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## Changes in electrokinetic index of buccal epithelium correlated with changes in some parameters of immunity and fecal microbiocenosis

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### Abstract

Previously we examined relationships between electrokinetic index of the buccal epithelium (EKI) and some functional and metabolic parameters of patients with chronic pyelonephritis in the phase of remission. The purpose of this study is the relationships between changes in EKI and some parameters of immunity, fecal microbiocenosis as well as urine in these same patients. **Materials and Methods.** Under a observations were 32 males and 10 females by age 24-76 years with chronic pyelonephritis in the phase of remission. We determined the rate of electronegative nuclei of buccal epithelium by intracellular microelectrophoresis (EKI), counted up leukocytogram and calculated its adaptation and strain indexes by IL Popovych, evaluated immune status on a set of I and II levels recommended by the WHO as well as fecal microbiocenosis, bacteriuria and leukocyturia by routine methods. After 9-11 days of balneotherapy (drinking of bioactive water Naftussya, applications of ozokerite, mineral pools) all testes repeated. **Results.** A number of parameters of immunity and fecal microbiocenosis have been identified, the changes of which correlate with changes in EKI. A turn-based exclusion in the multiple regression model includes changes in the Killing Index by Neutrophils of *E. coli*, levels in the blood of T-active and 0 Lymphocytes, rod-nuclear Neutrophils, Entropy of the Immunocytogram, Popovych's Strain Index of Leukocytogram as well as Leukocytesuria and content in feces *E. coli*.  $R=0,686$ ;  $R^2=0,470$ ; Adjusted  $R^2=0,342$ ;  $F_{(8,3)}=3,7$ ;  $p=0,004$ . **Conclusion.** Caused by balneotherapy, the increase in most patients in the EKI is accompanied by favorable

changes in parameters of immunity and fecal microbiocenosis, which justifies its use to assess the effectiveness of balneotherapy.

**Key words:** electrokinetic index of the buccal epithelium, immunity, fecal microbiocenosis, relationships.

## INTRODUCTION

Previously we examined relationships between EKI and some functional and metabolic parameters of 23 males with chronic pyelonephritis in the phase of remission. We have shown that Baevskiy's Adaptation Potential and Stange's Test together determines EKI on 28%. Vagal markers RMSSD and VLF as well as Baevskiy's Stress Index determines EKI on 31%. Plasma Cholesterol and Klimov's Atherogenicity Coefficient determines EKI on 56%. In summary model of multiple regression with stepwise excluding are currently two last parameters as well as Plasma Testosterone and relative Spectral Power VLF band HRV, which together determines EKI on 73% [4,9,10]. In the next study [8] under a observations were 32 males and 10 females by age 24-76 years with chronic pyelonephritis in the phase of remission, who were treated at the Truskavets' spa. All patients were divided into four clusters, homogeneous with changes in EKI. The largest cluster was 26 people, in which the EKI increased by  $2,36 \pm 0,22\%$ . In another 5 people, EKI increased by  $0,90 \pm 0,17\%$ , at the same time, in 7 patients, EKI remained unchanged, and only in 4 patients it decreased by  $1,38 \pm 0,65\%$ . Thus, in 74% of the subjects balneotherapy caused the so-called "youthful" effect, while in 17% it was ineffective, and in 9% the effect should be assessed as unfavorable. The method of discriminant analysis revealed 27 parameters (12 parameters of EEG. 2 HRV markers of vagal tone, plasma levels testosterone normalized by sex, cholesterol, creatinine, potassium, excretion of urea, urates and phosphates, parathyrin activity as well as 4 parameters of hemodynamics and Hench's expiratory test), the changes in which are characteristic of four qualitative-quantitative variations of changes in EKI. Thus, changes in EKI really reflects changes in neuro-endocrine regulation, metabolism and hemodynamics.

The purpose of this study is the relationships between changes in EKI and some parameters of immunity, fecal microbiocenosis as well as urine in these same patients.

## MATERIALS AND METHODS

Under a observations were 32 males and 10 females by age 24-76 years with chronic pyelonephritis in the phase of remission. At a receipt, we first determined them the EKI as rate of electronegative nuclei of buccal epithelium by intracellular microelectrophoresis on the device "Biotest" (Kharkiv State University), according to the method described [12,13,17].

Then in portion of capillary blood we counted up Leukocytogram and calculated its Adaptation Index as well as Strain Index by IL Popovych [1,14,15].

Immune status evaluated on a set of I and II levels recommended by the WHO as described in the manual [18]. For phenotyping subpopulations of lymphocytes used the methods of rosette formation with sheep erythrocytes on which adsorbed monoclonal antibodies against receptors CD3, CD4, CD8, CD22 and CD16 from company "Granum" (Kharkiv) with visualization under light microscope with immersion system. Subpopulation of T cells with receptors high affinity determined by test of "active" rosette formation. The state of humoral immunity judged by the concentration in serum of Immunoglobulins classes G, A, M (ELISA, analyser "Immunochem", USA) and circulating immune complexes (by polyethylene glycol precipitation method).

We calculated also the Entropy (h) of Immunocytogram (ICG) and Leukocytogram (LCG) using classical CE Shannon's formula [16]:

$$hICG = - [CD4 \cdot \log_2 CD4 + CD8 \cdot \log_2 CD8 + CD22 \cdot \log_2 CD22 + CD16 \cdot \log_2 CD16] / \log_2 4$$

$$hLCG = - [L \cdot \log_2 L + M \cdot \log_2 M + E \cdot \log_2 E + SNN \cdot \log_2 SNN + StubN \cdot \log_2 StubN] / \log_2 5$$

Parameters of phagocytic function of neutrophils estimated as described by SD Douglas and PG Quie [3] with moderately modification by MM Kovbasnyuk [7]. The objects of phagocytosis served daily cultures of Staphylococcus aureus (ATCC N 25423 F49) as typical specimen for Gram-positive Bacteria and Escherichia coli (O55 K59) as typical representative of Gram-negative Bacteria. Both cultures obtained from Laboratory of Hydro-Geological Regime-Operational Station JSC "Truskavets'kurort". Take into account the following parameters of Phagocytosis: activity (percentage of neutrophils, in which found microbes - Hamburger's Phagocytic Index PhI), intensity (number of microbes absorbed one phagocytes - Microbial Count MC or Right's Index) and completeness (percentage of dead microbes - Killing Index KI). On the basis of the recorded partial parameters of Phagocytosis, taking into account the Neutrophils (N) content of 1 L blood, we calculated the integral parameter - Bactericidal Capacity of Neutrophils (BCCN) by the formula [7,15]:

$$BCCN (10^9 \text{ Bact/L}) = N (10^9/\text{L}) \cdot \text{PhI} (\%) \cdot \text{MC} (\text{Bact/Phag}) \cdot \text{KI} (\%) \cdot 10^{-4}$$

For the Z-scores of eleven immune parameters, the integral immune index was calculated [21].

In addition, we determined the indicators of fecal microbiocenosis as well as bacteriuria and leukocyturia by routine methods.

After 9-11 days of balneotherapy (drinking of bioactive water Naftussya, applications of ozokerite, mineral pools) all testes repeated.

For statistical analysis used the software package "Statistica 5.5".

## RESULTS AND DISCUSSION

The screening of correlation between changes in EKI as factor (independent) variable and parameters of immunity, fecal microbiocenosis as well as urine as dependent variables revealed the following variables worthy of attention (Table 1).

Table 1. Matrix of correlation between changes in EKI and others variables

Variables	EKI
Killing Index vs Staph. aureus	,31
Integral Immune Index	,30
Killing Index vs E. coli	,26
Bactericidity vs E. coli	,26
T-active Lymphocytes	,25
Phagocytic Index vs E. coli	,21
Bactericidity vs Staph. aureus	,21
CD22 <sup>+</sup> B-Lymphocytes	,19
CD16 <sup>+</sup> NK-Lymphocytes	,19
Entropy of Immunocytogram	,17
Immunoglobulins A	,15
Rod-nuclear Neutrophils	,14
Segment-nuclear Neutrophils	,13
Popovych's Strain Index	,12
Bacteriuria	-,24
0-Lymphocytes	-,21
Leukocyturia	-,19
Phagocytic Index vs St. aureus	-,18
E. coli attenuated in faeces	-,16
E. coli total in faeces	-,13

It was found that balneotherapy induced changes in the EKI (recall, it increased in 74% of patients) was accompanied by unidirectional changes in 13 immune parameters as well as in the Integral Immune Index. The oppositely directed changes in markers of pyelonephritis (Bacteriuria and Leukocyturia) and dysbiosis (contents E. coli in faeces), as well as the level of functionally defective 0-Lymphocytes, also reflect the **beneficial** effects of balneotherapy. From this series only the Phagocytic Index vs St. aureus falls.

According to the formula:

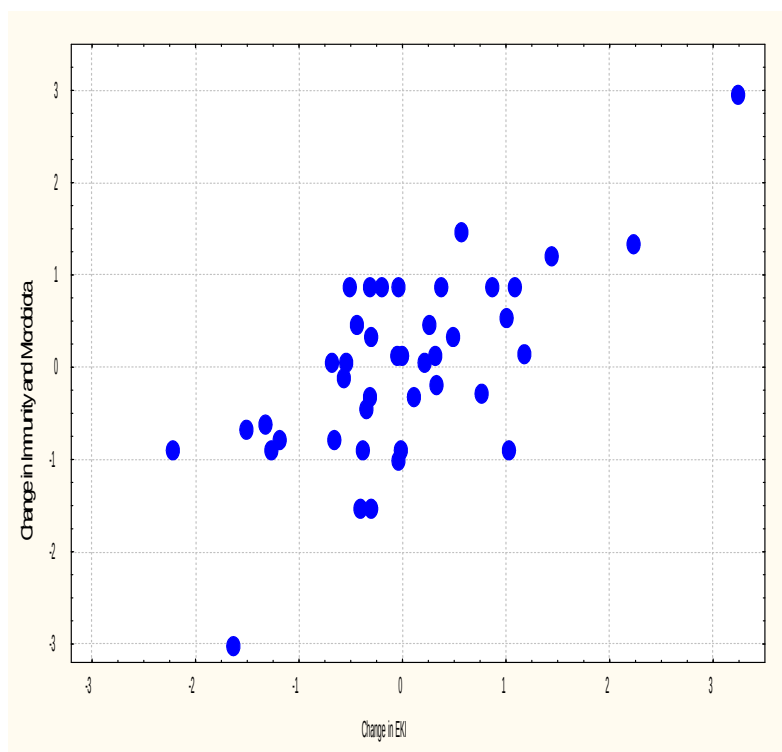
$$|r| \geq \frac{\exp[2t/(n-1,5)^{0,5}] - 1}{\exp[2t/(n-1,5)^{0,5}] + 1},$$

for a sample of 42 observations critical value of correlation coefficient module  $|r|$  at  $p < 0,05$  ( $t > 2,02$ ) is 0,31.

Nevertheless, the program included an unexpected set of variables in the final regression model after the turn-by-turn exclusion (Table 2 and Fig. 1).

Table 2. Regression Summary for Independent Variable: Change in EKI  
 $R=0,686$ ;  $R^2=0,470$ ; Adjusted  $R^2=0,342$ ;  $F_{(8,3)}=3,7$ ;  $p=0,004$ .

		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(33)}$	p-level
<b>Change in Variables</b>	r		Intercept	1,515	,277	5,48	$10^{-5}$
Killing Index vs E. coli, %	0,26	,668	,177	,0745	,0197	3,77	$10^{-3}$
T-active Lymphocytes, %	0,25	,343	,135	,1010	,0397	2,55	,016
Entropy of Immunocytogram	0,17	,447	,302	18,16	12,29	1,48	,149
Rod-nuclear Neutrophils, %	0,14	,280	,144	,3393	,1748	1,94	,061
Popovych's Strain Index	0,12	,160	,141	,760	,669	1,14	,264
0-Lymphocytes, %	-0,21	,471	,326	,0825	,0570	1,45	,157
Leukocyturia, $10^3$ Leuk/mL	-0,19	-,304	,154	-,02	,01	-1,98	,056
E. coli, $10^6$ /g faeces	-0,13	-,854	,208	-,0107	,0026	-4,11	$10^{-3}$



$R=0,686$ ;  $R^2=0,470$ ;  $\chi^2_{(8)}=23$ ;  $p=0,004$ ;  $\Lambda$  Prime=0,530

**Fig. 1. Scatterplot of canonical correlation between changes in Electrokinetic Index (line X) and parameters of Immunity and Microbiocenosis (line Y)**

The results we obtained in this and previous studies, show that the changes in EKI caused by balneotherapy, characterize its effectiveness. How not to mention here that when the device "Biotest" patenting his main purpose was stated rapid testing efficiency rehabilitation of human health, particularly in resort [12,13]! Ambiguous changes in the EKI coincide with the previously detected unclear changes in immune [7,19-21] and other [2] parameters of the patients in the Truskavets' Spa. We confirm the legality of the application of the method for assessing the general state of health [5,6,18].

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## ACCORDANCE TO ETHICS STANDARDS

Tests in patients are conducted in accordance with positions of Helsinki Declaration 1975, revised and complemented in 2002, and directive of National Committee on ethics of scientific researches. During realization of tests from all participants the informed consent is got and used all measures for providing of anonymity of participants.

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