<0.001) and 44.44% (p<0.001), with lower level of IL-10 – on 37.30% (p<0.001) respectively.

Table 1

Cytokine level production in patients with Colienteritis

Data	Control (n=30)	Study group (n=95)	Cytokine level production in patients	
			Production within the norm, n (%)	High, moderate production, n (%)
IL-1β, pg/ml	33.74±4.33	57.12±4.63 p<0.001	24 (25.26)	71 (74.74)
TNFα, pg/ml	35.05±3.27	63.08±5.32 p<0.001	24 (25.26)	71(74.74)
IL-10, pg/ml	13.95±2.11	8.74±0.82 p<0.001	36 (37.89)	Below the norm. n (%)
				n=59 (62.11%)

Notes. p – probability of differences of the parameters with the control group

Due to the fact that cytokine content in the control group considering polymorphic variants of HSP70-2 genes and IL-10 did not differ, division of the parameters of the body immune response, levels of adaptation-compensatory nonspecific reactions, cellular and immunological reactivity, reactive response of neutrophil granulocytes considering certain genotypes of the analyzed genes have not been performed.

The concentration of cytokines IL-1β, TNFα, IL-10 in the blood plasma of patients with Colienteritis considering allele condition of *HSP*70-2 (1267A→G) and IL-10 (C-592A) genes is presented in Table 2. The levels of pro-inflammatory pre-immune markers of the immune system functioning of the first generation (*IL-1β* and *TNFα*) prevailed reliably over those in practically healthy individuals irrespective of polymorphic variants of *HSP70*-2 gene (more substantial in carriers of *GG*-genotype): for *IL-1β* – on 38.35% in individuals with *A*-allele and twice as much in the carriers of *GG*-genotype ($p<0.001$) respectively; for *TNFα* – on 62.28% and 92.64% ($p<0.001$) respectively. Against the ground of increasing pro-inflammatory cytokines the content of anti-inflammatory *IL*-10 was on the contrary lower than that of the control group – on 29.18% ($p<0.05$) and 45.73% ($p=0.005$) respectively, with reliable difference between groups: those with *GG*-genotype had *IL*-10 level lower than that of those individuals with *A*-allele on 23.38% ($p_1=0.031$), and the content of *IL-1β* higher – on 45.05% ($p_1<0.001$) respectively (Table 2).

Single-factor dispersive analysis confirmed the association between the gene promoter of heat shock protein HSP70-2 (1267A→G) and increased level of IL-1β in the blood ($F=11.66$; $p<0.001$), and decreased concentration of IL-10 ($F=4.56$; $p=0.035$) (Table 2).

Table 2

Cytokine level in patients with Colienteritis depending on gene's *HSP*70-2 (1267A→G) allelic condition (M±m)

Data	Control group	Genotypes gene HSP70-2 in patients	
		AA+AG, n=51	GG, n=44
IL-1β, pg/ml	33.74±4.33	46.68±3.45 p<0.001	67.71±5.29 p.p_1<0.001
TNFα, pg/ml	35.05±3.27	56.88±5.23 p<0.001	67.52±6.30 p<0.001
IL-10, pg/ml	13.95±2.11	9.88±0.85 p<0.05	7.57±0.62 p=0.005 p_1=0.031

Notes. p – probability of differences of the parameters with the control group; p_1 - probability of differences of the parameters with carriers of AA+AG-genotypes

Cytokine content in the blood of patients with AGEC depending on the allele state of *IL-10* (*C-592A*) gene is presented in Table 3. Concentrations of *IL-1β* and *TNFα* in patients are reliably higher than those in the control group, irrespective of the genotype of *IL-10* (*C-592A*) gene – on 58.03-71.55% (p<0.001) and on 62.17-85.51% (p<0.001) respectively. As to the content of *IL-10,* reliably lower concentration was found in patients with AGEC and *A*-allele (*AA*-, *CA*-genotypes), in comparison with *GG*-carriers – on 26.95% and 20.64% (p<0.05).

Single-factor dispersive analysis confirmed the association between the gene promoter *IL-10* (*C-592A*) only with the reduced content of *IL*-10 (F=3.14, p=0.048).

Table 3

Cytokine level in patients with Colienteritis depending on gene's *IL-10* (*C-592A*) polymorphic variants (M±m)

Data	Control group	Genotypes gene *IL-10* in patients		
		AA, n=9	*CA*, n=36	*CC*, n=50
IL-1β, pg/ml	33.74±4.33	53.32±6.91 p<0.001	58.14±5.49 p<0.001	57.88±7.03 p<0.001
TNFα, pg/ml	35.05±3.27	56.84±4.56 p<0.001	60.63±8.15 p<0.001	65.02±6.79 p<0.001
IL-10, pg/ml	13.95±2.11	6.83±0.57 p=0.01	7.42±0.46 p<0.01	9.35±0.70 p=0.042 p_{AA}=0.007 p_{CA}=0.025

Notes. p – probability of differences of the parameters with the control group; p_{AA} - probability of differences of the parameters with AA-genotype carriers; p_{CA} - probability of differences of the parameters with CA-genotype carriers.

The results obtained are indicative of the availability of acute inflammatory process in patients, when pre-immune *IL-1β* and *TNFα* quickly increase in response to alteration, and synthesis of anti-inflammatory *IL*-10 is not yet able to increase compensatory to stimulate B-lymphocytes and mast cells, development of humoral response, inhibition of the activity of Th1 cells and cellular immune response, decreased proliferation of T-cells and production of pro-inflammatory cytokines. *TNFα* is one of the co-factors of activation, growth and maturation of T- and B-lymphocytes, endothelial cells, NK-cells, and fibroblasts. *IL-1β* interacts with Th2 and induces synthesis of *IL*-8, *IL*-6, *IL*-5, *IL*-4, *IL*-3, *IF*-γ, increases secretion of antibodies by B-lymphocytes, expression of *IL*-2 receptors [6-10]. *IL-1β* causes chemotaxis of macrophages, neutrophils, promotes their migration through the vascular endothelium into the focus of inflammation, where it activates synthesis of cytokines, prostaglandins, acute phase proteins, collagen and fibronectin, stimulates phagocytosis, causes degranulation of mast cells, and thus promotes development of exudative and proliferative phases of inflammatory reaction [11]. *IL-1β* produces an effect on immune-regulating processes: intensifies proliferation of CD4+-cells, growth and differentiation of B-cells, promotes activation of antibody production, stimulates hemopoiesis processes. Therefore, *IL-1β* causes triggering immune reactions, plays a key role in the development of inflammation, and is a mediator between the immune and nervous systems [11].

Conclusions: Changes of the body immune-inflammatory response in patients with colienteritis are characterized by increased synthesis of the first generation pro-inflammatory cytokines *IL-1β* and *TNFα* on 40,93% and 44,44% respectively against the ground of low production of anti-inflammatory *IL*-10 and are indicative of imbalance between high activity of the immunity cellular link and insufficient activity of the humoral link of the immune response.

The gene promoter of heat shock protein *HSP*70-2 (1267A→G) associates with increased level of *IL-1β* in the blood and decreased concentration of *IL*-10, more substantial in the carriers of GG-genotype on 45,05% and 23,38% respectively. On the contrary, promoter of *IL-10* (*C-592A*) gene associates only with reduced *IL*-10 content, most considerably in the carriers of the mutation *A*-allele on 26,95% and 20,64% respectively.

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