Vegetative, metabolic and immune accompaniments of changes in the electrokinetic index of the buccal epithelium under the influence of therapeutic factors

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Abstracts

Background. In previous studies, we have shown that electrokinetic index of buccal epithelium (EKI) correlated with some functional and metabolic parameters. Subsequent studies have shown that changes in EKI correlated with changes in some parameters of EEG, HRV, hemodynamics, metabolism, immunity and fecal microbiocenosis. Further research in this direction was continued on a significantly increased contingent of patients and with the involvement of new methods and factors of influence. This message starts the presentation of the obtained results. Material and methods. Under a observations were 44 men (49±15 years) and 30 women (51±13 years) without clinical diagnosis or with chronic pyelonephritis in the phase of remission (23 men). We registered caused by the various therapeutic factors changes in EKI, state of the vegetative and hormonal regulation as well as immunity and metabolism, then calculated relationships between changes. Results. In 49 patients the changes in EKI were in the range of ±2,5%, in 19 people EKI increased by more than 2,5% (M±SD=+4,0±1,6%), while in 9 people decreased by more than 2,5% (-4,2±1,7%). The canonical correlation between changes in EKI, on the one hand, and HRV and immunity parameters, on the other, is moderate: R=0,478; p=0,023. The method of discriminant analysis revealed 10 immune and 6 HRV parameters as well as triglycerides and cholesterol, whose changes are characteristic of multidirectional changes in EKI. Conclusion. Electrokinetic index of buccal epithelium responds to therapeutic factors in different directions, accompanied by characteristic changes in a number of parameters of HRV, immunity and metabolism.

Keywords: Electrokinetic index, HRV, hormones, immunity, metabolism, relationships.
INTRODUCTION

In previous pilot studies, we have shown that electrokinetic index of buccal epithelium (EKI) correlated with some functional and metabolic parameters [9,18,20]. Subsequent studies have shown that caused by balneofactors changes in EKI correlated with changes in some parameters of EEG, HRV, hemodynamics and metabolism [1,17] as well as immunity and fecal microbiocenosis [1,19]. Further research in this direction was continued on a significantly increased contingent of patients and with the involvement of new methods and factors of influence. This message starts the presentation of the obtained results.

MATERIALS AND METHODS

Under a observations were 44 men (49±15 years) and 30 women (51±13 years) without clinical diagnosis or with chronic pyelonephritis in the phase of remission (23 men).

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 [13,14,22,23,28,30].

Then estimated the state of the autonomous (vegetative) regulation by the method heart rate variability (HRV) [2,5,12,27], using a hardware-programmatic complex "CardioLab+HRV" (KhAI Medica, Kharkiv, Ukraine). Calculated as well Kerdö’s Vegetative Index [8].

At last we took venous blood samples for biochemical and immune tests. We determined content in plasma hormones: Cortisol, Testosterone and Triiodothyronine (by the ELISA with the use of analyzer “RT-2100C” and corresponding sets of reagents from “Алкор Био”, XEMA Co., Ltd and DRG International Inc.); nitric metabolites: creatinine (by Jaffe's color reaction by Popper's method); urea (urease method by reaction with phenolhypochlorite) and uric acid (uricase method); lipide spectrum of plasma: total cholesterol (by a direct method after the classic reaction by Zlatkis-Zack) and content of him in composition of α-lipoproteins (by the enzyme method after precipitation of noto-lipoproteins); praе-β-lipoproteins (expected by the level of triglycerides, by a certain meta-periodate method); β-lipoproteins (expected by a difference between a total cholesterol and cholesterol in composition α-and praе-β-lipoproteins). The analysis carried out according to instructions [10] with the use of analyzers "Reflotron" (BRD) and "Pointe-180" (USA) and corresponding sets of reagents.

Immune status evaluated on a set of I and II levels recommended by the WHO as described in the manual [21]. 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 CD56 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 “Immunochim”, USA) and circulating immune complexes (by polyethylene glycol precipitation method) as well as C-reactive protein (by the ELISA with the use of analyzer “RT-2100C”), Interleukin 1β and 6 (ELISA, analyzer “Stat Fax 303”, USA, reagents from “Vector-Best”, RF).

In portion of capillary the blood we counted up Leukocytogram and calculated its Adaptation Index-2 as well as Strain Index-2 by IL Popovych [4,11,24,25]:

\[
PSI-2 = \left(\frac{\text{Eosinoph}}{2,75-1}\right)^2 + \left(\frac{\text{StubNeutroph}}{4,25-1}\right)^2 + \left(\frac{\text{Monoc}}{6-1}\right)^2 + \left(\frac{\text{Leukocyt}}{5-1}\right)^2 \right] / 4
\]

We calculated also the Entropy (h) of Immunocytogram (ICG) and Leukocytogram (LCG) using IL Popovych’s formula [11] based on classical CE Shannon’s formula [29]:

\[
hICG = - \left[\text{CD4} \cdot \log_2 \text{CD4} + \text{CD8} \cdot \log_2 \text{CD8} + \text{CD22} \cdot \log_2 \text{CD22} + \text{CD22} + \text{CD16} \cdot \log_2 \text{CD16}\right] / \log_2 4
\]

\[
hLCG = - \left[\text{L} \cdot \log_2 \text{L} + \text{M} \cdot \log_2 \text{M} + \text{E} \cdot \log_2 \text{E} + \text{SNN} \cdot \log_2 \text{SNN} + \text{StubN} \cdot \log_2 \text{StubN}\right] / \log_2 5
\]
Parameters of phagocytic function of neutrophils estimated as described by SD Douglas and PG Quie [7] with moderately modification by MM Kovbasnyuk [16]. The objects of phagocytosis served daily cultures of Staphylococcus aureus (ATCC N25423 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’kuort”. 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 [11]:

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

After therapy: drinking of bioactive water Naftussya only (n=22), Naftussya and applications of ozokerite and mineral pools (n=13) or electrostimulation by device “VEB”[1] (n=39), all testes repeated.

For statistical analysis used the software package "Statistica 64".

RESULTS AND DISCUSSION

In this sample, as in previous ones, a variety of changes in EKI were found. In 49 patients the changes were in the range of ±2.5% and were considered insignificant, in 19 people EKI increased by more than 2.5% (M±SD=+4.0±1.6%), while in 9 people decreased by more than 2.5% (-4.2±1.7%). All three variants were observed in response to all three treatment regimens. This is consistent with the well-documented concept of the multivariate effect of therapeutic factors of the spa of ‘Truskavets’ on the parameters of the human and animal body due to individual reactivity [3,6,15,16,26,31-33].

According to the formula:

\[ |r| \geq \frac{1}{\left(\exp\left[2t/(n-1.5)^{0.5}\right] - 1\right)/\left(\exp\left[2t/(n-1.5)^{0.5}\right] + 1\right)}\]

for a sample of 74 observations critical value of correlation coefficient module |r| at p<0.05 (t>2.00) is 0.23; at p<0.01 (t>2.66) is 0.30; at p<0.001 (t>3.46) is 0.38.

Screening of correlations between changes in EKI and recorded parameters showed a direct correlation with phagocytosis activity by neutrophils vs E. coli, while inverse correlation with eosinophilia, leukocytogram strain index, and a number of HRV parameters (Table 1).

| Table 1. Factor loads and correlations for change in HRV&Immunity parameters and Electrokinetic Index |
|---------------------------------|---------|---------|
| **Left set**                    | R       | r       |
| Eosinophiles, %                 | 0.747   | -0.36   |
| Total Power HRV, msec²          | 0.542   | -0.26   |
| (VLF+ULF) HRV PS, msec²         | 0.532   | -0.25   |
| Popovych’s Strain Index-2       | 0.506   | -0.24   |
| ULF HRV PS, msec²               | 0.504   | -0.24   |
| LF/(LF+HF)                      | 0.483   | -0.23   |
| LF/HF                           | 0.475   | -0.23   |
| Phagocytose Index vs E. coli, %  | -0.535  | 0.26    |
| **Right set**                   | R       |         |
| Electrokinetic Index, %         | -1      |         |

If the LF/(LF+HF) and LF/HF indices uniquely reflect sympathetic tone and sympathetic-vagal balance, respectively, VLF and ULF bands still do not have a clear
physiological interpretation. Let's take advantage of the latest wonderful review Shaffer F & Ginsberg JP [27]. We quote. "There is uncertainty regarding the physiological mechanisms responsible for activity within the VLF (0.04÷0.0033 Hz) band. The heart’s intrinsic nervous system appears to contribute to the VLF rhythm and the sympathetic nervous system influences the amplitude and frequency of its oscillations. Very-low-frequency power may also be generated by physical activity, thermoregulatory, renin–angiotensin, and endothelial influences on the heart. Vagal activity may contribute to VLF power since parasympathetic blockade almost completely abolishes it. In contrast, sympathetic blockade does not affect VLF power and VLF activity is seen in tetraplegics, whose sympathetic nervous system innervation of the heart and lungs is disrupted. The VLF rhythm appears to be generated by the stimulation of afferent sensory neurons in the heart. This, in turn, activates various levels of the feedback and feed-forward loops in the heart’s intrinsic cardiac nervous system, as well as between the heart, the extrinsic cardiac ganglia, and spinal column. This experimental evidence suggests that the heart intrinsically generates the VLF rhythm and efferent sympathetic nervous system activity due to physical activity and stress responses modulates its amplitude and frequency”.

Because in our device ULF band (range 0.015÷0.003 Hz) is integrated into the lower zone of VLF band, what has been said about the latter also applies to the former. By the way, the relative power spectrum of these bands during the analysis were combined into an option (VLF+ULF). The presence in the factor structure of total power of HRV is explained by the fact that the shares of VLF and ULF bands are on average 47% and 6%, respectively.

The canonical correlation between changes in EKI, on the one hand, and HRV and immunity parameters, on the other, is moderate (Fig. 1).

Another approach to identifying autonomic, hormonal, immune, and metabolic accompaniments to EKI changes is discriminant analysis. The forward stepwise program included 19 parameters in the discriminant model. These are, in addition to EKI by definition, 10 immune and 6 HRV parameters as well as triglycerides and cholesterol levels standardized by sex and age (Tables 2 and 3).
Table 2. Discriminant Function Analysis Summary for Changes in HRV, Immune and Metabolic Variables
Step 19, N of vars in model: 19; Grouping: 3 grps. Wilks' Λ: 0.0546; approx. F(38) = 9.1; p<10^-6

| Variables currently in the model | EKI >+2.5% (19) | EKI +2.5% (-25) (49) | EKI <−2.5% (6) | Wilks Λ | Partial Λ | F-remove (2,53) | p-level | Tolerance |
|----------------------------------|------------------|-----------------------|-----------------|----------|-----------|----------------|---------|-----------|
| Electrokinetic Index, %          | +4.0             | +0.65                 | -4.2            | 0.299    | 0.183     | 118            | 10^-4   | 0.509     |
| Popovych's Adaptation Index-2    | +0.28            | +0.11                 | -0.16           | 0.060    | 0.904     | 2.83           | 0.068   | 0.657     |
| Polymorphonucl Neutrophils, %    | +1.2             | -0.7                  | -2.7            | 0.066    | 0.824     | 5.67           | 0.006   | 0.175     |
| Bactericidivity vs E. coli, 10^9 B/L | +18              | +13                   | +5              | 0.060    | 0.906     | 2.75           | 0.073   | 0.439     |
| Phagocytose Index vs E. coli, %  | +0.1             | 0.0                   | -1.9            | 0.063    | 0.867     | 4.05           | 0.023   | 0.597     |
| Interleukin-6, ng/L              | -0.15            | -0.02                 | -0.78           | 0.060    | 0.914     | 2.50           | 0.092   | 0.424     |
| Eosinophils, %                   | -0.34            | -0.24                 | +2.7            | 0.060    | 0.911     | 2.60           | 0.084   | 0.524     |
| Leukocytes, 10^9/L               | -0.04            | -0.19                 | +0.65           | 0.057    | 0.964     | 0.99           | 0.379   | 0.555     |
| Immunocytogram Entropy           | -1.1             | +4                    | +4              | 0.080    | 0.686     | 12.1           | 10^-4   | 0.363     |
| (VLF+ULF) HRV PS, msec^1         | -2604            | -335                  | -419            | 0.056    | 0.967     | 0.89           | 0.416   | 0.750     |
| Mode HRV, msec                   | -53              | -46                   | -20             | 0.072    | 0.763     | 8.25           | 0.001   | 0.467     |
| Baevsky’s Stress Index, units    | +31              | -11                   | +226            | 0.071    | 0.773     | 7.76           | 0.001   | 0.044     |
| AMo/MxDMn Index, units           | +50              | -17                   | +207            | 0.068    | 0.803     | 6.49           | 0.003   | 0.053     |
| Baevsky’s ARS Index, units       | +0.16            | -0.13                 | +1.53           | 0.058    | 0.947     | 1.49           | 0.235   | 0.474     |
| ULF HRV PS, %                    | +0.1             | -0.7                  | +6.4            | 0.056    | 0.979     | 0.58           | 0.565   | 0.636     |
| Triglycerides standardized, Z    | -0.33            | +0.14                 | -0.62           | 0.058    | 0.943     | 1.60           | 0.211   | 0.779     |
| Cholesterol standardized, Z      | -0.37            | -0.21                 | -0.45           | 0.065    | 0.837     | 5.17           | 0.009   | 0.375     |
| 0 Lymphocytes, %                 | -3.4             | -0.7                  | -2.7            | 0.080    | 0.684     | 12.2           | 10^-4   | 0.386     |
| Pan Lymphocytes, %               | -1.3             | +0.6                  | -0.3            | 0.059    | 0.927     | 2.09           | 0.133   | 0.193     |

Table 3. Summary of Stepwise Analysis for Changes in HRV, Immune and Metabolic Variables, ranked by criterion Lambda

| Variables currently in the model | F to enter | p-level | Wilks Λ | F-value | p-level |
|----------------------------------|------------|---------|---------|---------|---------|
| Electrokinetic Index, %          | 121        | 10^-6   | 0.227   | 121     | 10^-6   |
| Phagocytose Index vs E. coli, %  | 5.60       | 0.006   | 0.195   | 44.4    | 10^-6   |
| Baevsky’s Stress Index, units    | 4.81       | 0.011   | 0.171   | 32.6    | 10^-5   |
| Leukocytes, 10^9/L               | 3.13       | 0.050   | 0.157   | 25.9    | 10^-5   |
| ULF HRV PS, %                    | 2.65       | 0.078   | 0.145   | 21.7    | 10^-5   |
| 0 Lymphocytes, %                 | 2.34       | 0.105   | 0.136   | 18.8    | 10^-4   |
| Immunocytogram Entropy           | 5.32       | 0.007   | 0.117   | 17.9    | 10^-5   |
| Mode HRV, msec                   | 3.36       | 0.041   | 0.106   | 16.6    | 10^-5   |
| AMo/MxDMn Index, units           | 2.42       | 0.097   | 0.098   | 15.3    | 10^-4   |
| (VLF+ULF) HRV PS, msec^1         | 1.60       | 0.210   | 0.093   | 14.1    | 10^-4   |
| Bactericidivity vs E. coli, 10^9 Bac/L | 1.29   | 0.284   | 0.090   | 13.0    | 10^-4   |
| Polymorphonucl Neutrophils, %    | 2.15       | 0.125   | 0.084   | 12.3    | 10^-4   |
| Baevsky’s ARS Index, units       | 1.77       | 0.179   | 0.079   | 11.6    | 10^-4   |
| Cholesterol standardized, Z      | 1.94       | 0.152   | 0.074   | 11.1    | 10^-4   |
| Interleukin-6, ng/L              | 1.56       | 0.218   | 0.070   | 10.6    | 10^-4   |
| Eosinophiles, %                  | 1.50       | 0.232   | 0.066   | 10.1    | 10^-4   |
| Popovych’s Adaptation Index-2    | 1.40       | 0.255   | 0.063   | 9.63    | 10^-4   |
| Pan Lymphocytes, %               | 2.47       | 0.094   | 0.058   | 9.46    | 10^-4   |
| Triglycerides standardized, Z    | 1.60       | 0.211   | 0.055   | 9.15    | 10^-4   |

The rest of the registered parameters were outside the model, despite the fact that some of them carry identifying information (Table 4).
Table 4. Discriminant Function Analysis Summary. HRV, Endocrine, Immune and Metabolic Variables change in which currently not in the model

| Variables | EKI >+2,5 % | EKI <+2,5 % | EKI <+2,5 % | Wilks Λ | Partial Λ | F to enter | p-level | Tolerance |
|-----------|-------------|-------------|-------------|---------|-----------|------------|---------|-----------|
| Uric acid standardized, Z | +0.08 | -0.04 | -0.01 | 0.054 | 0.993 | 0.193 | 0.825 | 0.763 |
| Interleukin-1, ng/L | +0.02 | -0.27 | -0.65 | 0.055 | 0.999 | 0.016 | 0.984 | 0.679 |
| CD3⁺ T active Lymphocytes, % | +1.4 | -0.3 | -0.5 | 0.054 | 0.991 | 0.228 | 0.797 | 0.681 |
| Immunoglobulins M, g/L | -0.03 | -0.03 | -0.20 | 0.054 | 0.989 | 0.294 | 0.746 | 0.827 |
| Mier. Count vs St. aur., Bac/Phag | +5.3 | +2.7 | +1.2 | 0.053 | 0.978 | 0.598 | 0.553 | 0.721 |
| Mier. Count vs E. coli, Bact/Phag | +4.1 | +2.5 | 0.0 | 0.054 | 0.992 | 0.204 | 0.816 | 0.480 |
| 100(I-Pd/HR) as Kerdő Index, % | +7.0 | +5.8 | +2.1 | 0.054 | 0.982 | 0.519 | 0.594 | 0.536 |
| Stab Neutrophils, % | +0.6 | 0.0 | -0.2 | 0.054 | 0.989 | 0.598 | 0.553 | 0.721 |
| Cortisol, nM/L | +28 | +27 | -41 | 0.053 | 0.978 | 0.598 | 0.553 | 0.721 |
| MxDMn HRV, msec | -8 | -7 | -46 | 0.054 | 0.979 | 0.546 | 0.582 | 0.629 |
| Triiodothyronine, nM/L | -0.17 | -0.05 | -0.16 | 0.054 | 0.978 | 0.598 | 0.746 | 0.827 |
| LF HRV PS, % | -0.2 | -2.5 | -23 | 0.054 | 0.982 | 0.464 | 0.631 | 0.541 |
| Urea, mM/L | -0.82 | -0.10 | +0.15 | 0.054 | 0.985 | 0.228 | 0.412 | 0.659 |
| Immunoglobulins G, g/L | +0.66 | +0.94 | +1.36 | 0.053 | 0.966 | 0.903 | 0.412 | 0.779 |
| Immunoglobulins A, g/L | -0.09 | -0.07 | -0.05 | 0.054 | 0.997 | 0.088 | 0.916 | 0.659 |
| Monocytes, % | -0.1 | +0.3 | +0.5 | 0.054 | 0.994 | 0.151 | 0.861 | 0.327 |
| Leukocytegram Entropy | -2 | +3 | +29 | 0.055 | 0.999 | 0.032 | 0.969 | 0.169 |
| Popovyč’s Strain Index-2, points | -0.03 | -0.02 | +0.49 | 0.054 | 0.980 | 0.519 | 0.598 | 0.536 |
| LF HRV PS, % | -1.4 | +1.2 | +18.3 | 0.054 | 0.990 | 0.256 | 0.775 | 0.650 |
| LF/(LF+HF), % | -3 | -3 | +12 | 0.054 | 0.990 | 0.525 | 0.594 | 0.655 |
| LF/HF | -0.3 | -0.7 | +5.7 | 0.054 | 0.990 | 0.903 | 0.412 | 0.779 |
| LF HRV PS, msec² | ±608 | -159 | -28 | 0.053 | 0.978 | 0.575 | 0.566 | 0.796 |
| SDNN, msec | -14 | -3 | -9 | 0.054 | 0.995 | 0.128 | 0.880 | 0.117 |
| RMSSD HRV, msec² | -6.0 | -1.8 | -6.3 | 0.054 | 0.992 | 0.209 | 0.812 | 0.818 |
| HF HRV PS, msec² | -177 | -11 | -115 | 0.054 | 0.980 | 0.525 | 0.594 | 0.753 |
| C-Reactive Protein, mg/L | -0.28 | -0.15 | -0.35 | 0.054 | 0.992 | 0.220 | 0.803 | 0.012 |
| CD56⁺ NK Lymphocytes, % | -2.0 | -0.6 | -3.3 | 0.055 | 0.999 | 0.032 | 0.968 | 0.729 |
| Phagocytose Ind vs St. aur., % | -0.3 | +0.2 | -0.5 | 0.054 | 0.994 | 0.163 | 0.850 | 0.717 |
| CIC, units | -0.4 | +5.5 | -1.2 | 0.054 | 0.990 | 0.265 | 0.768 | 0.717 |
| Killing Index vs Staph. aureus, % | +5.2 | +6.6 | +5.3 | 0.054 | 0.983 | 0.450 | 0.640 | 0.388 |
| Killing Index vs E. coli, % | +3.7 | +6.9 | +2.5 | 0.055 | 0.999 | 0.013 | 0.987 | 0.390 |
| CD4⁺ T-helper Lymphocytes, % | +1.1 | -0.45 | +1.6 | 0.054 | 0.985 | 0.386 | 0.682 | 0.726 |
| Testosterone normalized, Z | -0.28 | -0.46 | -0.18 | 0.054 | 0.992 | 0.209 | 0.812 | 0.818 |
| CD2⁺ B Lymphocytes, % | +2.6 | +0.7 | +1.5 | 0.054 | 0.992 | 0.205 | 0.815 | 0.112 |
| CD8⁺ T-cytolytic Lymphocytes, % | +1.4 | +1.1 | +2.9 | 0.055 | 0.999 | 0.025 | 0.975 | 0.764 |
| Bactericidality vs St. aur., 10⁶ Bac/L | ±22 | +12 | +20 | 0.054 | 0.995 | 0.123 | 0.885 | 0.277 |
| AMo HRV, % | +5.5 | -0.2 | +8.9 | 0.053 | 0.979 | 0.564 | 0.572 | 0.601 |

Next, the 19-dimensional space of discriminant variables transforms into 2-dimensional space of a canonical roots. The canonical correlation coefficient is for Root 1 0.950 (Wilks' Λ=0.055; χ²(19)=180; p<10⁻⁷), for Root 2 0.665 (Wilks' Λ=0.558; χ²(18)=36.2; p=0.007). The major root contains 92% of discriminative capabilities, while the minor 8% only. The calculation of the discriminant root values for each person as the sum of the products of raw coefficients to the individual values of discriminant variables together with the constant (Table 5) enables the visualization of each patient in the information space of the roots (Fig. 2).
Table 5. Standardized and Raw Coefficients and Constants for Neuro-Immune Variables

| Variables currently in the model | Coefficients | Standardized | Raw |
|----------------------------------|--------------|--------------|-----|
| Electrokinetic Index, %          | Root 1       | Root 2       | Root 1 | Root 2 |
| Phagocytose Index vs E. coli, %  | -1,333       | 0,078        | -1,136 | 0,067  |
| Baevsky's Stress Index, units   | 2,380        | 0,411        | 0,014  | 0,002  |
| Leukocytes, 10⁹/L                | -0,093       | 0,359        | -0,080 | 0,307  |
| ULF HRV PS, %                   | 0,151        | 0,171        | 0,019  | 0,022  |
| 0 Lymphocytes, %                | 0,784        | -0,770       | 0,130  | -0,128 |
| Immunocytogram Entropy          | 0,834        | -0,730       | 0,025  | -0,022 |
| Mode HRV, msec                  | 0,750        | 0,019        | 0,0059 | 0,0001 |
| AMo/MxDMn Index, units          | -2,034       | 0,160        | -0,0099 | 0,0008 |
| Bactericidity vs E. coli, 10⁹ Bacteria/L | -0,032 | 0,0099 | 0,016 | -0,022 |
| Polymorphonucl Neutrophils, %   | -1,033       | 0,324        | -0,176 | 0,056  |
| Baevsky's ARS Index, units      | -0,349       | -0,077       | -0,126 | -0,028 |
| Cholesterol standardized, Z     | -0,695       | -0,001       | -0,929 | -0,002 |
| Interleukin-6, ng/L             | 0,471        | -0,084       | 0,376  | -0,067 |
| Eosinophils, %                  | -0,396       | 0,254        | -0,213 | 0,137  |
| Popovych's Adaptation Index-2, points | -0,388 | 0,156 | -0,662 | 0,266 |
| Panlymphocytes, %               | -0,647       | -0,065       | -0,130 | -0,013 |
| Triglycerides standardized, Z   | 0,111        | -0,375       | 0,126  | -0,426 |
| Constants                       | 1,220        | -0,332       | 1,028  | 0,793  |
| Cumulative Proportion           | 0,921        | 1            |       |       |

Table 6 presents the full Structural coefficients and average values (centroids) of Roots as well as changes in Variables, both included and not included in the model.

Table 6. Correlations Variables-Canonical Roots, Means of Roots and Changes in Variables

| Change in Variables                  | Correlations Variables-Roots | EKI >+2.5% | EKI ±2.5% | EKI <-2.5% |
|--------------------------------------|-----------------------------|------------|-----------|------------|
| Root 1 (92%)                         | Root 1                      | Root 2     |           |            |
| Electrokinetic Index, %              | -0,609                      | -0,011     | +4,0      | +0,65      | +7,38      |
| Popovych's Adaptation Index-2, points | -0,065                      | -0,014     | +0,28     | +0,11      | -0,16      |
| Polymorphonucleary Neutrophils, %    | -0,060                      | 0,022      | +1,2      | -0,7       | -2,7       |
| Stub Neutrophils, %                  |                            |            | +0,6      | 0          | -0,2       |
| CD3⁺ T active Lymphocytes, %         |                            |            | +1,4      | -0,3       | -0,5       |
| Bactericidity vs E. coli, 10⁹ Bacteria/L | -0,032                      | -0,009     | +18       | +13        | +5         |
| Phagocytose Index vs E. coli, %      | -0,121                      | -0,348     | +0,1      | 0          | -1,9       |
| Interleukin-6, ng/L                  | -0,029                      | -0,162     | -0,15     | -0,02      | -0,78      |
| Interleukin-1, ng/L                  |                            |            | +0,02     | -0,27      | -0,65      |
| Eosinophils, %                       | 0,111                       | 0,313      | -0,34     | -0,24      | +2,7       |
| Leukocytes, 10⁹/L                    | 0,033                       | 0,192      | -0,04     | -0,19      | +0,65      |
| Monocytes, %                         |                            |            | -0,1      | +0,3       | +0,5       |
| Immunocytogram Entropy               | 0,052                       | -0,139     | -11       | +4         | +4         |
| Leukocytogram Entropy                |                            |            | -2        | +3         | +29        |
| Popovych's Strain Index-2, points    |                            |            | -0,03     | -0,02      | +0,49      |
| LF HRV PS, %                         |                            |            | -1,4      | +1,2       | +18,3      |
| Parameter                                      | LF/(LF+HF), % | Urea, mM/L | (VLF+ULF) HRV PS, msec | LF HRV PS, msec | Mode HRV, msec | Baevsky’s Stress Index, units | AMo/MxDMn Index, units | Baevsky’s ARS Index, units | ULF HRV PS, % | Root 2 (8%) | Triglycerides standardized, Z | Cholesterol standardized, Z | Pan Lymphocytes, % | C-Reactive Protein, mg/L | CD56+ NK Lymphocytes, % | SDNN, msec | RMSSD HRV, msec | HF HRV PS, msec | Amplitude of Mode HRV, % | CD4+ T-helper Lymphocytes, % | CD22+ B Lymphocytes, % | Bactericidity vs St. aur., 10⁹ Bac/L |
|-----------------------------------------------|---------------|------------|------------------------|----------------|---------------|-----------------------------|------------------------|--------------------------|----------------|-------------|-----------------------------|-----------------------------|-------------------------|-------------------------|------------------------|----------|----------------|--------------|-------------------------|------------------------|------------------------|-------------------------|
|                                               | -3            | -0.82      | -0.12                  | -608          | -0.02         | 0.066                      | 0.036                  | 0.009                    | -0.049        | Root 1 | -0.343                     | -0.135                      | 0.029                   | -0.28                   | -2.0                   | -14       | -6.0           | -0.177       | 5.5                     | 1.1                    | 2.6                    | 22                     |
|                                               | -3            | -0.10      | -0.12                  | -335          | -0.53         | 0.369                      | 0.329                  | 0.158                    | +0.18          | Root 2 | -0.15                     | -0.37                       | 0.220                   | -0.15                   | -0.6                   | -3        | -1.8           | -0.09        | -0.2                    | -0.45                  | +0.7                    | 12                     |
|                                               | +12           | +0.15      | -0.12                  | -419          | -0.46         | +31                        | +50                    | +0.16                    | -0.13          | +1.53     | +0.14                      | -0.21                       | +0.1                    | +0.3                    | +0.16                  | -9        | -6.3           | -0.177       | 8.9                     | 1.6                    | +1.5                    | 20                     |
|                                               |               |            |                        |               |               |                            |                        |                          |                |           |                            |                             |                        |                    |                        |                        |          |                |              |                                |                                |                                |                          |

Fig. 2 shows the data in table 6, the therapeutic factor-induced increase in EKI is accompanied by an increase in the adaptation index and physiologically favorable or minimal changes in immunity and autonomic regulation, whereas in rare cases a decrease in EKI is also reduced in combination with adverse changes in immunity and autonomic regulation. Individuals with minor changes in EKI occupy an intermediate position along the axis of the first root.

Additional delimitation of clusters occurs along the axis of the second root. The lowest position of persons with insignificant changes in EKI reflects minimal changes in parameters, while a significant increase or decrease in EKI is accompanied by a significant decrease in triglycerides and cholesterol, zero-, total and NK-Lymphocytes and vagal tone - on the one hand, and increase in sympathetic tone, T-helper and B-lymphocytes level, as well as bactericidity against Staph. aureus - on the other hand.
In the information space of both discriminant roots, the members of all three clusters are very clearly delineated, as documented by the calculation of Mahalanobis distances (Table 7).

**Table 7. Squared Mahalanobis Distances between Clusters of change and F-values**

(df=19.5; for all $p<10^{-6}$)

| Clusters of change | EKI $\pm$2.5% | EKI $>2.5\%$ | EKI $<-2.5\%$ |
|--------------------|----------------|---------------|----------------|
| EKI $\pm$2.5%     | 0              | 24            | 52             |
| EKI $>2.5\%$      | 12.9           | 0             | 131            |
| EKI $<-2.5\%$     | 10.9           | 23.5          | 0              |

Retrospective classification according to the parameters given in table 8, infallible.

**Table 8. Coefficients and Constants for Classification Functions for Clusters of change in EKI**

| CLUSTERS | EKI $\pm$2.5% (49) | EKI $>2.5\%$ (19) | EKI $<-2.5\%$ (6) |
|----------|---------------------|-------------------|-------------------|
| Variables currently in the model | p=0.662 | p=0.257 | p=0.081 |
| Electrokinetic Index, % | 0.722 | 6.125 | -6.745 |
| Phagocytose Index vs E. coli, % | -0.137 | 0.631 | -3.391 |
| Baevsky’s Stress Index, units | -0.001 | -0.063 | 0.099 |
| Leukocytes, 10^9/L | -0.335 | 0.496 | -0.081 |
| ULF HRV PS, % | -0.017 | -0.073 | 0.167 |
| 0 Lymphocytes, % | -0.037 | -0.834 | 0.508 |
| Immunocytogram Entropy | -0.0014 | -0.1485 | 0.1086 |
| Mode HRV, msec | -0.0046 | -0.0318 | 0.0353 |
| AMo/MxDMn Index, units | 0.0002 | 0.0474 | -0.0642 |
| (VLF+ULF) HRV PS, msec^2 | 0.000012 | 0.000094 | -0.000385 |
| Bactericidity vs E. coli, 10^9 Bacteria/L | 0.0198 | -0.0539 | 0.0936 |
| Polymorphonuclear Neutrophils, % | 0.050 | 0.955 | -0.992 |
| Baevsky’s ARS Index, units | -0.012 | 0.533 | -0.930 |
| Cholesterol standardized, Z | -0.061 | 4.270 | -6.310 |
| Interleukin-6, ng/L | 0.017 | -1.838 | 2.372 |
| Eosinophils, % | 0.070 | 1.270 | -1.010 |
| Popovych’s Adaptation Index-2, points | 0.315 | 3.805 | -3.454 |
| Panlymphocytes, % | 0.114 | 0.700 | -0.793 |
| Triglycerides standardized, Z | 0.380 | -0.848 | 0.131 |
| Constants | -0.990 | -16.21 | -24.53 |

**CONCLUSION**

Electrokinetic index of buccal epithelium responds to therapeutic factors in different directions, accompanied by characteristic changes in a number of parameters of HRV, immunity and metabolism.

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ACCORDANCE TO ETHICS STANDARDS

Tests in patients are carried out 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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