POPOVYCH, Igor, GOZHENKO, Anatoliy, BOMBUSHKAR, Igor, ANCHEV, Anatoliy & ZUKOW, Walery. Sexual dimophism in plasma nitrogenous metabolites levels and some psycho-neuro-endocrine parameters. Quality in Sport. 2022;8(4):46-57. eISSN 2450-3118. DOI http://dx.doi.org/10.12775/QS.2022.08.04.005
https://apcz.umk.pl/QS/article/view/41503

The journal has had 20 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. 32582. Has a Journal's Unique Identifier: 201398. Scientific disciplines assigned: Economics and finance (Field of social sciences); Management and Quality Sciences (Field of social sciences). Punkty Ministerialne z 2019 - aktualny rok 20 punktów. Załącznik do komunikatu Ministra Edukacji i Nauki z dnia 21 grudnia 2021 r. Lp. 32582. Posiada Unikatowy Identyfikator Czasopisma: 201398. Przypisane dyscypliny naukowe: Ekonomia i finanse (Dziedzina nauk społecznych); Nauki o zarządzaniu i jakości (Dziedzina nauk społecznych).

Received: 16.11.2022. Revised: 19.11.2022. Accepted: 19.12.2022.

SEXUAL DIMOPHISM IN PLASMA NITROGENOUS METABOLITES LEVELS AND SOME PSYCHO-NEURO-ENDOCRINE PARAMETERS

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Abstract

Background. We previously showed that the factor structure of the relationships between the plasma levels of nitrogenous metabolites (uric acid, bilirubin, urea, and creatinine), on the one hand, and the parameters of anxiety and neuro-endocrine status, on the other hand, is significantly different in men and women of reproductive age and postmenopausal. The purpose of this study is to identify the metabolic and psycho-neuro-endocrine features of these three cohorts of people. **Materials and methods.** The object of observation were 31 males (24÷69 y) and 30 females, from among them 18 postmenopausal (45÷76 y) and 12 of reproductive age (30÷42 y), with dysfunction of neuro-endocrine-immune complex. In basal conditions we determined plasma levels of nitrogenous metabolites and adaptation hormones, estimated the severity of the trait and reactive anxiety, recorded the ongoing HRV and EEG. After 4 or 7 days, repeated testing was performed. **Results**. To achieve the goal, the registered parameters were subjected to discriminant analysis. Along with the quite expected nitrogenous metabolites, age, testosterone and calcitonin (both raw and sex- and age-standardized values), trait anxiety, Kerdö's Vegetative Index, one of the vagal markers (SDNN), as well as 10 EEG parameters emerged as characteristic features of the cohorts. In the information space of two discriminant roots, three cohorts are clearly demarcated. **Conclusion**. We interpret this as another proof of the existence of connections between nitrogenous metabolites and psycho-neuro-endocrine parameters in line with the concept of the functional-metabolic continuum.
Keywords: nitrogenous metabolites, adaptation hormones, anxiety, HRV, EEG, men, women.

INTRODUCTION

We previously showed that the factor structure of the relationship between the plasma level of nitrogenous metabolites (uric acid, bilirubin, urea, and creatinine), on the one hand,

and the parameters of anxiety and neuro-endocrine status, on the other hand, is significantly different in men and women of reproductive age and postmenopausal [4-8,26]. To the long-known sexual and age differences in the metabolism of uric acid, creatinine and urea [21], as well as sex hormones by definition, data on sexual and age differences in HRV and EEG parameters, as well as calcitonin [1,11,12,23-25,29] have been added relatively recently. The purpose of this study is to identify the metabolic and psycho-neuro-endocrine features of these three cohorts of people.

MATERIALS AND METHODS

The object of observation were employees of the clinical sanatorium "Moldova" and PrJSC “Truskavets’ Spa”: 31 males (24÷69 y) and 30 females, from among them 18 postmenopausal (45÷76 y) and 12 of reproductive age (30÷42 y). The volunteers were considered practically healthy (without a clinical diagnosis), but the initial testing revealed deviations from the norm in a number of parameters of the neuro-endocrine-immune complex (details follow) as a manifestation of maladaptation. Testing was performed twice with an interval of 4 ("Moldova") or 7 (“Truskavets’ Spa”) days.

We determined the plasma levels of the Bilirubin (by diazoreaction using the Jedrashik-Kleghorn-Grof method), Uric acid (by uricase method), Urea (by urease method by reaction with phenol hypochlorite) and Creatinine (by Jaffe's color reaction by Popper's method) as well as main adaptation hormones Cortisol, Testosterone, Aldosterone, Triiodothyronine and Calcitonin (by the ELISA with the use of corresponding sets of reagents from “Алкор Био”, XEMA Co. Ltd, and DRG International Inc.).

The analyzes were carried out according to the instructions described in the manual [14]. The analyzers “Pointe-180” ("Scientific", USA), “Reflotron” (Boehringer Mannheim, BRD) and “RT-2100C” (PRCh) were used.

To assess the parameters of heart rate variability (HRV) we recorded during 7 min electrocardiogram in II lead (software-hardware complex "CardioLab+HRV", KhAI-MEDICA, Kharkiv). For further analysis the following parameters HRV were selected [2,3,19,30]. Temporal parameters (Time Domain Methods): heart rate (HR), the mode (Mo), triangular index (TNN), the standard 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 than 50 msec (pNN_{50}). Spectral parameters (Frequency Domain Methods): power spectrum density (PSD) bands of HRV: high-frequency (HF, range 0,40÷0,15 Hz), low-frequency (LF, range 0,15÷0,04 Hz), very low-frequency (VLF, range 0,04÷0,015 Hz) and ultralow-frequency (ULF, range 0,015÷0,003 Hz). Derived indices were calculated: (VLF+LF)/HF, LF/HF, LFnu as well as Kerdoe’s Vegetative Index [13,20], for which HR and diastolic blood pressure were recorded synchronously with the "Omron M4-I" device (Netherlands).

Simultaneosly with ECG EEG recorded a hardware-software complex “NeuroCom Standard” (KhAI MEDICA, Kharkiv) monopolar in 16 loci (Fp1, Fp2, F3, F4, F7, F8, C3, C4, T3, T4, P3, P4, T5, T6, O1, O2) by 10-20 international system, with the reference electrodes A and Ref on tassels the ears. The duration of the epoch was 25 sec. Among the options considered the average EEG amplitude (μV), average frequency (Hz), frequency deviation (Hz) as well as absolute (μV^2/Hz) and relative (%) PSD of basic rhythms: β (35÷13 Hz), α (13÷8 Hz), θ (8÷4 Hz) and δ (4÷0,5 Hz) in all loci, according to the instructions of the device.

We calculated also for HRV and each locus EEG the Entropy (h) of normalized PSD using Popovych’s IL equations [17,27] based on classic Shannon’s CE [31] equation:

$$hHRV = -[PSDHF \cdot \log_2 PSDHF + PSDLF \cdot \log_2 PSDLF + PSDVLF \cdot \log_2 PSDVLF + PSDULF \cdot \log_2 PSDULF]/\log_2 4;$$

$$hEEG = -[PSD\alpha \cdot \log_2 PSD\alpha + PSD\beta \cdot \log_2 PSD\beta + PSD\theta \cdot \log_2 PSD\theta + PSD\delta \cdot \log_2 PSD\delta]/\log_2 4.$$

The levels of the trait and reactive anxiety estimated by STAI of Spielberger ChD [32] in modification of Khanin YL [28].

Results processed by using the software package "Statistica 6.4".

RESULTS AND DISCUSSION

To achieve the goal, the registered parameters were subjected to discriminant analysis [22] (method forward stepwise). The program included only 22 parameters in the discriminant model. Along with the quite expected nitrogenous metabolites, age, testosterone and calcitonin (both raw and sex- and age-standardized values), trait anxiety, Kerdö's Vegetative Index, one of the vagal markers, as well as 10 EEG parameters emerged as characteristic features of the cohorts (Tables 1 and 2).

Table 1. Discriminant Function Analysis Summary

Step 22, N of vars in model: 22; Grouping: 3 grps; Wilks' Λ: 0,0251; appr. $F_{(44)}$=23,7; $p<10^{-6}$

Variables currently in the model	Cohorts (n) and Means±SE			Parameters of Wilks' Statistics				
	Reproductive age Women (24)	Postmenopausal Women (36)	Men (62)	Wilks Λ	Partial Λ	F-remove (2,98)	p-level	Tolerancy
Age, years	36,6±0,9	57,5±1,6	47,4±1,6	0,042	0,597	33,1	10^{-6}	0,441
Uric acid, μM/L	252±18	261±14	296±8	0,027	0,934	3,46	0,035	0,605
Bilirubin, μM/L	11,4±0,7	11,5±0,7	14,2±0,5	0,026	0,959	2,07	0,131	0,751
Urea, mM/L	5,47±0,18	5,50±0,18	5,76±0,13	0,026	0,948	2,71	0,072	0,783
Testosterone, nM/L	3,76±0,77	3,23±0,23	13,5±0,8	0,072	0,348	91,8	10^{-6}	0,224
Testosterone, Z	1,26±0,70	0,78±0,36	0,01±0,24	0,043	0,582	35,2	10^{-6}	0,247
Calcitonin, ng/L	5,01±0,73	6,16±0,49	10,5±0,9	0,041	0,618	30,2	10^{-6}	0,164
Calcitonin, Z	-0,02±0,30	0,45±0,20	-0,50±0,13	0,039	0,640	27,6	10^{-6}	0,169
Cortisol, nM/L	333±26	287±15	298±16	0,027	0,914	4,59	0,012	0,706
Kerdö's Vegetat Ind	-2±6	-20±4	-13±3	0,029	0,860	7,99	0,001	0,607
SDNN HRV, msec	64,5±7,0	42,1±2,5	44,2±2,8	0,026	0,953	2,42	0,094	0,765
Amplitude β, μV	11,2±0,6	14,2±0,8	11,7±0,4	0,028	0,908	4,98	0,009	0,202
F3-β PSD, μV^2/Hz	59±5	128±12	64±5	0,028	0,890	6,04	0,003	0,327
F4-β PSD, μV^2/Hz	51±4	131±15	73±10	0,028	0,903	5,28	0,007	0,309
T5-β PSD, μV^2/Hz	61±6	120±20	65±5	0,026	0,971	1,45	0,241	0,382
T6-β PSD, μV^2/Hz	75±9	98±9	55±4	0,026	0,964	1,84	0,164	0,473
O2-β PSD, μV^2/Hz	90±11	122±11	77±7	0,027	0,916	4,47	0,014	0,362
Fp1-θ PSD, %	13,5±1,4	9,2±0,7	9,2±0,6	0,027	0,923	4,06	0,020	0,825
C4-θ PSD, μV^2/Hz	57±9	84±15	38±3	0,026	0,954	2,35	0,100	0,343
F4-α PSD, μV^2/Hz	85±15	167±35	89±11	0,028	0,894	5,80	0,004	0,241
Frequency δ, Hz	1,31±0,09	1,07±0,03	1,08±0,02	0,026	0,972	1,43	0,243	0,738
Trait Anxiety, points	42,8±1,7	44,1±1,3	39,6±1,0	0,027	0,916	4,51	0,013	0,753

Table 2. Summary of stepwise analysis of discriminant variables ranked by criterion Λ

Variables currently in the model	F to enter	p-level	Λ	F-value	p-level
Testosterone, nM/L	66,1	10^{-6}	0,474	66,1	10^{-6}
Testosterone, Z	58,9	10^{-6}	0,237	62,2	10^{-6}
Age, years	44,2	10^{-6}	0,135	67,1	10^{-6}
F4-β PSD, $\mu V^2/Hz$	13,5	10^{-5}	0,110	58,6	10^{-6}
Fp1-θ PSD, %	8,29	0,0004	0,096	51,3	10^{-6}
Calcitonin, ng/L	8,08	0,0005	0,084	46,6	10^{-6}
Calcitonin, Z	30,8	10^{-6}	0,054	53,1	10^{-6}
C4-θ PSD, $\mu V^2/Hz$	6,86	0,002	0,048	49,7	10^{-6}
Trait Anxiety, points	4,39	0,015	0,045	45,9	10^{-6}
Cortisol, nM/L	3,64	0,029	0,042	42,6	10^{-6}
Kerdö's Vegetative Index	3,65	0,029	0,039	40,0	10^{-6}
SDNN HRV, msec	3,08	0,050	0,037	37,6	10^{-6}
Amplitude β, μV	2,36	0,099	0,036	35,3	10^{-6}
Urea, mM/L	2,49	0,088	0,034	33,4	10^{-6}
Uric acid, μM/L	1,56	0,214	0,033	31,5	10^{-6}
Bilirubin, μM/L	2,04	0,135	0,032	29,9	10^{-6}
F3-β PSD, $\mu V^2/Hz$	2,06	0,132	0,031	28,6	10^{-6}
O2-β PSD, $\mu V^2/Hz$	2,14	0,123	0,029	27,4	10^{-6}
F4-α PSD, $\mu V^2/Hz$	4,08	0,020	0,027	26,9	10^{-6}
Frequency δ, Hz	1,30	0,278	0,027	25,7	10^{-6}
T6-β PSD, $\mu V^2/Hz$	1,37	0,258	0,026	24,6	10^{-6}
T5-β PSD, $\mu V^2/Hz$	1,45	0,241	0,025	23,7	10^{-6}

A number of variables, despite their recognizable properties, were outside the discriminant model, apparently due to duplication and/or redundancy of information (Tables 3-6).

Table 3. Metabolic and psycho-neuro-endocrine parameters not included in the model

Variables	Cohorts (n) and Means±SE			Parameters of Wilks' Statistics				
	Reproductive age Women (24)	Postmenopausal Women (36)	Men (62)	Wilks Λ	Partial Λ	F to enter	p-level	Tolerancy
Creatinine, μM/L	80±2	82±2	91±2	0,034	0,996	0,21	0,815	0,533
Triiodothyr., nM/L	2,40±0,24	2,04±0,10	2,01±0,11	0,034	0,990	0,47	0,626	0,486
LFnu, %	72,7±2,5	76,4±2,3	81,8±1,4	0,034	0,996	0,17	0,843	0,783
LF/HF HRV	3,67±0,57	4,71±0,50	7,51±0,88	0,034	0,991	0,46	0,635	0,795
(VLF+LF)/HF	10,7±2,6	11,7±1,3	18,3±1,9	0,034	0,988	0,58	0,561	0,733
TNN HRV, units	13,8±0,8	10,3±0,6	10,8±0,5	0,034	0,997	0,13	0,876	0,341
RMSSD HRV, msec	33,1±3,5	24,0±2,8	22,9±2,0	0,034	0,993	0,34	0,713	0,436
VLF PSD, $mcec^2$	1573±239	807±97	1020±119	0,034	0,986	0,70	0,499	0,457
LF PSD, $mcec^2$	1479±228	751±109	944±135	0,034	0,996	0,19	0,828	0,282
HF PSD, $mcec^2$	596±137	243±50	249±51	0,034	0,987	0,66	0,519	0,473
HF PSD, %	15,4±2,1	11,6±1,5	8,0±0,8	0,034	0,990	0,33	0,710	0,430
Reactive Anxiety, ps	23,2±1,5	23,0±1,2	25,2±1,2	0,034	0,999	0,03	0,968	0,083

Table 4. Parameters of beta-rhythm not included in the model

Variables	Cohorts (n) and Means±SE			Parameters of Wilks' Statistics				
	Reproductive age Women (24)	Postmenopausal Women (36)	Men (62)	Wilks Λ	Partial Λ	F to enter	p-level	Tolerancy
Fp1-β PSD, μV²/Hz	42±4	102±12	55±4	0,033	0,984	0,81	0,446	0,219
Fp2-β PSD, μV²/Hz	63±17	100±12	53±3	0,034	1,000	0,01	0,989	0,408
F7-β PSD, μV²/Hz	42±6	81±12	49±7	0,034	0,983	0,82	0,445	0,476
F8-β PSD, μV²/Hz	31±5	64±12	46±5	0,034	0,995	0,26	0,769	0,408
T3-β PSD, μV²/Hz	71±10	122±19	65±5	0,034	0,991	0,43	0,650	0,343
T4-β PSD, μV²/Hz	55±5	117±18	72±8	0,034	0,998	0,08	0,922	0,151
C3-β PSD, μV²/Hz	76±7	153±17	75±6	0,034	0,998	0,10	0,907	0,246
C4-β PSD, μV²/Hz	71±6	149±17	81±7	0,034	0,991	0,43	0,654	0,210
P3-β PSD, μV²/Hz	86±12	150±18	78±6	0,034	0,995	0,26	0,769	0,215
P4-β PSD, μV²/Hz	82±10	127±14	74±6	0,034	0,984	0,81	0,449	0,164
O1-β PSD, μV²/Hz	92±10	137±15	91±7	0,034	0,998	0,07	0,929	0,207

Table 5. Parameters of theta-rhythm not included in the model

Variables	Cohorts (n) and Means±SE			Parameters of Wilks' Statistics				
	Reproductive age Women (24)	Postmenopausal Women (36)	Men (62)	Wilks Λ	Partial Λ	F to enter	p-level	Tolerancy
Amplitude θ, μV	8,6±0,6	10,9±1,0	8,2±0,4	0,034	0,984	0,77	0,467	0,442
Fp2-θ PSD, %	11,8±0,9	8,9±0,6	9,0±0,7	0,034	0,999	0,05	0,949	0,552
F3-θ PSD, μV²/Hz	49±8	78±12	35±3	0,034	0,991	0,45	0,639	0,358
F4-θ PSD, %	12,0±0,9	9,7±0,8	9,6±0,7	0,034	0,984	0,78	0,462	0,496
F7-θ PSD, %	10,9±0,6	10,5±0,8	8,3±0,6	0,034	0,994	0,27	0,761	0,532
F7-θ PSD, μV²/Hz	23±4	66±12	25±5	0,034	0,994	0,31	0,731	0,727
F8-θ PSD, %	12,5±1,2	9,6±0,8	8,9±0,6	0,033	0,980	0,99	0,374	0,476
T3-θ PSD, μV²/Hz	36±4	68±12	26±2	0,034	0,989	0,52	0,598	0,390
C3-θ PSD, μV²/Hz	54±7	78±13	37±3	0,034	0,997	0,13	0,877	0,155
T5-θ PSD, μV²/Hz	36±6	63±12	31±3	0,034	0,997	0,17	0,846	0,295

Table 6. Parameters of delta- and alpha-rhythm and entropy not included in the model

Variables	Cohorts (n) and Means±SE			Parameters of Wilks' Statistics				
	Reproductive age Women (24)	Postmenopausal Women (36)	Men (62)	Wilks Λ	Partial Λ	F to enter	p-level	Tolerancy
Amplitude δ, μV	19±2	27±4	25±3	0,034	1,000	0,01	0,991	0,602
F8-δ PSD, μV²/Hz	105±31	495±201	552±206	0,034	0,998	0,08	0,927	0,716
T4-δ PSD, μV²/Hz	141±33	567±194	380±93	0,034	0,986	0,70	0,498	0,809
O2-δ PSD, μV²/Hz	122±22	233±63	328±91	0,034	0,985	0,72	0,488	0,715
Fp1-α PSD, μV²/Hz	61±11	152±33	82±9	0,034	0,983	0,82	0,444	0,051
F3-α PSD, μV²/Hz	84±14	188±39	96±12	0,034	0,999	0,03	0,968	0,083
F7-α PSD, μV²/Hz	42±6	140±39	44±5	0,034	0,991	0,43	0,649	0,213
T3-α PSD, μV²/Hz	72±11	156±34	75±10	0,034	0,998	0,10	0,909	0,208
T4-α PSD, μV²/Hz	73±12	133±29	64±7	0,034	0,997	0,16	0,851	0,130
Entropy F7	0,85±0,02	0,80±0,03	0,71±0,03	0,034	0,995	0,23	0,795	0,679
Entropy F8	0,85±0,03	0,76±0,04	0,72±0,03	0,034	0,998	0,10	0,909	0,208
Entropy T6	0,85±0,02	0,77±0,03	0,76±0,03	0,034	1,000	0,02	0,984	0,630

The identifying information contained in the 22 discriminant variables is condensed into two roots. The major root contains 86,4% of discriminatory opportunities (r^*=0,962; Wilks' Λ=0,025; $\chi^2_{(44)}$=400; $p<10^{-6}$), while minor root – 14,6% only (r^*=0,814; Wilks' Λ=0,338; $\chi^2_{(21)}$=118; $p<10^{-6}$).

Calculating the values of discriminant roots for each patient as the sum of the products of raw coefficients for individual values of discriminant variables together with the constant (Table 7) allows visualization of each patient in the information space of roots.

Table 7. Standardized and raw coefficients and constants for discriminant variables

Coefficients	Standardized		Raw	
Variables	Root 1	Root 2	Root 1	Root 2
Testosterone, nM/L	1,749	0,343	0,362	0,071
Testosterone, Z	-1,286	-0,492	-0,553	-0,212
Age, years	0,796	0,703	0,076	0,067
F4-β PSD, μV²/Hz	-0,428	0,468	-0,0057	0,0063
Fp1-θ PSD, %	-0,174	-0,313	-0,035	-0,063
Calcitonin, ng/L	1,555	-0,363	0,294	-0,069
Calcitonin, Z	-1,371	0,769	-1,216	0,682
C4-θ PSD, μV²/Hz	-0,376	0,059	-0,0068	0,0011
Trait Anxiety, points	-0,346	-0,039	-0,044	-0,005
Cortisol, nM/L	-0,008	-0,428	-0,0001	-0,0037
Kerdö's Vegetat Index	-0,224	-0,528	-0,009	-0,021
SDNN HRV, msec	-0,131	-0,262	-0,006	-0,011
Amplitude β, μV	0,570	-0,485	0,157	-0,133
Urea, mM/L	0,197	0,216	0,205	0,224
Uric acid, μM/L	-0,213	0,318	-0,0030	0,0046
Bilirubin, μM/L	0,154	-0,220	0,038	-0,054
F3-β PSD, μV²/Hz	-0,141	0,693	-0,0029	0,0141
O2-β PSD, μV²/Hz	-0,045	0,588	-0,0008	0,0102
F4-α PSD, μV²/Hz	-0,020	-0,815	-0,0002	-0,0061
Frequency δ, Hz	0,015	-0,240	0,057	-0,923
T6-β PSD, μV²/Hz	0,027	0,338	0,0006	0,0081
T5-β PSD, μV²/Hz	-0,182	-0,259	-0,0025	-0,0035
	Constants		-8,129	-2,337
	Eigenvalues		12,47	1,963
Cumulative Proportions			0,864	1

Reference (R) values of HRV parameters are taken from the instructions for "CardioLab+HRV", hormones - from the instructions for the kits, nitrogenous metabolites - from the handbook [21], EEG – from the data base of Truskavetsian Scientific School of Balneology [27].

In order to make a correct comparison, the individual actual values of the Variables (V) were transformed into Z-scores according to the classical equations [17,18,27]:

$Z = (V/R-1)/Cv = (V - R)/SD = 4•(V-R)/(Max - Min)$.

Following the algorithm of Truskavetsian Scientific School of Balneology [17,18,27], Table 8 shows the Z-scores of variables both included in the discriminant model and worthy of attention in view of their identifying information.

Table 8. Correlations between variables and roots, centroids of cohorts and Z-scores of variables and their clusters

Variables	Correlations Variables-Roots		Reproductive age Women (24)	Postmenopausal Women (36)	Men (62)
Root 1 (86,4 %)	**Root 1**	**Root 2**	-4,24	-3,04	**+3,41**
Bilirubin	**0,093**	-0,010	-0,06±0,16	-0,05±0,18	**+0,60±0,13**
LFnu HRV			+0,73±0,21	+0,80±0,16	**+1,26±0,10**
LF/HF HRV			+0,56±0,32	+0,86±0,24	**+2,28±0,42**
(VLF+LF)/HF HRV			+1,73±0,86	+1,22±0,35	**+2,96±0,45**
Mean			**+0,74±0,37**	**+0,71±0,27**	**+1,78±0,52**
Creatinine			+0,49±0,17	+0,47±0,14	**0,00±0,11**
Testosterone	**-0,062**	-0,033	+1,26±0,70	+0,78±0,36	**+0,01±0,24**
Trait Anxiety	**-0,071**	0,061	+1,38±0,49	+1,74±0,37	**+0,45±0,28**
Mean			**+1,04±0,28**	**+1,00±0,38**	**+0,15±0,15**
Uric acid			-0,34±0,18	-0,66±0,19	**-1,30±0,12**
HF band PSDr			-0,08±0,14	-0,07±0,19	**-0,57±0,06**
Mean			**-0,21±0,13**	**-0,37±0,30**	**-0,94±0,37**
Root 2 (13,6 %)	**Root 1**	**Root 2**	**-2,23**	**+1,77**	-0,16
Urea			**+0,49±0,20**	0,00±0,19	+0,11±0,13
SDNN HRV	-0,053	**-0,228**	**+0,14±0,24**	-0,42±0,08	-0,39±0,09
TNN HRV			**+1,05±0,8**	-0,37±0,24	-0,15±0,22
VLF band PSDa			**+0,16±0,33**	-0,55±0,15	-0,37±0,17
Frequency δ	-0,056	**-0,217**	**+0,69±0,34**	-0,18±0,10	-0,13±0,09
Fp1-θ PSDr	-0,060	**-0,197**	**+0,51±0,22**	-0,19±0,12	-0,20±0,10
Kerdö's Vegetative Index	0,010	**-0,183**	**+0,85±0,22**	+0,12±0,12	+0,41±0,11
Cortisol	-0,012	**-0,101**	**-0,33±0,23**	-0,76±0,13	-0,65±0,14
Triiodothyronine			**+0,39±0,24**	-0,32±0,20	-0,38±0,23
Mean			**+0,44±0,14**	**-0,30±0,09**	-0,19±0,10
Age	-0,001	**0,499**	-0,92±0,07	**+0,73±0,12**	-0,07±0,13
F3-β PSDa	-0,088	**0,375**	-0,37±0,09	**+0,90±0,23**	-0,28±0,09
F4-β PSDa	-0,037	**0,278**	-0,45±0,06	**+0,67±0,21**	-0,15±0,14
Amplitude β	-0,042	**0,219**	-0,23±0,18	**+0,61±0,22**	-0,10±0,11
T5-β PSDa	-0,050	**0,216**	-0,20±0,13	**+1,02±0,42**	-0,12±0,11
T6-β PSDa	-0,107	**0,166**	+0,02±0,13	**+0,33±0,12**	-0,26±0,06
F4-α PSDa	-0,040	**0,164**	-0,22±0,18	**+0,73±0,41**	-0,18±0,13
O2-β PSDa	-0,070	**0,161**	0,00±0,23	**+0,64±0,23**	-0,25±0,13
C4-θ PSDa	-0,083	**0,145**	+0,27±0,22	**+0,93±0,37**	-0,19±0,09
Calcitonin	-0,081	**0,139**	-0,02±0,30	**+0,45±0,20**	-0,50±0,13
Mean			**-0,21±0,10**	**+0,70±0,07**	-0,21±0,04

Further, the variables were grouped into several clusters.

The first cluster (Fig. 1) reflects a situation in which men have an upper limit level of bilirubin accompanied by increased levels of HRV markers of sympathetic tone, while women of both age groups have a completely normal level of bilirubin, and the levels of sympathetic markers are equally upper limit. The second cluster shows that in men a completely normal level of creatinine is accompanied by a completely normal level of testosterone and an upper borderline level of trait anxiety, while in women of both age groups creatinineemia is upper borderline, and the levels of testosterone and trait anxiety are moderately elevated. The third cluster illustrates the combination of hypouricemia in men with the lower limit level of HRV marker of vagal tone, while in women the level of uric acid is in the lower zone of the age norm, and the vagal tone fully corresponds to the age norm.

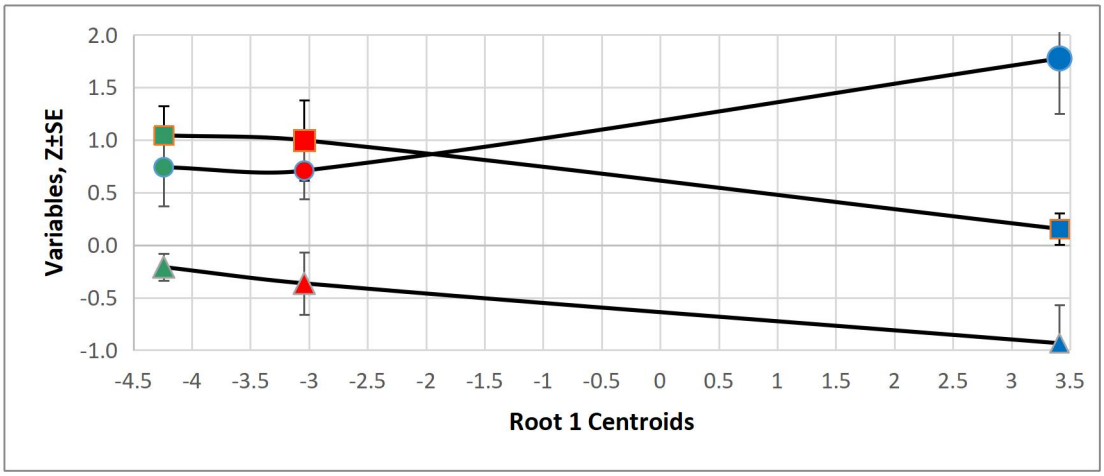

Fig. 1. Z-scores of bilirubin (circles), creatinine (squares), and urate (triangles) cluster variables (Y axis) and the centroids of the first root (X axis) of cohorts of women of reproductive age (green icons), postmenopausal women (red icons), and men (blue icons)

The other two clusters reflect differences between women of different ages (Fig. 2).

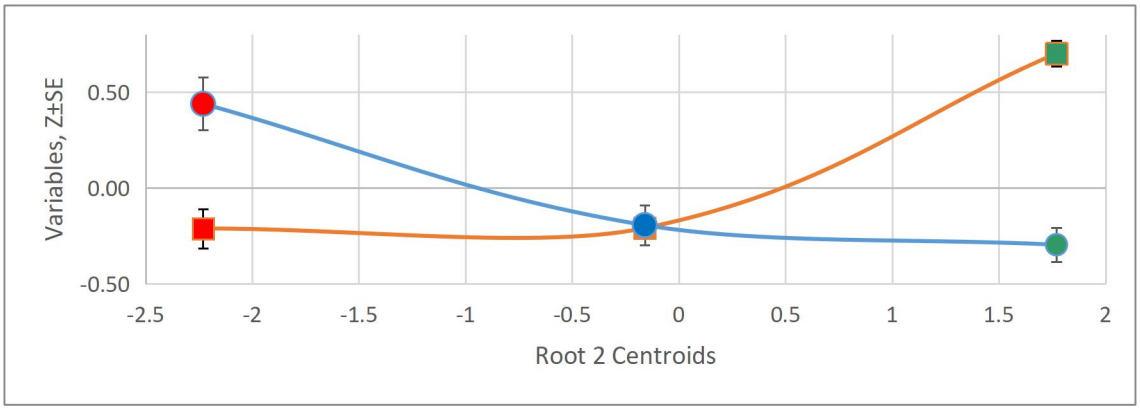

Fig. 2. Z-scores of urea (squares) and age (circles) cluster variables (Y axis) and the second root centroids (X axis) of cohorts of women of reproductive age (green icons), men (blue icons), and postmenopausal women (red icons)

In particular, in women of reproductive age, the upper-limit level of urea is accompanied by upper-limit or normal levels of vagal markers, delta-rhythm frequency, PSD of theta-rhythm in Fp1 locus and triiodothyronine, as well as the lower-limit level of cortisol. At the same time, in postmenopausal women, the level of urea is completely normal, other parameters are normal or at the lower limit, and cortisol level is reduced.

On the other hand, upper limit levels of calcitonin and a number of EEG parameters were found in postmenopausal women, while in younger women similar parameters are quite normal or lower limit. According to these parameters, men generally occupy an intermediate position.

In the information space of two discriminant roots, three cohorts are clearly demarcated (Fig. 3).

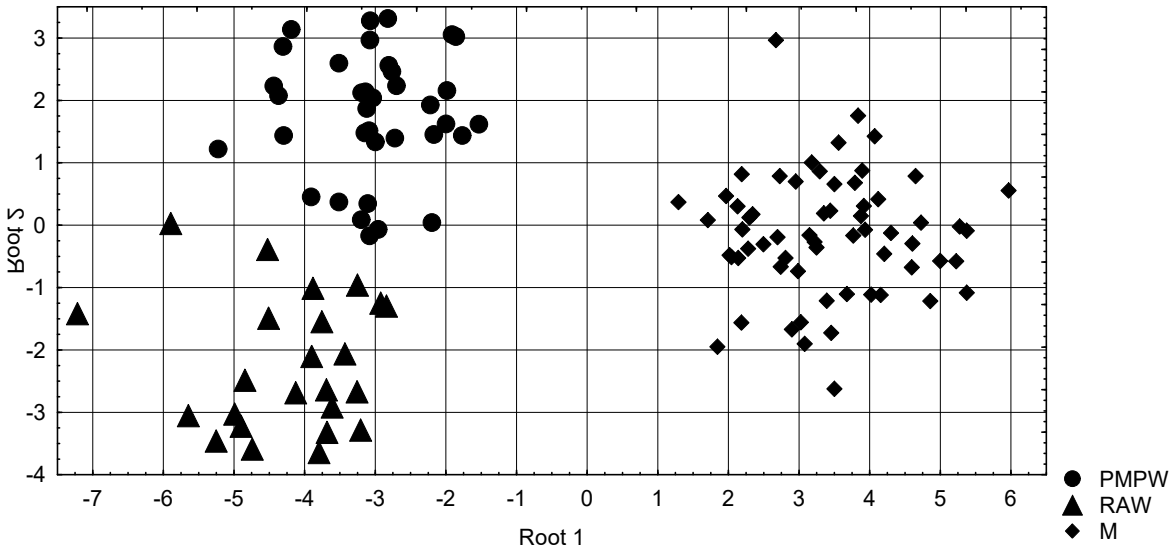

Fig. 3. Scattering of individual values of the first and second discriminant roots of postmenopausal women (PMPW), reproductive age women (RAW) and men (M)

The visual impression of a clear demarcation is documented by calculating the distances of Mahalanobis (Table 9).

Table 9. Squares of Mahalanobis distances between clusters (above the diagonal) and F-criteria (df=23,0) and p-levels (below the diagonal)

Cohorts	PMPW	RAW	Men
Postmenopausal Women (36)	0	17,4	45,3
Reproductive age Women (24)	9,4 10^{-6}	0	62,8
Men (62)	38,6 10^{-6}	40,7 10^{-6}	0

Selected discriminant variables were used to identify the affiliation of a patient to a particular cohort. This goal of discriminant analysis is realized with the help of classification functions (Table 10).

Table 10. Coefficients and constants of classification functions

Cohorts	Postmeno-pausal Women (36)	Reproduc-tive age Women (24)	Men (62)
Variables	p=,295	p=,197	p=,508
Testosterone, nM/L	2,557	1,837	4,753
Testosterone, Z	-5,773	-4,260	-8,930
Age, years	1,175	0,816	1,534
F4-β PSD, μV²/Hz	-0,016	-0,035	-0,065
Fp1-θ PSD, %	0,031	0,323	-0,073
Calcitonin, ng/L	0,940	0,860	2,971
Calcitonin, Z	-1,139	-2,402	-10,29
C4-θ PSD, μV²/Hz	-0,022	-0,018	-0,068
Trait Anxiety, points	0,607	0,679	0,333
Cortisol, nM/L	0,008	0,023	0,015
Kerdö's Vegetat Index	-0,082	0,013	-0,099
SDNN HRV, msec	0,132	0,185	0,117
Amplitude β, μV	2,195	2,540	3,464
Urea, mM/L	8,789	7,648	9,677
Uric acid, μM/L	0,047	0,032	0,018
Bilirubin, μM/L	0,719	0,888	1,066
F3-β PSD, μV²/Hz	0,026	-0,027	-0,019
O2-β PSD, μV²/Hz	0,096	0,056	0,071
F4-α PSD, μV²/Hz	-0,042	-0,017	-0,031
Frequency δ, Hz	19,15	22,77	21,30
T6-β PSD, μV²/Hz	0,017	-0,016	0,006
T5-β PSD, μV²/Hz	-0,092	-0,075	-0,102
Constants	-115,5	-102,1	-162,5

The classification accuracy is absolute (Table 11).

Table 11. Classifications matrix

	Rows: Observed classifications Columns: Predicted classifications			
Group	Percent Correct	PMPW p=,29508	RAW p=,19672	M p=,50820
PMPW	100	36	0	0
RAW	100	0	24	0
M	100	0	0	62
Total	100	36	24	62

CONCLUSION

It is significant that one or another nitrogenous metabolite was found in the composition of each cluster. Earlier we discovered peculiarities of relationships between plasma levels of nitrogenous metabolites and EEG&HRV parameters in patients with postradiation encephalopaty [9]. We interpret this as another proof of the existence of connections between nitrogenous metabolites and psycho-neuro-endocrine parameters in line with the concept of the functional-metabolic continuum [15,16].

ACKNOWLEDGMENT

We express sincere gratitude to administrations of clinical sanatorium "Moldova" and PrJSC "Truskavets' Spa" as well as TA Korolyshyn and VV Kikhtan for help in carrying out this investigation.

ACCORDANCE TO ETHICS STANDARDS

Tests in patients are carried out in accordance with positions of Helsinki Declaration 1975 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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