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Features of neuroendocrine-immune complex by various types of gall-bladder motility at men with chronic cholecystitis and pyelonephritis

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Abstract

Background. In our last study has formed three homogeneous cholekinetics groups, namely: normokinesia as well as hypertonic-hyperkinetic and hyperkinetic-hypertonic dyskinesia. The purpose of this study to identify the parameters of neuroendocrine-immune complex under which three types of the gall-bladder motility differ significantly from each other. **Material and methods.** The object of observation were the same ones 22 men aged 24-70 years old, who came to the spa Truskavets' for the treatment of chronic cholecystitis combined with pyelonephritis in remission. On the tone and motility of gall-bladder judged by its fasting and postprandial volume. In the daily urine determined content of electrolytes: phosphates, calcium, potassium and sodium. Estimation state of central and autonomous nervous systems carried out by electroencephalogram (EEG) and heart rate variability (HRV). Immune status evaluated on a set of I and II levels recommended by the WHO. **Results.** The method of discriminatory analysis revealed 23 parameters (12 immune, 7 EEG and 2 HRV as well as Parathyrine activity and Popovych's Adaptation Index), in the totality of which three types of cholekinetics are clearly delineated. **Conclusion.** The results confirm the pattern of the previously detected prior reactions of various systems of the organism to the balneofactors of the spa Truskavets'.

Key words: Cholekinetics, EEG, HRV, Immunity.

INTRODUCTION

Previously it has been shown that 10-12-days course of complex balneotherapy in the spa Truskavets' in men with chronic cholecystitis combined with pyelonephritis causes cholecystokinetic effect and activation depurative and excretory functions of the kidneys accompanied by modulation of neuroendocrine-immune complex, including a decrease in the levels of neuroendocrine markers of stress and increase killing by neutrophils Staphylococcus aureus and Escherichia coli [13].

The goal of next study [14] was clarification relationships between parameters of gall-bladder motility and neuroendocrine-immune complex and metabolism. Discovered 23 neural, metabolic and immune parameters, canonical correlation which with the parameters of gall-bladder motility is very strong. Thus, cholecystokinetic effect balneotherapy on spa Truskavets' may be the result of modulation of neuroendocrine-immune complex.

In another study we investigated relationships between parameters of gall-bladder motility and ongoing electroencephalogram (EEG) as well as heart rate variability (HRV) [8,15]. We maked conclusion that fasting gall-bladder volume is strong controlled by Vagal nerves as well as by neural structures generating β-rhythm, whereas early (5 min) postprandial volume is also strong controlled by structures generating θ- rhythm. Next (15 and 30 min) postprandial volumes significantly less liable to neural regulatory influences.

In our last study [7] by method of cluster analysis has formed three homogeneous cholekinetics groups, namely: normokinesia as well as hypertonic-hyperkinetic and hyperkinetic-hypertonic dyskinesia. The method of discriminant analysis revealed 13 parameters of daily urine and three parameters of blood, as well as body weight and height, electrokinetic index and Kerdoe's index, in the totality of which three types of cholekinetics are clearly delineated.

The purpose of this study to identify the parameters of neuroendocrine-immune complex under which three types of the gall-bladder motility differ significantly from each other.

MATERIAL AND METHODS

The object of observation were the same ones 22 men aged 24-70 (mean 49,1±2,5) years old, who came to the spa Truskavets' (Ukraïna) for the treatment of chronic cholecystitis combined with pyelonephritis in remission. The survey was conducted twice, before and after balneotherapy (drinking bioactive water Naftussya, ozokerite applications, mineral baths).

About the endocrine status was judged by the rations of electrolyte urine: $(Ku/Nau)^{0,5}$, $(Pu/Cau)^{0,5}$ and $(Pu \bullet Cau)^{0,5}$, that characterize Mineraocorticoid, Parathyrine and Calcitonine activity respectively [5,10].

For estimation state of autonomous nervous system we recorded ECG in standard lead II hardware-software complex "Cardiolab+VSR" (KhAI Medica, Kharkiv, Ukraine). For further analysis the following parameters HRV were selected. Temporal parameters (Time Domain Methods): the standart deviation of all NN intervals (SDNN), the square root of the mean of the sum of the squares of differences between adjacent NN intervals (RMSSD), the percent of interval differences of successive NN intervals greater then 50 ms (pNN_{50}), Triangulary Index (HRV TI); heart rate (HR), moda (Mo), the amplitude of moda (AMo), variational sweep (MxDMn). Spectral parameters (Frequency Domain Methods): power spectral density (PSD) bands of HRV - high-frequency (HF, range 0,4÷0,15 Hz), low-frequency (LF, range 0,15÷0,04 Hz), very low-frequency (VLF, range 0,04÷0,015 Hz) and ultra low-frequency (ULF, range 0,015÷0,003 Hz) [1,3,6].

Then EEG recorded a hardware-software complex "NeuroCom Standard" (KhAI Medica, Kharkiv, Ukraine). Among the options considered the average EEG amplitude (μV), average frequency (Hz), frequency deviation (Hz), index (%) and coefficient of asymmetry (%) of basic rhythms: β (35÷13 Hz),

α (13÷8 Hz), θ (8÷4 Hz) and δ (4÷0,5 Hz), according to the instructions of the device. In addition, we calculated Laterality Index.

In portion of capillary blood counted up Leukocytogram and calculated its Adaptation Index as well as Strain Index by IL Popovych [2,16].

About phagocytic function of neutrophils judged by activity (percentage of neutrophils, in which found microbes - Phagocytic index), intensity (number of microbes absorbed one phagocytes - Microbial Count) and completeness (percentage of dead microbes - Killing Index) phagocytosis museum cultures Staphylococcus aureus (ATCC N 25423 F49) and Escherichia coli (O55 K59) from laboratory Truskavetsian hydrogeological regime-operational station [4,11].

On the basis of the recorded partial parameters of Phagocytosis, taking into account the Neutrophils content of 1 L blood, we calculated the integral parameter - Bactericidal Capacity of Neutrophils (BCCN) by the formula [10]:

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

Immune status evaluated on a set of I and II levels recommended by the WHO. For phenotyping subpopulations of lymphocytes used the methods of rosette formation and indirect immunofluorescent binding reaction monoclonal antibodies [11,17] from company "Sorbent" (RF) with visualization under fluorescent microscope. T-cellular immunity assessed by the following parameters: blood levels of a subpopulation of “active”, theophilline resistance and theophilline sensitive T-lymphocytes as well as T-lymphocytes phenotype of $CD3^+CD4^+$(helpers). State of killer link of immunity estimated by the content of $CD3^+CD8^+$-lymphocytes (T-killers) and $CD16^+$-lymphocytes (natural killers). The state of humoral immunity judged by the content of EAC and $CD19^+$ B-lymphocytes and by concentration in serum of immunoglobulins classes G, A, M (radial immunodiffusion method) as well as circulating immune complexes (with polyethylene glycol precipitation method), using standardized methods described in manual [12].

Results processed by methods of discriminant analyses [9], using the software package "Statistica 5.5".

RESULTS AND DISCUSSION

At the first stage by method of cluster analysis has formed three homogeneous cholekinetics groups, namely: normokinesia (N) as well as hypertonic-hyperkinetic (T^+K^+) and hyperkinetic-hypertonic (K^+T^+) dyskinesia [7].

At the second stage, by the method of discriminant analysis 23 recognizable variables (12 immune, 7 EEG and 2 HRV as well as Parathyrine activity and Popovych’s Adaptation Index) were detected (Table 1).

Discriminant information is condensed in two roots. First root have 87,1% of recognition capabilities (r_1*=0,979; Wilks' Λ=0,009; $\chi^2_{(46)}$=140; $p<10^{-6}$), while the minor root remains 12,9% (r_2*=0,880; Wilks' Λ=0,225; $\chi^2_{(22)}$=45; p=0,003).

Table 1. Discriminant Function Analysis Summary
Step 23, N of variables in model: 23; Grouping: 3 groupps
Wilks' Lambda: 0,0093; approx. $F_{(46)}=7,7$; $p<10^{-6}$

Variables currently in model	Wilks' Λ	Partial Λ	F-rem (2,19)	p	Tolerancy	F to enter	p	Λ	F-value	p
δ-rhythm Asymmetry	,023	,410	13,7	10^{-3}	,222	4,47	,018	,821	4,47	,018
Eosinophiles blood level	,016	,575	7,0	,005	,193	4,08	,024	,682	4,22	,004
$CD8^+$ T-Lymphocytes	,046	,201	37,7	10^{-6}	,091	3,81	,031	,570	4,21	,001
α-rhythm Frequency	,028	,337	18,7	10^{-4}	,141	4,41	,019	,463	4,46	10^{-3}
Popovych's Adaptation Index	,041	,227	32,4	10^{-6}	,043	6,02	,008	,043	5,11	10^{-6}
$CD19^+$ B-Lymphocytes level	,020	,473	10,6	10^{-3}	,134	4,40	,025	,023	6,22	10^{-6}
ULF PSD HRV	,011	,833	1,9	,177	,263	1,57	,224	,142	4,14	10^{-5}
Leukocytes blood level	,016	,596	6,5	,007	,076	2,76	,077	,301	4,12	10^{-4}
Bactericidity vs. Staph. aureus	,025	,379	15,6	10^{-4}	,026	2,53	,095	,262	4,05	10^{-4}
α-rhythm Laterality	,025	,378	15,6	10^{-4}	,098	2,39	,108	,175	4,45	10^{-5}
Active T-Lymphocytes level	,026	,360	16,9	10^{-4}	,205	4,08	,030	,032	5,60	10^{-6}
β-rhythm Laterality	,028	,334	18,9	10^{-4}	,168	1,83	,178	,157	4,30	10^{-5}
Microbian Count for E. coli	,011	,871	1,4	,269	,130	2,64	,088	,120	4,21	10^{-5}
$(Pu/Cau)^{0,5}$ Parathyrine Activity	,027	,349	17,8	10^{-4}	,173	1,41	,261	,070	4,50	10^{-6}
Segmented Neutrophiles level	,027	,342	18,3	10^{-4}	,072	1,18	,324	,064	4,33	10^{-5}
$CD16^+$ Lymphocytes level	,015	,632	5,5	,013	,275	3,67	,043	,017	6,73	10^{-6}
pNN_{50} HRV	,037	,253	28,0	10^{-5}	,107	2,68	,082	,348	4,17	10^{-4}
β-rhythm Asymmetry	,016	,592	6,5	,007	,227	4,75	,020	,011	7,63	10^{-6}
β-rhythm Frequency	,011	,820	2,1	,152	,320	2,08	,152	,009	7,74	10^{-6}
IgM serum level	,021	,449	11,7	10^{-3}	,163	2,27	,122	,103	4,23	10^{-5}
α-rhythm Asymmetry	,015	,626	5,7	,012	,192	4,36	,023	,078	4,65	10^{-6}
Killing Index for Staph. aureus	,020	,465	10,9	10^{-3}	,101	2,90	,068	,400	4,30	10^{-4}
Microb. Count for Staph. aur.	,018	,511	9,1	,002	,056	4,98	,013	,201	4,51	10^{-5}

Applying the already mentioned algorithm [7] and Raw coefficients (Table 2), we visualized each patient on the plane of two discriminatory roots (Fig. 1).

Table 2. Standardized and Raw Coefficients and Constants for Canonical Variables

Variables currently in model	Standardized Coefficients for Canonical Variables		Raw Coefficients for Canonical Variables	
	Root 1	Root 2	Root 1	Root 2
δ-rhythm Asymmetry	-1,661	,109	-,104	,007
Eosinophiles blood level	-1,382	-,696	-1,113	-,560
$CD8^+$ T-Lymphocytes blood level	2,979	,607	,959	,195
α-rhythm Frequency	-2,136	-,636	-2,577	-,767
Neutrophiles Killing Ind. for St. aur	-1,909	-1,521	-,301	-,240
pNN_{50} HRV	2,696	-,156	,220	-,013
Leukocytes blood level	-,376	-2,587	-,361	-2,481
Bacterocidity of Neutrop. vs. St. aur	3,746	3,590	,131	,125
Microbian Count for Staph. aureus	-2,328	-2,121	-,280	-,255
α-rhythm Laterality	2,479	-,784	,116	-,037
β-rhythm Laterality	-1,958	,614	-,085	,027
ULF PSD HRV	-,160	-,886	-,001	-,006
Microbian Count for E. coli	,865	,593	,114	,078
IgM serum level	-1,859	-,292	-6,038	-,948
α-rhythm Asymmetry	1,238	,784	,133	,084
$(Pu/Cau)^{0,5}$ as Parathyrine Activity	-1,977	-,163	-3,570	-,295
Segmented Neutrophiles blood lev.	-3,060	-,365	-,689	-,082
Popovych Adaptation Index	4,285	-,529	8,701	-1,073
Active T-Lymphocytes blood level	1,779	-,344	,401	-,078
$CD19^+$ B-Lymphocytes blood level	-1,863	,889	-,910	,434
$CD16^+$ Lymphocytes blood level	-1,180	-,070	-,510	-,030
β-rhythm Asymmetry	1,113	-,884	,078	-,062
β-rhythm Frequency	-,399	,727	-,097	,176
		Constants	**79,17**	**26,86**

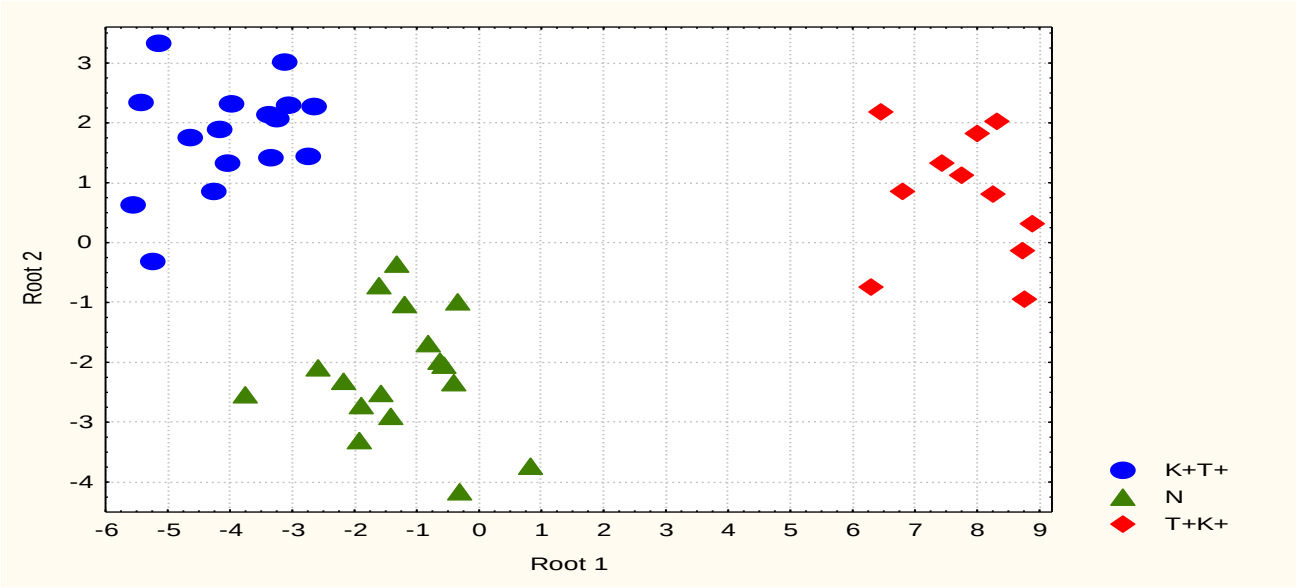

Fig. 1. Individual scores of discriminant roots of neuroendocrine and immune parameters of different clusters

The localization of representative points of patients with **hypertonic-hyperkinetic** dyskinesia along the first root axis in its extreme right region reflects the **minimal** values in cohorts parameters

(Table 3) that correlate with the root **negatively** while the **maximal** levels of **positively** correlating with it parameters. The shift of two others clusters to the left reflects the increase or decrease of the respectively parameters, herewith the representative points of patients with both **normokinesia** and **hyperkinetic-hypertonic** dyskinesia are partially mixed despite the statistically significant distance between the clusters.

On the other hand, along the axis of the second root, the second (**normokinetic**) cluster occupies an extreme bottom position, while the other two are placed at approximately the same level (centroides: +0,8 and +1,8 for **hypertonic-hyperkinetic** and **hyperkinetic-hypertonic** clusters respectively). Such a disposition reflects the **minimal** values in cohorts parameters that correlate with the second root **positively** while the **maximal** levels of **negatively** correlating with it parameters.

In general, all three clusters are quite clearly delineated. Squared Mahalanobis Distance between normokinetic and hyperkinetic-hypertonic clusters make up 25 ($F=3,9$; $p<10^{-3}$), between normokinetic and hypertonic-hyperkinetic 98 ($F=12,1$; $p<10^{-6}$), between the two dyskinetic 150 ($F=18,2$; $p<10^{-6}$).

Table 3. Neuroendocrine and Immune Accompaniments of Gall-bladder Motility Types

Variables currently in model	K^+T^+ (n=16)	Norm (n=17)	T^+K^+ (n=11)
Root 1 (87,1% Discriminant Properties) Centroides	-4,0	-1,3	**+7,8**
δ-rhythm Asymmetry, %	52±5	37±3	**30±6**
Killing Index of Neutrophiles for Staph. aureus, %	57±2	52±2	**49±2**
$CD19^+$ B-Lymphocytes blood level, %	24,7±0,5	24,1±0,6	**23,1±0,7**
α-rhythm Frequency, Hz	10,4±0,2	10,7±0,3	**10,1±0,2**
α-rhythm Asymmetry, %	17,3±2,5	17,5±3,0	**13,3±2,7**
β-rhythm Frequency, Hz	19,0±1,3	18,8±1,0	**18,0±1,5**
Parathyrin Activity as $(Pu/Cau)^{0,5}$	2,36±0,09	2,29±0,17	**2,26±0,17**
IgA serum level, g/l	1,60±0,11	1,54±0,12	**1,33±0,16**
α-rhythm Laterality, %	-15±7	-5±6	**+5±6**
pNN_{50} HRV, %	6,0±1,5	10,1±3,1	**16,4±6,1**
Microbian Count for E. coli, microbes/phagocyte	60±2	59±2	**64±1**
Popovych's Leukocytary Adaptation Index, points	1,22±0,16	0,98±0,15	**1,38±0,21**
Active T-Lymphocytes blood level, %	26,3±1,1	27,0±0,9	**27,1±2,0**
Root 2 (12,9% Discriminant Properties) Centroides	+1,8	**-2,2**	+0,8
$CD8^+$ T-Lymphocytes blood level, %	26,2±0,6	**23,8±0,9**	26,8±1,0
$CD16^+$ Lymphocytes blood level, %	11,2±0,7	**9,6±0,5**	10,6±0,6
Segmented Neutrophiles blood level, %	51,6±1,5	**47,8±1,5**	52,2±1,5
β-rhythm Laterality, %	+6±7	**-5±5**	+6±8
Bactericidity of Neutrophiles vs Staph. aur., 10^9/l	105±7	**93±8**	101±10
IgM serum level, g/l	1,39±0,09	**1,31±0,08**	1,33±0,09
Microbian Count for Staph. aur., micr./phagocyte	61±3	**59±2**	61±1
Total T-Lymphocytes blood level, %	48,3±1,3	**45,4±1,1**	48,8±2,1
Eosinophiles blood level, %	2,6±0,3	**4,2±0,4**	2,0±0,4
Leukocytes blood level, 10^9/l	5,44±0,18	**6,58±0,40**	6,01±0,47
ULF PSD HRV, $msec^2$	82±28	**177±59**	52±22
β-rhythm Asymmetry, %	19±3	**26±5**	22±5

Selected 7 parameters **EEG**, 2 **HRV**, 12 **Immune** as well as **Parathyrine activity and Popovych's Adaptation Index** can be used to identify the belongings of a particular patient to one or another cholekinetic cluster. This is achieved through the calculation of classification functions on the basis of the obtained Coefficients and Constants (Table 4).

Table 4. Coefficients and Constants of Classification Functions for Gall-bladder Motility Types

Variables currently in model	K^+T^+	Norm	T^+K^+
δ-rhythm Asymmetry	8,39	8,08	7,16
α-rhythm Frequency	259,0	255,1	229,4
α-rhythm Laterality	-8,19	-7,73	-6,79
α-rhythm Asymmetry	-14,62	-14,60	-13,14
β-rhythm Laterality	6,23	5,89	5,20
β-rhythm Asymmetry	-5,03	-4,57	-4,05
β-rhythm Frequency	5,49	4,52	4,17
ULF PSD HRV	0,23	0,25	0,22
pNN_{50} HRV	-17,82	-17,17	-15,21
Parathyrine Activity as $(Pu/Cau)^{0,5}$	352,5	344,0	310,7
Popovych's Leukocytary Adaptation Index	-708,4	-680,4	-604,7
Leukocytes blood level	139,9	148,8	138,1
Eosinophiles blood level	110,29	109,50	97,73
Segmented Neutrophiles level	69,92	68,37	61,88
Killing Ind. of Neutrophiles for St. aureus	38,90	39,04	35,60
Microbian Count for Staph. aureus	35,41	35,67	32,37
Microbian Count for E. coli	-9,55	-9,55	-8,28
Bactericidity of Neutrophils vs. St. aureus	-17,57	-17,71	-16,15
Active T-Lymphocytes blood level	-29,98	-28,58	-25,17
$CD8^+$ T-Lymphocytes blood level	-81,31	-79,48	-70,19
$CD16^+$ Lymphocytes blood level	51,22	49,95	45,24
$CD19^+$ B-Lymphocytes blood level	59,36	55,14	48,19
IgM serum level	491,9	479,2	421,6
Constants	-4649	-4534	-3764

Accuracy of classification is absolute (Table 5).

Table 5. Classification Matrix. Rows: Observed classifications; Columns: Predicted classifications

Clusters	% Correct	K^+T^+	Norm	T^+K^+
K^+T^+	100	**16**	0	0
Norm	100	0	**17**	0
T^+K^+	100	0	0	**11**
Total	100	16	17	11

The results presented in this and previous [7] articles confirm the pattern of the previously detected [5,10,18] prior reactions of various systems of the organism to the balneofactors of the spa Truskavets'.

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ACCORDANCE TO ETHICS STANDARDS

This study was approved by the local ethical committee of Truskavets' Scientists Assotiation. 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. For all authors any conflict of interests is absent.

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