Kyrylenko Iryna G., Fediaieva Svitlana I., Miesoiedova Vita, Popadynets' Oleksandr O., Żukow Xawery. Vegetative, metabolic and immune accompaniments of changes in the electrokinetic index of the buccal epithelium under the influence of therapeutic factors. Journal of Education, Health and Sport. 2022;12(1):344-354. eISSN 2391-8306. DOI http://dx.doi.org/10.12775/JEHS.2022.12.01.030
https://apcz.umk.pl/JEHS/article/view/JEHS.2022.12.01.030
https://zenodo.org/record/5992134

The journal has had 40 points in Ministry of Education and Science of Poland parametric evaluation. Annex to the announcement of the Minister of Education and Science of December 21, 2021. No. 32343. Has a Journal's Unique Identifier: 201159. Scientific disciplines assigned: Physical Culture Sciences (Field of Medical sciences and health sciences); Health Sciences (Field of Medical Sciences and Health Sciences).

Punkty Ministerialne z 2019 - aktualny rok 40 punktów. Załącznik do komunikatu Ministra Edukacji i Nauki z dnia 21 grudnia 2021 r. Lp. 32343. Posiada Unikatowy Identyfikator Czasopisma: 201159. Przypisane dyscypliny naukowe:Nauki o kulturze fizycznej (Dziedzina nauk medycznych i nauk o zdrowiu); Nauki o zdrowiu (Dziedzina nauk medycznych i nauk o zdrowiu).

Received: 02.01.2022. Revised: 17.01.2022. Accepted: 31.01.2022.

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 notα-lipoproteins); prae-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 prae-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 "Immunochem", 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]:

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

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]:

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

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

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 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 [11]:

BCCN (10^9 Bact/L) = N (10^9/L)•PhI (%)•MC (Bact/Phag)•KI (%)•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:

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

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

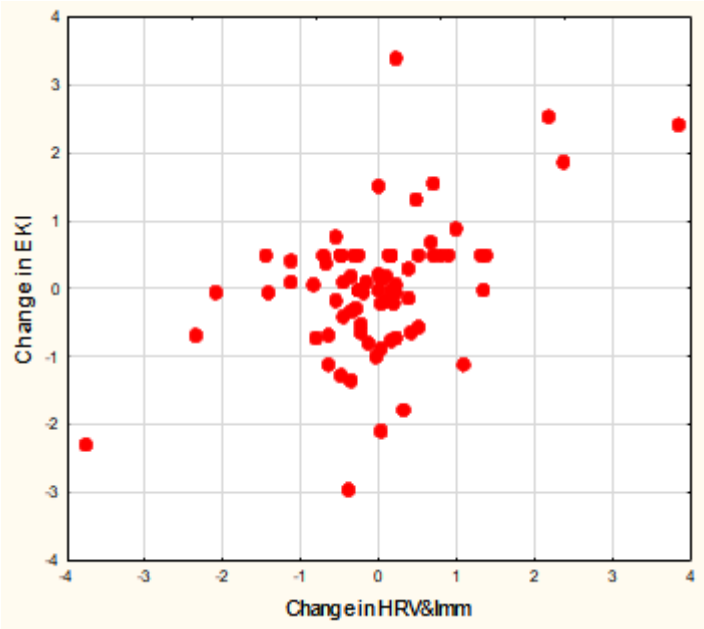
Left set	R	r
Eosinophiles, %	0,747	-0,36
Total Power HRV, msec^2	0,542	-0,26
(VLF+ULF) HRV PS, msec^2	0,532	-0,25
Popovych's Strain Index-2	0,506	-0,24
ULF HRV PS, msec^2	0,504	-0,24
LF/(LF+HF)	0,483	-0,23
LF/HF	0,475	-0,23
Phagocytose Index vs E. coli, %	-0,535	0,26
Right set	**R**	
Electrokinetic Index, %	-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).



R=0,478; R^2=0,229; $\chi^2_{(8)}$=17,7; p=0,023; Λ Prime=0,771

Fig. 1. Scatterplot of canonical correlation between change in HRV and Immune parameters (X-line) and Electrokinetic index (Y-line)

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 change in which currently in the model	EKI >+2,5% (19)	EKI ±2,5 (49)	EKI <-2,5% (6)	Wilks Λ	Partial Λ	F-remove (2,53)	p-level	Tolerancy
Electrokinetic Index, %	**+4,0**	+0,65	**-4,2**	0,299	0,183	118	10^{-6}	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
Bactericidity 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	**-11**	+4	**+4**	0,080	0,686	12,1	10^{-4}	0,363
(VLF+ULF) HRV PS, msec^2	**-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	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	44,2	10^{-6}
Baevsky's Stress Index, units	4,81	0,011	0,171	32,6	10^{-6}
Leukocytes, 10^9/L	3,13	0,050	0,157	25,9	10^{-6}
ULF HRV PS, %	2,65	0,078	0,145	21,7	10^{-6}
0 Lymphocytes, %	2,34	0,105	0,136	18,8	10^{-6}
Immunocytogram Entropy	5,31	0,007	0,117	17,9	10^{-6}
Mode HRV, msec	3,36	0,041	0,106	16,6	10^{-6}
AMo/MxDMn Index, units	2,42	0,097	0,098	15,3	10^{-6}
(VLF+ULF) HRV PS, msec^2	1,60	0,210	0,093	14,1	10^{-6}
Bactericidity vs E. coli, 10^9 Bac/L	1,29	0,284	0,090	13,0	10^{-6}
Polymorphonucl Neutrophils, %	2,15	0,125	0,084	12,3	10^{-6}
Baevsky's ARS Index, units	1,77	0,179	0,079	11,6	10^{-6}
Cholesterol standardized, Z	1,94	0,152	0,074	11,1	10^{-6}
Interleukin-6, ng/L	1,56	0,218	0,070	10,6	10^{-6}
Eosinophiles, %	1,50	0,232	0,066	10,1	10^{-6}
Popovych's Adaptation Index-2	1,40	0,255	0,063	9,63	10^{-6}
Pan Lymphocytes, %	2,47	0,094	0,058	9,46	10^{-6}
Triglycerides standardized, Z	1,60	0,211	0,055	9,15	10^{-6}

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	Parti-al Λ	F to enter	p-level	Tole-rancy
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
Micr. Count vs St. aur., Bac/Phag	+5,3	+2,7	+1,2	0,053	0,978	0,598	0,553	0,721
Micr. Count vs E. coli, Bact/Phag	+4,1	+2,5	0,0	0,054	0,992	0,204	0,816	0,480
100(1-Pd/HR) as Kerdö Index, %	+7,0	+5,8	+2,1	0,054	0,982	0,519	0,594	0,536
Stub Neutrophils, %	+0,6	0,0	-0,2	0,054	0,989	0,284	0,754	0,703
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
VLF 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
Leukocytogram Entropy	-2	+3	+29	0,055	0,999	0,032	0,969	0,169
Popovych'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, $msec^2$	-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, msec	-6,0	-1,8	-6,3	0,054	0,992	0,209	0,812	0,818
HF HRV PS, $msec^2$	-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
$CD22^+$ 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
Bactericidity vs St. aur., 10^9 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; $\chi^2_{(38)}$=180; $p<10^{-6}$), for Root 2 0,665 (Wilks' Λ=0,558; $\chi^2_{(18)}$=36,2; p=0,007). The major root contains 92% of discriminative capabiliities, 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

Coefficients	Standardized		Raw	
Variables currently in the model	Root 1	Root 2	Root 1	Root 2
Electrokinetic Index, %	-1,333	0,078	-1,136	0,067
Phagocytose Index vs E. coli, %	-0,346	-0,507	-0,310	-0,454
Baevsky's Stress Index, units	2,380	0,411	0,014	0,002
Leukocytes, 10^9/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
(VLF+ULF) HRV PS, $msec^2$	-0,146	-0,234	-0,00004	-0,00006
Bactericidity vs E. coli, 10^9 Bacteria/L	0,460	-0,232	0,0136	-0,0069
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
Eosinophiles, %	-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
	Eigenvalues		9,208	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**	**-4,01**	**+0,65**	**+7,38**
Electrokinetic Index, %	**-0,609**	-0,011	**+4,0**	+0,65	**-4,2**
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	**-0,2**
$CD3^+$ T active Lymphocytes, %			**+1,4**	-0,3	**-0,5**
Bactericidity vs E. coli, 10^9 Bacteria/L	**-0,032**	-0,009	**+18**	+13	**+5**
Phagocytose Index vs E. coli, %	**-0,121**	-0,348	+0,1	0,0	**-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, 10^9/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**

LF/(LF+HF), %			**-3**	-3	**+12**
Urea, mM/L			**-0,82**	-0,10	**+0,15**
(VLF+ULF) HRV PS, msec^2	**0,043**	-0,120	**-2604**	-335	**-419**
LF HRV PS, msec^2			**-608**	-159	**-28**
Mode HRV, msec	**0,020**	0,029	**-53**	-46	**-20**
Baevsky's Stress Index, units	**0,066**	0,369	+31	-11	**+226**
AMo/MxDMn Index, units	**0,036**	0,329	+50	-17	**+207**
Baevsky's ARS Index, units	**0,029**	0,158	+0,16	-0,13	**+1,53**
ULF HRV PS, %	**0,049**	0,220	+0,1	-0,7	**+6,4**
Root 2 (8%)	**Root 1**	**Root 2**	+0,90	**-0,59**	+1,98
Triglycerides standardized, Z	0,005	**-0,343**	-0,33	**+0,14**	-0,62
Cholesterol standardized, Z	0,003	**-0,135**	-0,37	**-0,21**	-0,45
0 Lymphocytes, %	0,029	**-0,204**	-3,4	**-0,7**	-2,7
Pan Lymphocytes, %	0,032	**-0,152**	-1,3	**+0,6**	-0,3
C-Reactive Protein, mg/L			-0,28	**-0,15**	-0,35
CD56$^+$ NK Lymphocytes, %			-2,0	**-0,6**	-3,3
SDNN, msec			-14	**-3**	-9
RMSSD HRV, msec			-6,0	**-1,8**	-6,3
HF HRV PS, msec^2			-177	**-11**	-115
Amplitude of Mode HRV, %			+5,5	**-0,2**	+8,9
CD4$^+$ T-helper Lymphocytes, %			+1,1	**-0,45**	+1,6
CD22$^+$ B Lymphocytes, %			+2,6	**+0,7**	+1,5
Bactericidity vs St. aur., 10^9 Bac/L			+22	**+12**	+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.

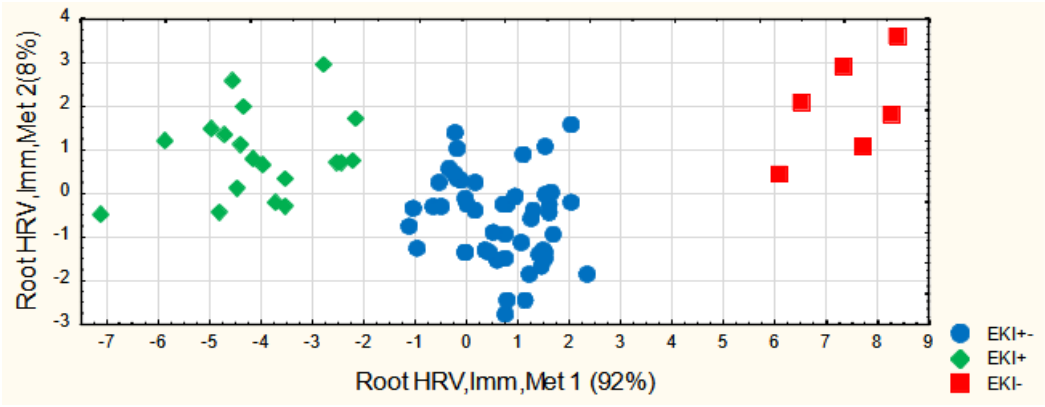


Fig. 2. Individual values of discriminant roots of changes in parameters that are characteristic of different variants of changes in the electrokinetic index

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 ±2,5 %	EKI >+2,5%	EKI <-2,5%
EKI ±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 ±2,5% (49)	EKI >2,5% (19)	EKI <2,5% (6)
Variables currently in the model	p=,662	p=,257	p=,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
Polymorphonucleary 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.

ACKNOWLEDGMENT

We express sincere gratitude to VY Musiyenko, TA Korolyshyn as well as administration of clinical sanatorium "Moldova" for help in recording tests.

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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