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TRACE ELEMENTS CONCENTRATIONS ASSOCIATION WITH SCHIZOPHRENIA SYMPTOMS; A CROSS-SECTIONAL STUDY IN CROATIA

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SUMMARY

Background: *Significant inconsistencies exist in findings on association of bio-elements (BE) concentrations and schizophrenia. Hypothesis of this research was that different concentrations of BE are associated with different psychopathological schizophrenia symptoms.*

Subjects and methods: *This cross-sectional study was performed from 2014 to 2016 at Psychiatric Hospital "Sveti Ivan" and University Psychiatric Hospital "Vrapče", Zagreb, Croatia, on the consecutive sample of 67 patients diagnosed with schizophrenia. BE concentrations were measured by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) at the Institute for Medical Research and Occupational Health in Zagreb. Severity of schizophrenia symptoms was assessed on Brief Psychiatric Rating Scale (BPRS).*

Results: *After adjustment for all preplanned possible confounding variables, the first canonical correlation between BE and BPRS dimensions variates were statistically significant (Rc^2=0.73; P=0.006). The first pair of canonical variates is defined by BPRS negative dimension (and marginally by positive symptoms and lack of resistance), and copper (Cu), lead (Pb), lithium (Li) and cobalt (Co) (marginally by cadmium (Cd) and nickel (Ni)).*

Conclusions: *Concentrations of different BE are associated with different schizophrenia symptoms. Maximal correlation between BPRS and BE may be achieved with the weighted linear composite of negative schizophrenia symptoms and copper (Cu), lead (Pb), lithium (Li) and cobalt (Co).*

Key words: *schizophrenia - bio-elements - trace elements - BPRS*

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INTRODUCTION

Trace elements are required for numerous metabolic and physiological processes in the human body. They play a part in the synthesis and structural stabilization of proteins and nucleic acids, and are required by living organisms in minute amounts (that is less than 0.1 percent by volume), usually as part of a vital enzyme, a cell-produced catalytic protein (Encyclopædia Britannica 2016). Therefore, imbalances in the optimum levels of trace elements may adversely affect biological processes, and are associated with many diseases (Mertz 1981, Muralidhar 2004, Kawahara et al. 2017).

Differences in concentrations of bio-elements (BE) may also play a role in the pathogenesis of schizophrenia. For example, it is known that nutritive deprivation in early stage of life increases a risk of illness (Berry 1994). Also, oxidative stress and thinking disorders, which are common to people with schizophrenia, may be result of different concentration of bio-elements (Bitanihirwe 2010).

Schizophrenia, like most psychiatric diagnoses, remains a syndromic concept.

However, the assumption that the clinical syndromes defined in this way represent valid disease entities with distinct underlying causes and pathogenesis is increasingly being seen as having impeded research. Indeed, psychiatric diagnoses have the unusual feature of being simultaneously too broad and too narrow (Owen et al. 2014). The diagnosis of schizophrenia is based on the simultaneous presentation of two types of symptoms that reflect a psychotic disturbance: "positive" symptoms that include delusions, hallucinations, and bizarre thoughts, and negative symptoms that include social withdrawal with affective flattening, poor motivation, and apathy (Sawa et al. 2002). Symptoms of schizophrenia should not be discussed as one clinical entity, but as an integral part of an overall syndrome approach.

Pyramidal model of schizophrenia may help to understand how concentrations of different BE are associated with different psychopathological schizophrenia symptoms. It shows symptom profiles and helps

to clarify the heterogeneity of schizophrenia toward syndrome-specific treatments (Kay et al. 1990). Negative, positive, and depressive features constituted divergent points of a triangular base, and excitement made up a separate vertical axis. Thus, this pyramidal set of axes encompasses the diversity in symptoms and may reflect separate pathological processes in schizophrenia. Paired syndromes could account for symptoms of the paranoid (positive-depressive), disorganized (positive-negative), and catatonic (negative-depressive) diagnostic subtypes. The transversal positions in this model suggested polarized dimensions in schizophrenia, including a prognostic axis (depression-cognitive dysfunction). Schizophrenic subtypes derive from a hybrid between unrelated but co-occurring dimensions that may define the fundamental elements of psychopathology (Kay & Sevy 1990).

Significant inconsistencies exist in the findings on association of BE concentrations and schizophrenia (Devanarayanan. et al. 2016, Liu et al. 2015). Majority of studies have analyzed the association of BE with schizophrenia compared to the healthy population (Yanik et al. 2004, Liu et al. 2015, Cai et al. 2015, Rahman et al. 2009), but this approach risks to fail to acknowledge the illness complexity and different pathophysiological and psychological basis of different schizophrenia symptoms.

The role of copper in schizophrenia is not well established and there are conflicting reports about copper levels in schizophrenia cases. Some investigators have demonstrated normal copper levels in cerebrospinal fluid and in the post-mortem brains, whereas, others have documented reduction in copper levels in hair of schizophrenia patients (Shore et al. 1983, Kornhuber et al. 1994, Tada et al.1986). In contrast to these findings, recent studies have reported elevated copper levels in blood and hair samples in patients with schizophrenia, and have attributed it to alteration in nutrition status and socioeconomic factors (Yanik et al. 2004, Rahman et al. 2009). Research of Liu (2015) demonstrated that copper, selenium and manganese were associated with an increased risk of schizophrenia. The study showed that lower levels of selenium, copper and higher levels of manganese were found in schizophrenia patients compared with healthy controls (Liu et al. 2015). In the study conducted by Rahman et al. (2009) the concentration of Zn and Ca in the hair of patients with schizophrenia was significantly lower, whereas the concentration of Cu and Cd was significantly higher. The authors, methodologically not entirely correctly, conclude that a significant lower concentration of Zn and Ca as well as a significant higher concentration of Cu and Cd can be used as prognostic means to diagnose and treat the illness, and that the illness can be treated with supplements.

Only a few studies tried to associate the BE concentrations with schizophrenia symptoms structure and severity. The recent study of Devanarayanan et al. (2016) concluded that copper, CRP, total cholesterol and LDL-Cholesterol concentrations are elevated in schizophrenia cases. Total cholesterol and triacylglycerol concentrations were significantly associated with severity of the psychopathology, whereas CRP and copper were not associated with severity score in schizophrenia cases. Further studies are needed to investigate whether drugs which reduces copper, CRP and cholesterol levels will defer the onset of those risk of schizophrenia, or reduce the severity of psychopathology in those already diagnosed with schizophrenia.

Contradictions in findings of previous studies may partially be caused by the fact that they considered schizophrenia as a single entity and miss to assess the differences between different symptoms and clinical features. The aim of this study was to examine whether differences in concentrations of different BE are associated with different psychopathological schizophrenia symptoms. The findings could help to clarify the heterogeneity of schizophrenia and to illuminate the path toward syndrome-specific treatments.

SUBJECTS AND METHODS

Study design

This cross-sectional study was conducted from May 2014 to May 2016 in Psychiatric Hospital “Sveti Ivan” and “University Psychiatric Hospital Vrapče”, Zagreb, Croatia. Ethics committees of two participating institutions approved the study protocol, and all the patients signed the informed consent. The study was done in accordance with World Medical Association Declaration of Helsinki 2013 (14). The study was not registered at any public registry.

Study population

The targeted population were the patients diagnosed with schizophrenia (ICD-10: F20) of both sexes, age 18-70 years with permanent residence in City of Zagreb (Croatian capital), who were hospitalized in one of the two participating institutions. Subjects with schizophrenia were diagnosed by two independent psychiatrists according to the criteria of ICD-10 . We included 67 participants, 42 female and 25 male. Exclusion criteria were: alcohol or drugs dependence, and taking medications that contain BE under the course of the study. We chose a consecutive sample of patients in the order of their admission.

Needed sample size

Power analysis was done before the start of the enrollment. Data and assumptions needed were taken from Vidović et al. (2013). A sample size of n=61 achieves 80% power to detect a difference of 0.35 from the null hypothesis correlation of 0.00 using a two-sided test with a significance level of 0.05. Expecting up to 15% of incorrectly collected data the initially needed sample size was determined to be n=72. We did not make a specific power analysis for the canonical correlation, but

we decided to include the maximum of n/5 variables, where "n" is the needed sample size calculated for the correlation analysis as presented above, counting on the fact that the reliability of our measurement of BE concentrations was relatively high (Tabachnick & Fidell 2013). However, we did not test the BE concentration measurements reliability by re-test or some other method, so this assumption remained unproved. Power analysis was done in PASS 13 Power Analysis and Sample Size Software (2014). NCSS, LLC. Kaysville, Utah, USA.

Outcome

Our two outcomes were measured by the Brief Psychiatric Rating Scale (BPRS). We used the standard 18 item version (Overall 1974). It was administered by psychiatrist who rated the severity of each symptom on the scale ranging from 1 (not present) to 7 (extremely severe). Our primary outcome was the result on each of five BPRS core dimensions: positive, negative, affective symptoms, activation, and resistance that we defined accordingly to Shafer meta-analysis conducted in 2005 (Shafer 2005). Positive symptoms dimension was defined as the sum of the following BPRS core items: unusual thought content, hallucinations, conceptual disorganization, and grandiosity. Negative symptoms dimension was defined as the sum of scores on blunted affect, emotional withdrawal, and motor retardation. Affective symptoms dimension was defined by anxiety, depression, guilt, and somatic concern. We defined the activation dimension by excitement, tension, and mannerisms-posturing, and resistance dimension by hostility, uncooperativeness, and suspiciousness. As disorientation was highly saturated by both positive and negative symptoms dimensions, we decided to exclude it. Our secondary outcome was the BPRS total score calculated as the sum of all items. The total score may range from 18 to 126 with higher values indicating more severe symptoms. We used this outcome for the sake of comparability with previous studies.

Independent variables

Independent variables were concentrations of BE measured by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Approximately 0.5 to 1 gram of hair was taken from the area located between the scalp and the occipital region, cut off and prepared for analysis according to scientific standards. Individual hair samples had a length of 1 cm, were mixed for 10 minutes in a solution of ethylether and acetone (3: 1 ratio), washed three times with redistilled H_2O, dried at 85°C for one hour, immersed one hour in 5% EDTA (ethylenediaminetetraacetic acid), washed again in redistilled H_2O, dried at 85°C for 12 hours and wet disintegrated in a HNO_3/H_2O_2 solution in a plastic tube and analyzed on a multielement profile. Hair dyeing data and shampoo used are considered before each analysis for correction of results.

Possible confounders

Possible confounding factors that we tried to control were patients' age and sex, education defined as the highest finished school and categorized into three categories: primary school, secondary school and university; work status before the last hospitalization categorized into: employed including pupils, students and housekeepers, unemployed and retired or in disability pension; body mass index calculated as body weight in kilograms divided by body height in square meters and categorized as normal (18-25.0), overweight (25.0-29.9) or obese (≥30.0); smoking of tobacco and alcohol consumption, hair dying, usage of non-herbal shampoo and nutrition supplements, treatment with 1st, 2nd generation antipsychotics or clozapine, and treatment with antidepressants and benzodiazepines and other medicines.

Statistical analysis

We planned all analysis and all possible confounders in advance. By the study protocol it was planned to remove all BE and confounding variables whose maximum correlation with any BPRS dimension will be $r_{max}<0.1$. In all instances, we used two-tailed tests with 0.05 significance level. Mean BE concentrations ≥100 were presented as the whole numbers, those that were ≥10 with one decimal, ≥1 with two, ≥0.01 with three, ≥0.001 with four and those whose mean concentrations were <0.001 with precision at five decimals. Regardless of precision of presentation of numbers, the calculations were always performed at statistical program defaults. In all instances, we presented the measures of dispersion like standard deviation or interquartile range with one decimal point higher precision than the measures of central tendency like mean or median. Normality of distributions was checked by D' Agostino Skewness, Kurtosis and Omnibus test, but only Omnibus test results were presented. Hotelling T^2 test based on the Mahalanobis distance of each point from the variable means as implemented in NCSS 10 was used for the multivariate outlier detection. We defined the univariate outliers as the cases who deviated from the mean for ≥3.29 standard deviations (Tabachnick & Fidell 2013), or as the cases who deviated ±1.5 interquartile range from the lower (1st) or the highest (4th) quartile. We did a sensitivity analysis by repeating the canonical correlation analysis with seven multivariate outliers omitted. To improve linearity of correlations and normality of distributions, a natural logarithmic transformation of original BE concentrations was done. Multicollinearity was checked on the bivariate level by the inspection of correlation matrix (r≥0.90) (Tabachnick & Fidell 2013) and on the multivariate level by the inspection of tolerance ($1-R^2$) ≤0.1 and equivalently variance inflation factors (VIF) >10 defined as reciprocal of tolerance or: $1/(1-R^2)$ where R^2 is squared multiple correlation of the every variable and all other variables, and by the variance condition number >30 defined as

the condition index with the largest value and calculated as the square root of the largest eigenvalue divided by the smallest eigenvalue. We did the introductory analysis by partial Pearson moment-product correlations adjusted for age, sex, education, work status, body mass index (kg/m^2), smoking, alcohol consumption, hair dyeing, using a non-herbal shampoo, using nutrition supplements, treatment with all particular antipsychotics, with particular benzodiazepines, and with mood stabilizers and antidepressants as the groups with no differentiation of particular drugs. In the study protocol, we planned to do a multiple imputation by the fully conditional specification of the iterative Markov chain Monte Carlo method and do the sensitivity analysis of our findings if we have the missing data in more than 10% of participants. This did not happen, so all of analyses were done on the cases with complete data only ("list-wise deletion"). The main analysis was done by canonical correlation. We have not applied any correction for the inflation of alpha error caused by the multiple testing because all of the analyses were preplanned and we interpreted only one, multivariate analysis. Statistical data analysis was done by NCSS 10 Statistical Software (2015). NCSS, LLC. Kaysville, Utah, USA.

RESULTS

We assessed 121 patients for eligibility, and recruited 75 (Figure 1). Data on BPRS was not properly collected for one 30 years old woman. In 7 (9.5%) patients we did not properly collect data on BE concentrations. The final sample consisted of 67 patients diagnosed with schizophrenia (ICD-10: F20). Patients' age ranged from 21 to 70 years, and 42 (62.7%) of patients were female and 25 were male (Table 1).

According to D' Agostino omnibus test[16] none of the BE concentrations were normally distributed. Consequently, concentrations' means and medians were different in all BE (Table 2). We detected seven multivariate and 25 univariate outliers. After the logarithmic transformation, normality of variables significantly improved and no multivariate outliers remained. However, correlations between natural log-transformed BE concentrations that deviated significantly from the linear one were: positive BPRS dimension and lithium (Li) (P<0.001), and sodium (Na) (P=0.021); resistance BPRS dimension and cobalt (Co) (P=0.010), zinc (Zn) (P=0.013), strontium (Sr) (P=0.032), barium (Ba) (P=0.001), lead (Pb) (P=0.024); activation BPRS dimension and magnesium (Mg) (P=0.030). Univariate multicollinearity defined as correlations >0.90 within BE was indicated between magnesium (Mg) and calcium (Ca) (r=0.92). Multivariate multicollinearity defined as tolerance ≤0.1, VIF >10 and/or condition numbers >30 was indicated in cases of barium (Ba) (VIF=14.9), calcium (Ca) (VIF=12.3), strontium (Sr) (VIF=21.6). We removed these three BE from the final analysis.

Table 1. Participants characteristics (n=67)

	n	(%)
Age, median (IQR)	35	(30-47)
Sex		
female	42	(62.7)
male	25	(37.3)
Education		
primary	14	(20.9)
secondary	47	(70.1)
university	6	(9.0)
Work status		
employed	21	(31.3)
unemployed	16	(23.9)
retired or disability pension	30	(44.8)
Body mass index (kg/m^2), median (IQR)	25	(22-29)
Body mass index (kg/m^2)		
normal (18-25.0)	34	(50.7)
overweight (25.0-29.9)	20	(29.9)
obese (≥30.0)	13	(19.4)
Smoking tobacco	39	(58.2)
Alcohol consumption	19	(28.4)
Hair dyeing	16	(23.9)
Using a non-herbal shampoo	10	(14.9)
Nutrition supplements	14	(20.9)
Antipsychotics		
monotherapy	23	(34.3)
combination of two drugs	33	(49.3)
combination of three or more drugs	11	(16.4)
1st generation antipsychotics	32	(47.8)
promazine	14	(20.9)
haloperidol	9	(13.4)
fluphenazine	13	(19.4)
zuclopentixol	3	(4.5)
levomepromazine	2	(3.0)
2nd generation antipsychotics	51	(76.1)
olanzapine	16	(23.9)
quetiapine	11	(16.4)
aripiprazole	9	(13.4)
risperidone	10	(14.9)
paliperidone	6	(9.0)
ziprasidone	3	(4.5)
amisulprid	2	(3.0)
clozapine	21	(31.3)
Benzodiazepines	52	(77.6)
diazepam	29	(43.3)
lorazepam	14	(20.9)
zolpidem	11	(16.4)
other*	9	(13.4)
Mood stabilizers†	19	(28.4)
Antidepressants‡	11	(16.4)

Abbreviations: IQR = interquartile range

* Other benzodiazepines: clonazepam, 6 (8.0%), alprazolam 2 (2.7%), nitrazepam 2 (2.7%), flurazepam 1 (1.3%), midazolam 1 (1.3%); † Mood stabilizers: sodium valproate 12 (16.0), lamotrigine 6 (8.0), carbamazepine 2 (2.7%), lithium 2 (2.7%); ‡ Antidepressants: selective serotonin reuptake inhibitors (SSRI) 6 (8.0%), serotonin-norepinephrine reuptake inhibitors (SNRI) 3 (4.0%), noradrenergic and specific serotonergic (NaSSA) 3 (4.0%), serotonin modulators and stimulators (SMS) 1 (1.3%), melatonergic agonists and 5HT2c antagonists 1 (1.3%)

Table 2. BE concentrations and test of normality of distributions (n=67)

	Mean	(SD)	Median	IQR	Min	Max	Normality, P
Aluminium (Al)	3.7	(2.86)	2.8	(1.91-5.43)	0.7	17.0	<0.001
Barium (Ba)	0.625	(0.7967)	0.255	(0.1675-0.7637)	0.027	3.808	<0.001
Cadmium (Cd)	0.0160	(0.06054)	0.00629	(0.00257-0.01263)	0.0000	0.5000	<0.001
Calcium (Ca)	107	(133.0)	59	(36.0-105.0)	17	649	<0.001
Chromium (Cr)	0.0613	(0.05011)	0.04488	(0.02992-0.06598)	0.0141	0.2946	<0.001
Cobalt (Co)	0.0470	(0.13809)	0.00909	(0.00398-0.02543)	0.0002	0.9473	<0.001
Copper (Cu)	12	(6.0)	10	(9.0-13.0)	7	55	<0.001
Iron (Fe)	10	(9.0)	7	(6.0-11.0)	4	51	<0.001
Lead (Pb)	0.577	(1.2762)	0.227	(0.1473-0.436)	0.032	9.369	<0.001
Lithium (Li)	0.0171	(0.04253)	0.00833	(0.00463-0.01646)	0.0027	0.3327	<0.001
Magnesium (Mg)	94	(107.0)	60	(30.0-103.0)	12	466	<0.001
Manganese (Mn)	0.206	(0.2703)	0.097	(0.059-0.2691)	0.024	1.767	<0.001
Molybdenum (Mo)	0.0270	(0.01179)	0.02474	(0.01994-0.0316)	0.0039	0.0784	<0.001
Nickel (Ni)	0.139	(0.1449)	0.080	(0.0515-0.169)	0.004	0.787	<0.001
Potassium (K)	30	(17.0)	24	(19.0-37.0)	8	82	<0.001
Silicon (Si)	0.0175	(0.01915)	0.01189	(0.00734-0.02363)	0.0033	0.1456	<0.001
Sodium (Na)	30	(54.0)	20	(13.0-31.0)	6	448	<0.001
Strontium (Sr)	1.4	(1.80)	0.7	(0.37-1.82)	0.1	8.6	<0.001
Vanadium (V)	0.0220	(0.00977)	0.01897	(0.0163-0.02301)	0.0117	0.0656	<0.001
Zinc (Zn)	173	(50.0)	157	(146.0-191.0)	77	350	<0.001

Abbreviations: Normality P = D' Agostino omnibus test of normality of distributions statistical significance

Table 3. Partial correlations of natural log-transformed BE concentrations with BPRS dimensions results, adjusted for all confounders (n=67)

	Total BPRS		BPRS symptoms dimensions									
			Positive		Negative		Affective		Activation		Resistance	
	r	p	r	p	r	p	r	p	r	p	r	p
Aluminium (Al)	0.07	0.658	0.01	0.970	-0.23	0.166	0.04	0.802	0.33	0.046	0.22	0.191
Barium (Ba)	-0.03	0.849	-0.02	0.908	-0.20	0.236	-0.06	0.731	-0.02	0.899	0.31	0.06
Cadmium (Cd)	-0.08	0.649	-0.03	0.852	-0.28	0.094	-0.33	0.041	0.16	0.349	0.39	0.016
Calcium (Ca)	0.11	0.521	0.05	0.749	-0.07	0.675	0.13	0.437	0.01	0.941	0.39	0.016
Chromium (Cr)	-0.13	0.432	-0.16	0.354	-0.27	0.101	-0.14	0.391	0.15	0.376	0.07	0.680
Cobalt (Co)	-0.26	0.115	-0.12	0.474	-0.39	0.015	-0.26	0.109	-0.23	0.161	0.19	0.252
Copper (Cu)	-0.23	0.169	-0.22	0.180	-0.46	0.003	-0.15	0.370	-0.04	0.833	0.11	0.512
Iron (Fe)	0.10	0.552	0.04	0.807	0.03	0.858	-0.04	0.792	0.27	0.11	0.18	0.278
Lead (Pb)	-0.15	0.380	-0.20	0.236	-0.47	0.003	-0.06	0.707	-0.07	0.688	0.35	0.029
Lithium (Li)	0.13	0.428	0.21	0.201	0.29	0.073	-0.04	0.814	-0.02	0.911	-0.02	0.885
Magnesium (Mg)	0.07	0.663	-0.03	0.871	-0.05	0.788	0.07	0.657	0.00	0.979	0.44	0.006
Manganese (Mn)	-0.03	0.881	-0.09	0.595	-0.15	0.377	-0.08	0.648	0.18	0.288	0.17	0.323
Molybdenum (Mo)	0.05	0.772	0.08	0.622	-0.07	0.673	0.03	0.841	0.03	0.880	0.18	0.289
Nickel (Ni)	-0.08	0.628	-0.04	0.796	-0.34	0.037	-0.17	0.299	0.15	0.381	0.22	0.188
Potassium (K)	-0.12	0.463	-0.14	0.405	-0.09	0.595	-0.26	0.119	0.07	0.662	-0.00	0.997
Silicon (Si)	-0.36	0.040	-0.24	0.152	-0.52	0.001	-0.28	0.100	-0.03	0.877	-0.09	0.574
Sodium (Na)	-0.19	0.261	-0.26	0.110	-0.10	0.560	-0.21	0.201	0.01	0.968	-0.07	0.682
Strontium (Sr)	-0.03	0.852	-0.06	0.731	-0.18	0.284	0.04	0.808	-0.03	0.849	0.25	0.124
Vanadium (V)	-0.09	0.574	-0.13	0.444	-0.16	0.351	-0.06	0.735	0.13	0.424	-0.07	0.693
Zinc (Zn)	-0.18	0.288	0.03	0.853	-0.28	0.085	-0.27	0.102	-0.15	0.389	0.05	0.764

r = Pearson-product moment correlations were adjusted for age, sex, education, work status, body mass index (kg/m^2), smoking, alcohol consumption, hair dyeing, using a non-herbal shampoo, using a nutrition supplements, treatment with particular antipsychotics, benzodiazepines, mood stabilizers and antidepressants; p = statistical significance

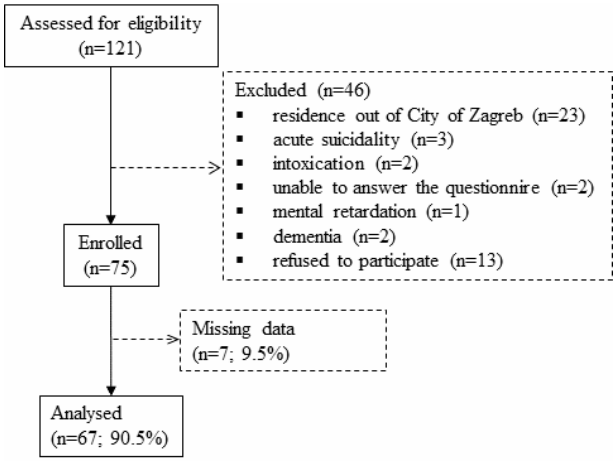

Figure 1. Study flow

In table 3 we presented the partial correlations of natural log-transformed BE concentrations with total BPRS score and particular BPRS dimensions, adjusted for all pre-planned possible confounders (Table 3). None of BE had the maximum correlation with any BPRS dimension $r_{max}<0.1$ so none was removed by this preplanned criterion. Five of pre-planned possibly confounding variables were not correlated with any BPRS dimension and were removed from the final canonical correlation analysis as irrelevant: body mass index ($r_{max}=-0.09$), alcohol consumption ($r_{max}=0.08$), treatment with fluphenazine ($r_{max}=0.08$), paliperidone ($r_{max}=-0.08$), and zolpidem ($r_{max}=-0.08$).

After adjustment for all preplanned possible confounding variables, the first canonical correlation between BE and BPRS dimensions variates was statistically significant ($R_c=0.85$, $R_c^2=0.73$; $F(75,114)=1.69$; $P=0.006$; Wilks' Lambda=0.028). Total of 13% of BPRS dimensions variation was explained by the first BE canonical variate, and 9.2% of variation in BE. Total of 6.7% of BE concentrations variation was explained by the first BPRS dimensions canonical variate, and 17.9% of BPRS dimensions. The first pair of canonical variates was defined by BPRS negative dimension, and marginally by positive symptoms and lack of resistance, and copper (Cu), lead (Pb), lithium (Li) and cobalt (Co), marginally by cadmium (Cd) and nickel (Ni) (Table 4). These two canonical variates give the highest correlation between BPRS dimensions and BE (Figure 2).

DISCUSSION

Our study showed that maximal correlation between BPRS and BE may be achieved with the weighted linear composite of negative schizophrenia symptoms and copper (Cu), lead (Pb), lithium (Li) and cobalt (Co). Positive symptoms were not correlated with BE. Those findings are in line with pyramidical model of schizophrenia which presumes that schizophrenic subtypes derive from a hybrid between unrelated but co-occurring dimensions of symptoms (Kay & Sevy 1990).

Table 4. Partial correlations of BE and BPRS dimensions with the 1st canonical variates adjusted for all confounders (n=67)

	1st canonical variates	
	BPRS	BE
BE		
Aluminium (Al)	-0.23	-0.27
Cadmium (Cd)	-0.25	-0.30
Cobalt (Co)	-0.35	-0.40
Copper (Cu)	-0.44	-0.51
Iron (Fe)	-0.03	-0.03
Lead (Pb)	-0.41	-0.48
Lithium (Li)	0.39	0.46
Magnesium (Mg)	-0.24	-0.28
Manganese (Mn)	-0.12	-0.14
Molybdenum (Mo)	-0.04	-0.05
Nickel (Ni)	-0.26	-0.30
Silicon (Si)	-0.25	-0.29
Sodium (Na)	-0.11	-0.13
Vanadium (V)	-0.08	-0.10
Zinc (Zn)	-0.20	-0.23
BPRS		
Positive	0.33	0.28
Negative	0.81	0.69
Affective	0.20	0.17
Activation	0.03	0.03
Resistance	-0.30	-0.25

Abbreviations: BPRS = Brief psychiatric rating scale five dimensions 1st canonical variate;
BE = bio-elements 1st canonical variate
Correlations were adjusted for age, sex, education, work status, body mass index (kg/m^2), smoking, alcohol consumption, hair dyeing, using a non-herbal shampoo, using a nutrition supplements, treatment with particular antipsychotics, benzodiazepines, mood stabilizers and antidepressants

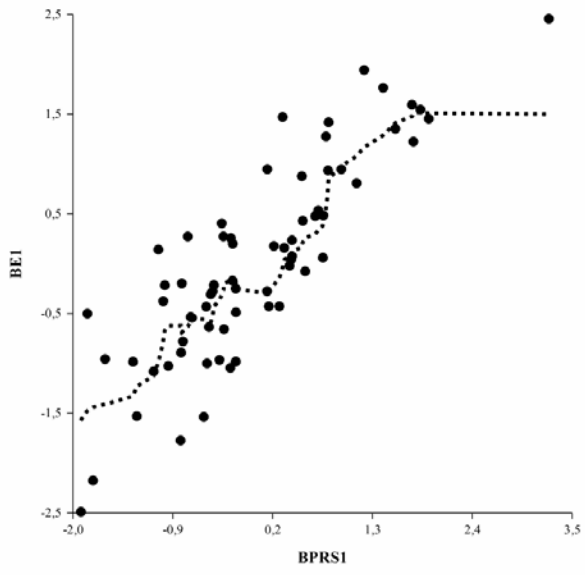

Figure 2. Score plot of the 1st BE and BPRS canonical variates; dotted line represents the moving median with 5 rows step

Our finding of negative correlation of copper (Cu) concentration with total BPRS and negative symptoms was in line with the findings of Wang 1988, Zhai 1990, Luo 1991. It was different to Rahman et al. 2009 and Connemann et al. 2010 finding that the concentration of copper (Cu) is increased in patients diagnosed with schizophrenia compared to the healthy population. Finally, in Tada et al. 1986 study no significant differences in copper (Cu) concentration was found between patients with schizophrenia and healthy control.

Our finding of negative correlation of lead (Pb) concentration with negative symptoms and resistance, as well as marginal negative correlation with positive symptoms and lack of resistance, is in line with findings from Zhai (1990), who detected lower lead (Pb) concentration in schizophrenic patients than in healthy controles. We observed the similar pattern in cases of barium (Ba), cadmium (Cd), copper (Cu), manganese (Mn), and nickel (Ni) but some of these correlations were not significant. Similarly, concentration of cadmium (Cd) was significant and negative with affective symptoms, and significant but positive with resistance.

While our findings show negative correlation of lithium (Li) concentration with negative symptoms and marginal correlation with positive symptoms and lack of resistance, in Zhai 1990 study no significant differences in lithium (Li) concentration were found between schizophrenic patients and control group.

We found negative correlation of cobalt (Co) concentration with negative symptoms and resistance, as well as marginal correlation with positive symptoms and lack of resistance. Interestingly, Tada et al. 1986 did not detect cobalt in patients with schizophrenia due to limitations of technology at disposal.

Future studies

Future studies should examine different schizophrenia types because eventual differences in concentrations of bioelements in different schizophrenia types may be yet another cause of noticed controversies in the results of previous studies. Future studies should try to assess the eventual causal relationships between BE and different schizophrenia symptoms. One step in this direction would be to examine the correlation of BE concentrations with the effectiveness of treatment of different symptoms.

Limitations of the study

Our study has several serious and several less serious limitations. First, although we tried to control different possible confounding factors we did not measure and control many others whose effect may be important, like: duration of pharmacological treatment and dosages of drugs used, concomitant somatic illnesses pharmacotherapies, everyday dietary habits, professional exposure, exposure in early childhood, geographically determined exposure, psychiatric comorbidities, etc. Second, our study was conducted in a large, specialized psychiatric hospital in a wealthy urban area, so the external validity of our findings may be imperfect for the small regional hospitals or other psychiatric settings. Third, as our data were collected in two centers, our results should not be generalized to the general Croatian population without caution. Fourth, both cross-sectional design of our study and canonical correlation as the main analysis prevented us from any causal inferences. Fifth, internal validity of our study might be jeopardized because of the wide age range while both BE concentrations and BPRS dimensions may be associated with age. Sixth, we used the five BPRS dimensions defined by Shafer meta-analysis and because of the sample size limitation we were not able to do a reliable factor or principal component analysis on our own sample. Seven, we have not controlled the seasonality while it may affect BE concentrations. Eight, BPRS was measured by only one physician for particular patient, and only two physicians in total. Therefore, we could not estimate the reliability of these estimations. Ninth, we did not validate our findings on the independent sample while the sample size we used was relatively low for the applied analysis and the number of variables examined.

CONCLUSION

Concentrations of different BE are associated with different psychopathological schizophrenia symptoms. Maximal correlation between BPRS and BE may be achieved with the weighted linear composite of negative schizophrenia symptoms and copper (Cu), lead (Pb), lithium (Li) and cobalt (Co). Considering all the limitations of this study, we hope that these findings could help to clarify the heterogeneity of schizophrenia and to illuminate the path toward syndrome-specific treatments.

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Contribution of individual authors:

Ivana Todorić Laidlaw: conception and design of the study; acquisition and analysis of data; drafting the manuscript and tables; writing the final version of the manuscript;

Ninoslav Mimica, Berislav Momčilović, Jasna Jurasović & Sandra Caratan: conception and design of the study, acquisition of data; drafting and writing the final version of the manuscript;

Igor Filipčić, Sandra Vuk Pisk & Žarko Bajić: data analysis, drafting the manuscript and table, writing the final version of the manuscript;

Stipe Drmić: conception and design of the study, analysis of data; drafting the manuscript and tables; writing the final version of the manuscript.

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