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Role of endometrial concentrations of heavy metals (cadmium, lead, mercury and arsenic) in the aetiology of unexplained infertility

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ABSTRACT

Objective: To determine the role of endometrial concentrations of heavy metals (cadmium, lead, mercury and arsenic) in the aetiology of unexplained infertility.
Study design: Thirty-three women with unexplained infertility and 32 fertile women were recruited. Endometrial biopsies were collected during the putative window of implantation (cycle days 20–24). The concentrations of cadmium, lead, mercury and arsenic were measured in endometrial biopsy specimens using atomic absorption spectrometry.
Results: Cadmium was detected in 91% (30/33) of women with unexplained infertility, compared with 34% (11/32) of fertile women. The median endometrial cadmium concentration was 19.58 (interquartile range 1.46–30.23) μg/l in women with unexplained infertility, compared with 0.00 (interquartile range 0.00–0.40) μg/l in fertile women. Lead was detected in 15% (5/33) of women with unexplained infertility and 3% (1/32) of fertile women. Mercury and arsenic were not detected in any endometrial samples from either group.
Conclusion: A significant difference in endometrial cadmium concentration was found between women with unexplained infertility and fertile women. This suggests that cadmium may be a contributing factor in the aetiology of unexplained infertility.

Introduction

Infertility is defined as failure to become pregnant for 12 months or more despite regular unprotected sexual intercourse [1]. It has been estimated that 15% of couples suffer from infertility worldwide [2]. Despite recent advances in diagnostic modalities, the aetiology of many cases of infertility remains unknown [3]. According to the World Health Organization, approximately 25% of couples are diagnosed with unexplained infertility [4].

As a consequence of global increases in industrial pollution over the last century, all living organisms are exposed to environmental pollutants such as heavy metals, organic hydrocarbons and pesticides. Cadmium, lead, mercury and arsenic are widely distributed in the environment and are known to be reproductive toxicants [5]. A growing body of evidence suggests that environmental factors have several adverse effects on reproductive health, including defective steroidogenesis, impaired semen quality, suppressed oocyte maturation, ovulation failure, defective implantation, miscarriage and birth defects [6,7].

Embryo implantation requires a delicate dialogue between the embryo and the mother, and a receptive endometrium is a prerequisite [8]. Reduced endometrial receptivity has been reported in an increasing number of women with unexplained infertility [9]. Therefore, an inadequate endometrium can be considered as a main fertility-determining factor [10]. Although various factors, including environmental pollutants, have been identified as possible causes of infertility [6], their effects on endometrial structure and function, including implantation, have received little research attention. A few studies have investigated the direct effect of cadmium on endometrial cells. An animal study found that intra-uterine instillation of cadmium inhibited the onset of implantation without affecting ovulation [11]. Tsutsumi et al. [12] reported that cadmium stimulates decidualization of the endometrium, and may disrupt the endometrial environment. Heavy metals have also been shown to promote the production of reactive oxygen species (ROS), resulting in increased lipid peroxidation, damaged DNA, altered gene expression, distributed membrane and apoptosis [13]. Increased ROS activity and apoptosis have an adverse effect on endometrial receptivity

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and embryo implantation [14]. As a consequence of these adverse effects, heavy metals may play a role in the pathophysiology of unexplained infertility.

The aim of this study was to explore the relationship between endometrial concentrations of the most ubiquitous heavy metal toxicants (lead, cadmium, mercury and arsenic) and unexplained infertility.

Materials and methods

Subjects

Thirty-three women with unexplained infertility who attended the Department of Obstetrics and Gynaecology, Inonu University, School of Medicine between January 2011 and November 2012 were recruited into this study. Women were considered to be infertile if they had not conceived after 12 months of contraceptive-free regular intercourse. Thirty-two women with at least one liveborn infant and no history of infertility or spontaneous abortion were enrolled from an unselected population as the control group over the same period.

The study protocol was approved by the Ethical Review Board of Inonu University, School of Medicine, and was designed and performed in accordance with the Declaration of Helsinki. All patients provided signed informed consent for participation in this study.

All study subjects were required to be aged between 18 and 40 years and non-smokers. Women who smoked, drank alcohol, had used an intra-uterine device at some time in their life, and had taken any hormonal medication within the previous 3 months were excluded from the study. None of the women had diabetes mellitus, metabolic disease, malabsorption syndrome or clinical evidence (history and examination) of recent or current infection, and none of the patients had undergone any gynaecological surgery (except diagnostic laparoscopy).

After completion of a detailed questionnaire regarding demographic and socio-economic data, all infertile couples underwent a full infertility investigation [15]. Men underwent at least two semen analyses according to the World Health Organization criteria [16], and serum levels of testosterone, luteinizing hormone and follicle-stimulating hormone (FSH) were measured. Women underwent hormonal assessment to evaluate their ovulatory cycles, thyroid function, and circulating levels of prolactin and androgen. Ovarian reserve was analysed using serum FSH level and antral follicle count on the third day of the menstrual cycle. Resumption of ovulation was defined as midluteal progesterone level >5 ng/ml, and fallopian tubal patency was assessed by hysterosalpingography. One couple with abnormal results for the aforementioned tests was excluded from this study. The remaining infertile couples, who had normal results to the diagnostic protocol, were considered to have unexplained infertility.

Measurements of metal concentrations

Endometrial samples were collected using a Pipelle curette during the putative window of implantation (cycle days 20–24) after 20 days of sexual abstinence. Samples were stored in an Eppendorf tube at −80 °C until assay. Wet tissue (150 mg) was placed in a propylene tube, and reagent-grade nitric acid:hydrochloric acid (1:1 mixture) was added. The mixture was digested in a water bath at 90 °C for 2 h. Samples were diluted to 10 ml with deionized water. An atomic absorption spectrophotometer (PerkinElmer AAnalyst 800, PerkinElmer Inc., Waltham, MA, USA) was used to determine the endometrial concentrations of cadmium, lead, mercury and arsenic. Detection limits were 0.01, 0.01, 0.6 and 0.05 μg/l, respectively. The correlation coefficients for the calibration curves of cadmium and lead were 0.994429 and 0.999171, respectively. The laboratory was blinded to subjects' clinical data, and two replicates were performed per sample.

Statistical analysis

Data were stored and analysed using Statistical Package for Social Sciences Version 11.0 (SPSS Inc., Chicago, IL, USA). A sample size calculation was performed with a significance level (alpha) of 0.05 and power (% chance of detecting) of 80%. After analysis, a sample size of 60 (30 per group) was required. Normality of the distribution was assessed using the Kolmogorov–Smirnov test. Variables with a skewed distribution were presented as median and interquartile range (IQR), and variables with a normal distribution were presented as mean ± standard deviation (SD). Chi-squared test with Yates' correction and Fisher's exact test were used as indicators. Groups were compared using Student's *t*-test for parametric data and the Mann–Whitney *U*-test for non-parametric data. Odds ratios (OR) and 95% confidence intervals (CI) were used to describe the association between unexplained infertility and heavy metals. In addition, age and body mass index were adjusted for multivariate analysis. $p < 0.05$ was considered to indicate statistical significance.

Results

The demographic features and clinical characteristics of women with unexplained infertility and fertile controls are presented in Table 1. None of the women had a history of occupational exposure to cadmium, lead, mercury or arsenic. There were no differences in educational status or family income between the groups. Smoking habits of male partners were similar in the two groups.

Cadmium was detected in 91% (30/33) of women with unexplained infertility, compared with 34% (11/32) of fertile women. Detection of cadmium in an endometrial biopsy sample was associated with 19-fold higher risk for unexplained infertility (crude OR 19.1, 95% CI 4.7–76.9) ($p < 0.001$ (Table 2). The association persisted after adjusment for age and body mass index (adjusted OR 18.9, 95% CI 4.5–79.9) ($p < 0.001$). The median endometrial cadmium concentration was 19.58 (IQR 1.46–30.23) μg/l in women with unexplained infertility, compared with 0.00 (IQR 0.00–0.40) μg/l in fertile women ($p < 0.001$) (Fig. 1).

Lead was detected in 15% (5/33) of women with unexplained infertility, compared with 3% (1/32) of fertile women ($p = 0.33$) (Table 2). Lead concentrations were 0.12, 0.12, 0.24, 0.29 and 0.32 μg/l per 150 mg endometrial tissue in the five women with unexplained infertility, compared with 0.03 μg/l per 150 mg endometrial tissue in the fertile woman. Mercury and arsenic were not detected in any endometrial samples from either group.

Comments

This study found a significant difference in the endometrial cadmium concentration between women with unexplained infertility and fertile women. The endometrial lead concentration did not differ significantly between the two groups. Mercury and arsenic were not detected in any endometrial samples from either group.

Several studies have reported an association between cadmium and both male and female infertility [17,18]. A recent study showed that environmentally relevant levels of cadmium and lead are associated with modest changes in reproductive hormone levels [19]. In a prospective cohort study, Buck Louis et al. [20] found that heavy metal exposure (i.e., cadmium and lead) is associated with a longer duration to conceive. Furthermore, a study conducted on hospital samples of the general population with infertility found

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