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Canonical analysis of concentrations of toxic metals in endometrium of women with gynecological disorders

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ABSTRACT

Objectives: Among the main adverse environmental factors, we usually distinguish the impact of heavy metals, especially Cd and Pb. Apart from the carcinogenic and toxic properties, their potential, stimulating estrogen receptors effect (metaloestrogens) is strongly emphasized; as well as participation in gene regulation mechanisms (epigenetic).
The aim of this study is to examine if there is a special scheme of concentrations of heavy metals accumulated in the female endometrium in certain pathologies: endometrial hyperplasia, endometrial cancer, endometrial polyps and miscarriages. The control group consisted of patients with abnormal uterine bleeding or functional bleeding with correct histopathological findings.

Material and methods: The study was performed on 92 women. Microwave induced plasma atomic emission spectrometry was used to assess metals concentrations in tissue material and the results of obtained concentrations were converted to µg/kg. The calculations were performed using discriminant and canonical analyses and revealed four discriminant functions.

Results: The results showed that metal's tissue concentrations vary in different types of histopathological diagnosis and the scheme of concentrations might be characteristic for analyzed diagnosis. Pb and Al has the most substantial impact on discrimination.

Conclusions: Endometrium may accumulate toxic metals such as: Pb, Cd, Ni, Mn, Cu, Zn, Al, Cr.
It can be assumed that there are characteristic distributions of toxic metals concentrations for individual histopathological diagnoses.

Key words: toxic metals; endometrium; metals scheme

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INTRODUCTION

There is an ongoing interest in analyses of various contaminants in biological material ranging from plasma, serum, hair, nails to soft and hard tissues. These analyses not only have certain value for basic research, but they can also serve as a biomarker of human exposure to environmental pollution or occupational risks [1]. Particular interest is given to their potential prognostic or diagnostic value.

So called "heavy metals" are one of the most studied groups of environmental contaminants with number of elements evidenced to display toxic effects on reproduction [2]. The contractual group includes non-essential toxic metals such cadmium (Cd) and lead (Pb) which can induce spontaneous abortion and have been implicated in fertility issues, endometriosis and reproductive cancer [3]. The other, *e.g.*, zinc (Zn), copper (Cu), manganese (Mn) and chromium (Cr) are pivotal for reproduction but can lead to harmful outcomes both, at deficiency and at increased levels [4, 5]. The additional attention is also paid to metalloids such as arsenic (As) and light metals such as aluminum (Al). The latter is ubiquitous in different foodstuffs and widely used in medicines [6]. It can exert metalloestrogenic action as observed *in vitro*, and lead to endocrine alterations evidenced *in vivo* [7, 8].

Continuous development of analytical procedures enables analyses of various biological material and determination of wide range of chemical elements. Apart from fluid samples, recently several studies directly assessing metal concentrations in tissues collected from women reproductive trait have been performed [9–13].

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The studies concerning the role of toxic metals in female human reproductive organs has been performed in our department since 2015. The aim of the present study was to use the results accumulated in our database on concentrations of selected metals in human endometrium (differentiated by histological conditions) and chorionic tissue in evaluation whether they could have any prognostic value [12, 13]. Therefore, statistical calculations based on discriminant and canonical analyses were performed.

MATERIAL AND METHODS

This paperwork is an additional statistical analysis of data largely already published. Thus, the calculations were performed retrospectively on previously partly published data [12, 13]. It included concentrations of lead (Pb), cadmium (Cd), nickel (Ni), manganese (Mn), copper (Cu), chromium (Cr), Zinc (Zn) and aluminum (Al) in human endometrium [11, 12]. The following groups were divided: normal endometrium (HP1, n = 28), endometrial hyperplasia (HP2, n = 15), endometrial cancer (HP3, n = 5), miscarriages (HP4, n = 19) and endometrial polyps (HP5, n = 25).

All patients have undergone curettage of the uterine cavity due to abnormal uterine bleeding, miscarriage or other suspicious changes such as endometrial hyperplasia or endometrial polyp. After that histopathological examination was conducted.

Microwave induced plasma atomic emission spectrometry was used to assess metals concentrations in tissue material and the results of obtained concentrations were converted to µg/kg.

The calculations were performed using discriminant and canonical analyses by Statistica 10.0 software (StatSoft, U.S.A.). Assumptions for the model concept were Tolerance: 0,01, Probability enter = 1, Probability removed = 0, Steps = 8. Canonical analyses revealed four discriminant functions. $p < 0.05$ was considered as statistically significant.

RESULTS

`The studies revealed different concentrations of metals in tissues with different histopathological diagnoses (Tab. 1).

When all metals were introduced to discriminant function analysis, Zn, Cd and Cr were found not to meet the model's concept and had the smallest contribution to the discrimination between groups of diagnosis. Therefore, these elements were excluded from further analyses. The discrimination of histopathological diagnosis by five metals that were included in the model (Cu, Pb, Ni, Mn and Al) was highly significant (Wilks' Lambda: 0,09580, $p < 0.05$). Pb and Ni have the smallest partial Wilks' Lambda of 0.37 and 0.53, respectively, thus the highest discriminant power for this model (Tab. 2). The high tolerance values were observed for Cu and Mn, indicating that 70 and 60% of information

Table 1. Concentrations of metals in tissue material [µg/kg]

	Endometrium without histopathological changes	Endometrial hyperplasia	Endometrial cancer	Miscarriages	Endometrial polyp
Cadmium	4.26	4.83	3.72	3.09	6.41
Lead	12.4	12.95	4.88	1.38	16.4
Nickel	19.24	6.61	5.43	3.4	11.19
Aluminum	11.34	12.19	8.61	6.73	20.46
Chromium	21.27	23.21	7.32	11.89	15.05
Zinc	24.56	8.66	7.17	12.81	13.74
Manganese	29.6	81.61	157.06	17.62	75.25
Copper	67.71	133.33	173.42	79.13	94.43

Table 2. Discriminant function analysis summary for variables: copper (Cu), lead (Pb), nickel (Ni), manganese (Mn), aluminum (Al) with histological diagnosis as a grouping variable

n = 47	Discriminant Function Analysis Summary. Grouping: 5 groups of diagnosis Wilks' Lambda: ,096 approx. F (20.126) = 6.5285 p < 0.0000					
	Wilks' Lambda	Partial Lambda	F-remove (4.38)	p value	Tolerance	1-Toler. (R-Sqr.)
Copper	0.15	0.63	5.62	< 0.01	0.72	0.28
Lead	0.26	0.37	15.95	< 0.001	0.33	0.67
Nickel	0.18	0.53	8.31	< 0.001	0.45	0.55
Manganese	0.15	0.63	5.67	< 0.01	0.61	0.39
Aluminum	0.13	0.75	3.09	< 0.05	0.49	0.51

Table 3. Statistical significance of the four discriminant functions (Root 1–4)

Roots removed	Chi-Square Tests with Successive Roots Removed					
	Eigen-value	Canonical R	Wilks' Lambda	Chi-Sqare	df	p value
1	2.81	0.86	0.10	96.16	20	< 0.001
2	0.95	0.70	0.36	41.35	12	< 0.001
3	0.26	0.46	0.71	13.99	6	< 0.05
4	0.11	0.32	0.90	4.41	2	> 0.05

df — discriminant functions

Table 4. Mean values of canonical variables in the groups of histopathological diagnoses for three statistically significant discriminant functions (Roots 1–3).

Group	Means of canonical variables		
	Root 1	Root 2	Root 3
HP1	1.16	0.24	0.79
HP2	–2.74	0.54	–0.15
HP3	0.19	–4.01	–0.34
HP4	1.21	0.30	–0.46
HP5	–1.15	–0.59	0.29

HP1 — endometrium without histopathological changes; HP2 — endometrial hyperplasia, HP3 — endometrial cancer; HP4 — miscarriages; HP5 — endometrial polyp

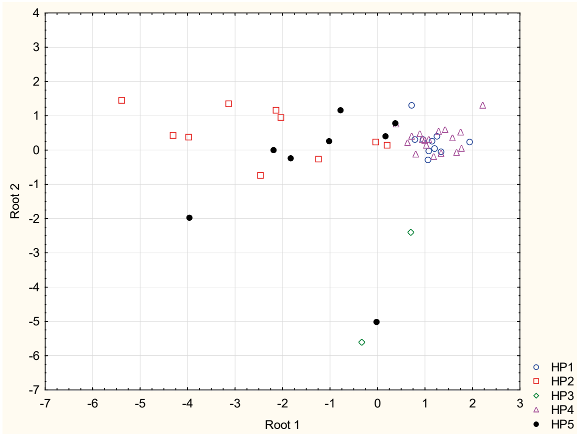

Figure 1. The configuration of points representing histopathological diagnosis in the system specified by the variable (axes) of discrimination; HP1 — endometrium without histopathological changes; HP2 — endometrial hyperplasia, HP3 — endometrial cancer; HP4 — miscarriages; HP5 — endometrial polyp

Table 5. Correlation coefficients (factor loadings) input variables with variables discriminatory

Variable	Factor structure matrix. Correlations variables — canonical roots		
	Root 1	Root 2	Root 3
Copper	–0.46	–0.60	0.39
Lead	–0.53	0.05	0.52
Nickel	0.02	0.01	0.88
Manganese	–0.15	–0.77	–0.02
Aluminum	–0.18	–0.14	0.31

brought by these variables, respectively, was not provided by the other variables included in the model (Tab. 2).

Then canonical analysis was performed to give four discriminant functions (Roots). Three of them were statistically significant ($p < 0.05$) (Tab. 3).

The first function discriminates most of all endometrial hyperplasia from the miscarriage group and endometrium without pathological changes and has the highest discriminant power (2.81). Furthermore, Pb and Cu play the most crucial role in this discrimination. The second function refers principally to endometrial cancers mainly based on Mn and Cu concentrations. The third one (with the lowest discriminant power and with the lowest statistical significance) distinguishes the miscarriage group and endometrium without pathological changes (Tab. 4 and 5).

Taking into account the whole studied group, different concentrations of metals have the cardinal impact on discrimination between histopathological diagnosis.

Pb had the greatest impact on classifying a tissue sample to histopathological diagnosis in case of endometrial hyperplasia, endometrium from miscarriages and endometrial polyps. In case of endometrial cancer such value was observed for Al (Tab. 6).

As indicated, 67% of tissue samples were correctly classified to their histopathological diagnosis groups, based on the discriminant analysis.

DISCUSSION

The present study has revealed using discriminant analysis that based on metals concentrations in tissue, it is possible to classify it sample to the histopathological diagnosis (67% corrected classified samples). It is suggested that there may be a different scheme of metals concentrations in order to reach a different histopathological diagnosis. These might

Table 6. Coefficients classification functions

Variable	Classification functions; grouping: histopathological diagnosis				
	HP1 p = 0.21277	HP2 p = 0.19149	HP3 p = 0.04255	HP4 p = 0.38298	HP5 p = 0.17021
Copper	0.03	0.08	0.08	0.02	0.06
Lead	0.04	0.96	–0.01	0.08	0.51
Nickel	0.06	–0.23	0.01	–0.01	–0.10
Manganese	–0.01	–0.02	0.04	–0.003	–0.004
Aluminum	0.01	–0.19	–0.21	–0.001	–0.07
Constant	–3.22	–12.46	–15.26	–1.32	–7.87

HP1 — endometrium without histopathological changes; HP2 — endometrial hyperplasia, HP3 — endometrial cancer; HP4 — miscarriages; HP5 — endometrial polyp

be primary or secondary changes. It is unknown whether concentration of metals in endometrium is primary findings that may cause tissue alteration or secondary accumulation due to histopathological lesions.

The first canonical function that discriminated mainly endometrial hyperplasia samples from endometrium without pathological changes and miscarriages group was based on Pb and Cu concentrations. Pb is considered as metaloestrogen — metal that can join estrogens receptors and stimulate it. It may have estrogenic properties, which are the main cause of endometrial hyperplasia [14].

There is evidence that also Cu may play a role in endometrial hyperplasia [15]. Chianeh and Rao suggest it may occur through vascular endothelial growth factor (VEGF) and endothelial nitric oxide synthase (eNOS) [16, 17]. It is pointed out that angiogenesis requires endothelial growth and differentiation.

Second canonical function referred to endometrial cancer samples is based on Mn and Cu. There are considerable findings revealing that oxygen stress is one of the most important causes of cancerogenic process [18, 19]. Mn and Cu are cofactors of stress oxygen dismutase (SOD) enzyme which takes part in defense mechanism, and this might be a reason that these two are so important in that discrimination. More studies provide extensive evidence that SOD activity is decreased in malignant cancers [20]. On the other hand, some authors suggest it depends on a type, stage and grade of a cancer. It is also assumed that SOD level may fluctuate during cancerogenesis [21, 22].

Taking the chart into consideration, HP5 and HP1 are almost in quite the same place, so these two groups are the most difficult to discriminate from each other based on metals concentrations. Assuming the chorion is created from endometrium, and it is new young tissue (till first 12 weeks of pregnancy), it may be concluded that it would differ from normal endometrium in the smallest extant. Other 3 groups are spread on the chart which indicates that the concentrations of toxic metals may be important.

All in all, it seems that the crucial metal in whole discrimination process is Pb while in case of endometrial cancer — Al. Pb is well known toxic and cancerogenic metal. It may play a decisive role also in endometrial cancerogenesis. Al, in turn, is considered as metalloestrogen and it seems it can stimulate for example, oestrogen receptors of MCF7 human breast cancer cells through the ligand binding or oestrogen-regulated reporter gene expression [23]. Additionally, the studies revealed that bladder and lung cancer is associated with aluminum smelting [24, 25].

In literature, there are evidence for specific, characteristic toxic metals concentrations in different types of diseases. Yu Yuan [26] discovered that five metals: titanium, arsenic, selenium, aluminum, and barium are significantly present in the blood of patients with coronary heart disease. Xiji Huang et al. [27] presented that asthma prevalence in adults is associated with urinary chromium, chromium, selenium, molybdenum, cadmium, and uranium concentration. Moreover, urinary lead, barium, iron, zinc, nickel, manganese and rubidium were negatively associated with asthma and interestingly when authors compare urinary metals in different subgroups, the associations of above 13 metals with asthma prevalence were nearly the same [27].

In our study, discriminant model enabled to classify 67 % of our samples to their correct histopathological diagnosis. It is possible that metals concentrations in blood, urine or tissue may serve us as a prognostic, additional examination in a diagnostic process.

Additional research with larger amounts of tissue samples is required to support our findings. Another limitation of the study is fact, that not all known toxic metals have been tested in the tissues, such as mercury. There may be more trace elements related to the metal's schemes.

CONCLUSIONS

1. Endometrium may accumulate toxic metals such as: Pb, Cd, Ni, Mn, Cu, Zn, Al, Cr.

2. Metal's scheme of concentrations varies in different types of histopathological diagnosis.
3. It can be assumed that there are characteristic distributions of toxic metals concentrations for individual histopathological diagnoses.
4. Among the metals taken into account, concentrations of Pb and Al has the biggest impact in canonical differentiation.

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Conflict of interest

All authors declare no conflict of interest.

REFERENCES

1. Rzymski P, Niedzielski P, Poniedziałek B, et al. Free-ranging domestic cats are characterized by increased metal content in reproductive tissues. Reprod Toxicol. 2015; 58: 54–60, doi: 10.1016/j.reprotox.2015.08.004, indexed in Pubmed: 26321139.
2. Rzymski P, Tomczyk K, Rzymski P, et al. Impact of heavy metals on the female reproductive system. Annals of Agricultural and Environmental Medicine. 2015; 22(2): 259–264, doi: 10.5604/12321966.1152077.
3. Zhao R, Wu Y, Zhao F, et al. The risk of missed abortion associated with the levels of tobacco, heavy metals and phthalate in hair of pregnant woman: A case control study in Chinese women. Medicine (Baltimore). 2017; 96(51): e9388, doi: 10.1097/MD.0000000000009388, indexed in Pubmed: 29390543.
4. Ajayi OO, Charles-Davies MA, Arinola OG. Progesterone, selected heavy metals and micronutrients in pregnant Nigerian women with a history of recurrent spontaneous abortion. Afr Health Sci. 2012; 12(2): 153–159, doi: 10.4314/ahs.v12i2.12, indexed in Pubmed: 23056021.
5. Apostoli P, Catalani S. Metal ions affecting reproduction and development. Met Ions Life Sci. 2011; 8: 263–303, indexed in Pubmed: 21473384.
6. Bratakos SM, Lazou AE, Bratakos MS, et al. Aluminium in food and daily dietary intake estimate in Greece. Food Addit Contam Part B Surveill. 2012; 5(1): 33–44, doi: 10.1080/19393210.2012.656289, indexed in Pubmed: 24779693.
7. Darbre PD. Metalloestrogens: an emerging class of inorganic xenoestrogens with potential to add to the oestrogenic burden of the human breast. J Appl Toxicol. 2006; 26(3): 191–197, doi: 10.1002/jat.1135, indexed in Pubmed: 16489580.
8. Wozniak M, Murias M. [Xenoestrogens: endocrine disrupting compounds]. Ginekol Pol. 2008; 79(11): 785–790, indexed in Pubmed: 19140503.
9. Rzymski P, Rzymski P, Tomczyk K, et al. Metal status in human endometrium: relation to cigarette smoking and histological lesions. Environ Res. 2014; 132: 328–333, doi: 10.1016/j.envres.2014.04.025, indexed in Pubmed: 24834829.
10. Tanrıkut E, Karaer A, Celik O, et al. Role of endometrial concentrations of heavy metals (cadmium, lead, mercury and arsenic) in the aetiology of unexplained infertility. Eur J Obstet Gynecol Reprod Biol. 2014; 179: 187––190, doi: 10.1016/j.ejogrb.2014.05.039, indexed in Pubmed: 24966000.
11. Guyot E, Solovyova Y, Tomkiewicz C, et al. Determination of Heavy Metal Concentrations in Normal and Pathological Human Endometrial Biopsies and In Vitro Regulation of Gene Expression by Metals in the Ishikawa and Hec-1b Endometrial Cell Line. PLoS One. 2015; 10(11): e0142590, doi: 10.1371/journal.pone.0142590, indexed in Pubmed: 26600472.
12. Rzymski P, Niedzielski P, Rzymski P, et al. Metal accumulation in the human uterus varies by pathology and smoking status. Fertil Steril. 2016; 105(6): 1511–1518.e3, doi: 10.1016/j.fertnstert.2016.02.006, indexed in Pubmed: 26921623.
13. Rzymski P, Niedzielski P, Poniedziałek B, et al. Identification of toxic metals in human embryonic tissues. Arch Med Sci. 2018; 14(2): 415–421, doi: 10.5114/aoms.2015.53915, indexed in Pubmed: 29593817.
14. Martin MB, Reiter R, Pham T, et al. Estrogen-like activity of metals in MCF-7 breast cancer cells. Endocrinology. 2003; 144(6): 2425–2436, doi: 10.1210/en.2002-221054, indexed in Pubmed: 12746304.
15. Kenneth M, Wells FM. Recent Advances in Histopathology. Advances in Endometrial Pathology. 2001; 19: 17–35.
16. Shizukuda Y, Tang S, Yokota R, et al. Vascular endothelial growth factor-induced endothelial cell migration and proliferation depend on a nitric oxide-mediated decrease in protein kinase Cdelta activity. Circ Res. 1999; 85(3): 247–256, doi: 10.1161/01.res.85.3.247, indexed in Pubmed: 10436167.
17. Chianeh YR, Rao P. Role of Copper and Vascular Endothelial Growth Factor (VEGF) on Endometrial Angiogenesis. JKIMSU. 2013; 2(2): 6–17.
18. Kulbacka J, Saczko J, Chwiłkowska A. [Oxidative stress in cells damage processes]. Pol Merkur Lekarski. 2009; 27(157): 44–47, indexed in Pubmed: 19650429.
19. Sun Yi. Free radicals, antioxidant enzymes, and carcinogenesis. Free Radic Biol Med. 1990; 8(6): 583–599, doi: 10.1016/0891-5849(90)90156-d.
20. Toyokuni S, Tanaka T, Nishiyama Y, et al. Induction of renal cell carcinoma in male Wistar rats treated with cupric nitrilotriacetate. Lab Invest. 1996; 75(2): 239–248, indexed in Pubmed: 8765324.
21. Jaruga P, Zastawny TH, Skokowski J, et al. Oxidative DNA base damage and antioxidant enzyme activities in human lung cancer. FEBS Lett. 1994; 341(1): 59–64, doi: 10.1016/0014-5793(94)80240-8, indexed in Pubmed: 8137923.
22. Sun Yi. Free radicals, antioxidant enzymes, and carcinogenesis. Free Radic Biol Med. 1990; 8(6): 583–599, doi: 10.1016/0891-5849(90)90156-d.
23. Darbre PD, Bakir A, Darbre PD, et al. Aluminium, antiperspirants and breast cancer. J Inorg Biochem. 2005; 99(9): 1912–1919, doi: 10.1016/j.jinorgbio.2005.06.001, indexed in Pubmed: 16045991.
24. Thériault G, Tremblay C, Cordier S, et al. Bladder cancer in the aluminium industry. Lancet. 1984; 1(8383): 947–950, doi: 10.1016/s0140-6736(84)92399-7, indexed in Pubmed: 6143877.
25. Andersen A, Dahlberg BE, Magnus K, et al. Risk of cancer in the Norwegian aluminium industry. Int J Cancer. 1982; 29(3): 295–298, doi: 10.1002/ijc.2910290312, indexed in Pubmed: 7068278.
26. Yuan Yu, Xiao Y, Feng W, et al. Plasma Metal Concentrations and Incident Coronary Heart Disease in Chinese Adults: The Dongfeng-Tongji Cohort. Environ Health Perspect. 2017; 125(10): 107007, doi: 10.1289/EHP1521, indexed in Pubmed: 29064788.
27. Huang X, Xie J, Cui X, et al. Association between Concentrations of Metals in Urine and Adult Asthma: A Case-Control Study in Wuhan, China. PLoS One. 2016; 11(5): e0155818, doi: 10.1371/journal.pone.0155818, indexed in Pubmed: 27191859.