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Plasma and urinary levels of lead and cadmium in patients with endometriosis

Antonio Schiattarella[1], Nicola Colacurci[1], Maddalena Morlando[1], Franco Pietro Ammaturo[1], Giuliana Genovese[2], Nadia Miraglia[2], Pasquale De Franciscis[1]

[1] Department of Woman, Child and General and Specialized Surgery, University of Campania "Luigi Vanvitelli"
[2] Occupational Medicine Area-Hygiene, Occupational and Forensic Section, Department of Experimental Medicine - University of Campania "Luigi Vanvitelli"

ABSTRACT

Purpose: An etiopathogenetic link between heavy metals and endometriosis has been demonstrated both in in-vivo and in-vitro studies, because of their ability to interfere with oestrogen action. The aim of this study was to analyse serum and urinary levels of lead and cadmium among patients suffering from endometriosis.

Material and methods: 126 patients undergoing laparoscopy for unexplained infertility were divided in three groups: 80 endometriosis-free women (group A), 22 patients with mild endometriosis (group B), 24 women with severe endometriosis (group C).

Main findings: significantly lower Pb plasma levels in group C compared to group B and controls, a non-statistically significant trend towards higher values of serum Cd levels in patients with endometriosis versus controls, no differences in urinary Cd levels among the studied groups, urinary Pb levels higher in group C versus group B and controls.

Conclusions: lower serum lead levels in patients with severe endometriosis compared to controls may indicate a metabolically active role of the endometriotic nodule.

Keywords: endocrine disruptors, heavy metals, lead, cadmium, endometriosis.

SOMMARIO

Obiettivo: studi in vivo e in vitro hanno dimostrato un legame eziopatogenetico tra l'endometriosi e i metalli pesanti, per la capacità di questi ultimi di interferire con l'azione degli estrogeni. Lo scopo di questo studio è quello di analizzare i livelli sierici e urinari di piombo e cadmio tra le pazienti con endometriosi.

Materiali e metodi: 126 pazienti sottoposte a laparoscopia per infertilità inspiegata sono state divise in tre gruppi: 80 donne senza endometriosi (gruppo A), 22 pazienti con endometriosi lieve (gruppo B) e 24 donne con endometriosi severa (gruppo C).

Risultati: i livelli plasmatici di piombo nel gruppo C sono risultati più bassi rispetto al gruppo B e ai controlli mentre i livelli sierici di cadmio hanno mostrato una tendenza verso valori più elevati, statisticamente non significativa, nelle pazienti con endometriosi rispetto ai controlli. Inoltre, nessuna differenza è stata riscontrata nei livelli di cadmio nelle urine per i gruppi in esame mentre i livelli di piombo urinario sono risultati più elevati nel gruppo C rispetto al gruppo B e ai controlli.

Conclusioni: i livelli inferiori di piombo sierico nelle pazienti con endometriosi severa rispetto ai controlli possono indicare un ruolo metabolicamente attivo del nodulo endometriosico.

Corresponding Author: Prof. Pasquale De Franciscis
pasquale.defranciscis@unicampania.it

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INTRODUCTION

Endometriosis is a chronic, estrogen-dependent disease characterized by the anomalous presence of endometrial glands and stroma outside the uterine cavity. It is a common gynaecological disorder as well as a major cause of infertility and chronic pelvic pain [1]. Even if several causes have been proposed [2], no single theory is able to explain all aspects of such a multifactored syndrome [3]. It is well recognized that disease onset and progression involve steroid-related mechanisms, including hormone-related changes of the endometrium and peritoneal cavity [4,5], excess estrogen production by ectopic endometriotic lesions, and alterations in ovarian steroidogenesis [6]. As a consequence, current medical treatments are based on ovarian suppressive agents [7-9].

Growing evidence suggests that endocrine disrupting chemicals (EDCs) may be etiologically involved in the development and severity of disease and may affect endometriosis risk because they are able to interfere with many aspect of oestrogen action, including their ability to alter hormone synthesis, modulate receptors, or act as agonists or antagonists [10]. The researches focusing on EDCs and endometriosis are focused on persistent chemicals, including dioxin-like compounds, organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs). Few and heterogeneous data are available regarding heavy metal, pollutants with high a global annual emission rate, not only resistant to decomposition in natural conditions, but may able to bioaccumulate and biomagnify in the food chains [11].

Despite directives and regulations mandating reductions and limits of their release, the environmental exposure to heavy metals is still high and occurs mainly via inhalation of contaminated air (or tobacco smoke) or consumption of contaminated food and water [12]. Although lead (Pb) in petrol has dramatically decreased over the last decades, it is still widely used in industrial branches and remains the most abundant metal on earth. Pb toxicity consists of inhibition of cellular enzymes, binding of sulfhydryl groups or dissociation of biological active metal ions from metalloenzymes [13]. Pb is a powerful disruptor of adrenal and ovarian steroidogenesis, inhibiting synthesis and activity of progesterone, 17-hydroxyprogesterone, 17,20-dihydroxyprogesterone, deoxycorticosterone, corticosterone and 21-deoxycortisol in a dose-dependent manner [14]. Interestingly, its effects on 17β-estradiol, testosterone and cortisol are biphasic, with stimulatory effects after low-levels exposure and inhibitory effects after high-level exposure: lead exposure results in inhibition of cytochrome P-450 aromatase activity and estrogen receptors in the granulosa cells of the ovarian follicles [15,16]. Cadmium (Cd) use is associated with refining of zinc, lead, and copper ores, mining, soil fertilizers, plastic stabilizers, pigment production, and nickel-cadmium battery production, other than tobacco consumption [17]. Cd may interfere with hormonal functions by binding at both the nuclear estrogen receptor [18] and G-protein coupled receptor 30 (GPR30) [19] and indirectly by P450 side-chain cleavage or through the low density lipoprotein receptor [20]. Experimentally, Cd has shown effects on estrogenic activity [18,21], including toxic effects to the ovary [21]. Together with estrogen, Cd increased estrogen receptor alpha mRNA expression beyond either estrogen or cadmium alone [20]. Indeed, different mechanisms are known to protect the human organism from the toxic effects of heavy metals such as the metallothioneins (MT I - II), synthesized by the liver, able to bind Cd and Pb ions [22].

Although experimental and laboratory data theorize a link between exposure to heavy metals and the pathogenesis of endometriosis, currently there are no clinical data for Pb and those concerning Cd are poor [23,24]. The purpose of the study was to evaluate the differences of blood and urinary levels of Pb and Cd between women with endometriosis and healthy controls.

MATERIALS AND METHODS

This was a diagnostic case control study of patients suffering from endometriosis and endometriosis-free subjects referred to the outpatient infertility clinic at one referral center. Local Institutional Review Board approved the study. Written informed consent was obtained by all patients. The study was conducted in accordance with principles of Helsinki Declaration. Inclusion criteria were age between 20 and 40 years, regular menstrual cycle (24 to 35 day), scheduled laparoscopy for infertility. Exclusion criteria were: hormonal therapy, in vitro fertilisation technique (IVF) and pelvic surgery within the previous 3 months, Body Mass Index (BMI) > 30 kg/m2, uterine myomatosis, endocrine and metabolic diseases, and any potential risk of exposure to heavy metals according to a specific questionnaire investigating the potential environmental and occupational exposure to lead and cadmium [25,26].

The questionnaire was divided into three parts:

a) Environmental exposure: this section collected information regarding the residence area of the patients (in terms of environmental pollution and traffic) and voluptuary habits, such as tobacco smoking and alcohol consumption, as well as the frequency of practicing hobbies involving the use of chemicals potentially containing BPA (boating and vessels maintenance, woodworking/bricolage/decoupage, gardening and agricultural products cultivation). The diet (fish consumption) and the frequency of using canned food and plastic containers were also investigated;

b) Occupational exposure: this section inquired current and previous working activity and seniority, the use of Personal Protective Equipments, the occurrence of occupational (no injury) accidents potentially involving BPA exposure (e.g. chemicals spillage);

c) Health State (part of this section was compiled by Authors): information about current and previous pathologies, clinical-instrumental diagnosis of endometriosis, surgical interventions, hormonal therapies, etc. were registered.

Laparoscopy was performed in the proliferative phase of the cycle. Endometriosis was staged according to the revised classification of the American Fertility Society (rAFS classification). To measure Pb and Cd concentrations, all patients were asked to provide a urine and blood samples before laparoscopy. A K2EDTA Vacutainer® 5 mL tube was used for Pb determination and BD Vacutainer® tube was used for Cd determination, samples were stored at the temperature of -20°C until analysis. Urine sample was collected in disposable containers and a 10 ml aliquot was immediately transferred into a glass test tube and kept in the fridge at -20°C until analysis. For blood sample, 1.5 mL of blood was transferred into 1.5 ml Eppendorf Tubes® and the remaining blood was centrifuged at 3.000 rpm for 10 min at room temperature (20-25°C) and then the supernatant (plasma) was collected. The two samples were stored at -20°C before analysis. All biological samples were transported to the laboratory in thermal bags within 4 h from collection and analysed within a month. Serum concentrations of metals were determined by flame atomic double beam absorption spectrometry with graphite furnace (Analyst 800, PerkinElmer, Italy). The serum samples were 1:10-diluted with nitric acid 0,2% to determine Cd level and blood samples were 1:20-diluted with Triton X-100 0,1% to identify Pb level. Statistical analysis was made using SPSS for Windows (version 15.0, SPSS, Chicago, IL). Data were shown as means ± standard deviation. Comparisons between the two groups were assessed with Student's t test. A p value <0.05 was considered statistically significant.

RESULTS

One hundred eighty-five women from the Campania area in Southern Italy were recruited for the study. Once the exclusion criteria were applied, 126 participants were enrolled. According to laparoscopic outcomes patients were divided in three groups, homogeneous for clinical characteristics (**table I**): 80 women (mean age 36.1 ± 9.4 years) with no evidence endometriosis and of other pelvic pathology (group A: control group), 22 women (mean age 31.6 ± 6.3) with a histologically confirmed diagnosis of endometriosis stage I-II (minimal/mild endometriosis) (group B), 24 women (median age 35.3 ± 6.7) with a histologically confirmed diagnosis of endometriosis stage III-IV (moderate/severe endometriosis) (group C). We observed significantly lower Pb plasma levels in controls and group B compared to group B, with slightly increased values in group A versus group B.

Table 1.
Characteristics of women in the study

	Age	BMI	Serum estradiol level (pg/mL)
Group A (n=80)	36.1 ± 9.4	24.1 ± 3.5	62.1 ± 15.5
Group B (n=22)	31.6 ± 6.3	25.1 ± 2.6	68.2 ± 13.4
Group C (n=24)	35.3 ± 6.7	24.5 ± 2.9	67.3 ± 14.3

Otherwise, serum Cd levels showed a non-statistically significant trend towards higher values in patients with endometriosis versus controls, especially in those with the most severe forms (group C). As regards urinary Cd levels there were no statistically significant differences among three groups of patients; urinary Pb levels were higher in patients of group C than patients of group B and controls. Results are shown in **table II**.

Table II.
Urinary and serum levels of Pb and Cd

	Group A	Group B	Group C
Pb S	9,33 ± 2,63*	8,10 ± 3,28*	5,22 ± 3,76
Pb U	2,03 ± 1,01	3,01 ± 1,32	5,73 ± 2,93**
Cd S	0,85 ± 0,57	0,97 ± 0,20	1,42 ± 0,28
Cd U	1,92 ± 0,97	1,60 ± 0,52	2,64 ± 0,50

* p < 0.05 vs endometriosis stage III-IV
** p < 0.05 vs endometriosis stage I-II and controls

DISCUSSION

An etiopathogenetic link between heavy metals and endometriosis has been demonstrated both in in-vivo and in-vitro studies [14–16,18–21,17]. Although limited by the small samples and the wide variability of values, our patients were free from potential bias such as dietary, environmental, professional and voluntary exposure to heavy metals; therefore, they may be useful for interpreting the relationship between endocrine disruptors and endometriosis. Regarding the data about Pb, the tendency towards lower values found in women affected by endometriosis is in contrast with a causal link with the pathology. In this regard, it is possible to provide two hypotheses. Firstly, our experience could reflect a condition of limited exposure and therefore different from that of the 1980s when an etiopathogenetic link between lead and endometriosis was hypothesized. Indeed, since 1992 lead pollution has decreased by means of regulations limiting the concentrations of the metal allowed in the industries and its elimination in gasoline used for automotive use [27]; accordingly, our findings show lower Pb levels than those of 30 years ago, even when compared to European reference values in a non-professionally exposed population (blood Pb 70 µg/l) [28]. However, our data reflect a condition of exposure limited to areas of origin of the studied population; therefore, multicentric studies should be performed in order to analyse different exposure conditions. As secondary hypothesis, a heavy metal accumulation in the endometriotic lesions can be theorized based on the model of its tissue distribution kinetic. In fact, after the penetration of Pb into the organism by the gastrointestinal tract or by inhalation, it enters into the bloodstream, before at plasma level and then quickly penetrate into erythrocytes, linking hemoglobin and other cellular components, until the equilibrium is established about at 97-99% [29]. Because of plasmatic free fraction the initial tissue distribution of the metal is 60% in the bone tissue, 25% in the hepatic tissue and 4% in the kidney, then a redistribution occurs with amount in the bones up to 90%. In fact, while half-life of metal in the blood is about two months with a steady-state reached within 6 months, in the bone it is about 20-30 years [29]. It can be hypothesized that endometriotic lesions could participate (like other tissues) to the intracellular compartmentalization of Pb according to the extent of the disease; therefore even in the presence of blood levels lower than healthy controls, an indirect etiopathogenetic link between the pathology and exposure to lead may not be excluded. Moreover, since endometriotic lesions in the early stages of the disease are metabolically active and able not only to absorb micro and macromolecules but also to produce numerous substances [30], they could be associated with higher serum Pb level than in advanced endometriosis lesions.

As regards Cd, the trend towards higher values found in women with endometriosis, especially in advanced stages, suggests a causal relationship with the disease according to a mechanism of endocrine disruption. Accordingly, previous laboratory studies showed that higher blood levels are correlated with a higher proestrogenic activity due to the ability of Cd to increase the transcription levels of the genes through estrogen receptor activation [31]. In our study, Cd levels in endometriosis stage III-IV are higher than for non-occupational exposure (blood Cd 1 µg/l). Heterogeneity in the urinary and serum Cd findings may in part be explained by the different biologic media, given that blood Cd is reflective of recent exposures (weeks), whereas urinary Cd is representative of long term exposure [17]. Specifically, Cd in urine represents cumulative exposure in the renal cortex of the kidney [32] generally reflective of exposure over a decade, although there are some recent concerns about the interpretation of urine cd concentrations at low-moderate levels [33].

Bias of our data have to be underlined: Pb concentrations below the limit of detection (LOD) in 26 samples (4 in group A, 5 in group B, 17 in group C), Cd concentrations below the LOD in 36 samples (3 in group A, 1 in group B, 33 in group C), and the possibility of undiagnosed endometriosis among controls, even if the prevalence of undiagnosed disease is likely to be small. Further studies are needed to demonstrate the relationship between the pathology and exposure to heavy metals, in order to reduce many methodologic biases. These include the difficulty to distinguish between ubiquitous exposure and occupational exposure, possible exposure combined with different classes of EDCs, the absence of biomarkers, the limited sample sizes, the difficulty to characterize and evaluate the individual risk of exposure to endocrine disruptor [11]. The final challenge should be to prevent the disease, assess the toxicological risk, have an epidemiological picture of environmental pollution, develop exposition biomarkers, and identify metabolic conditions and genetic polymorphisms as potential susceptibility factors in the population to the effects of specific EDCs.

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