Elevated levels of whole blood nickel in a group of Sri Lankan women with endometriosis: a case control study

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Abstract

Background: Endometriosis is characterized by the persistence of endometrial tissue in ectopic sites outside the uterine cavity. Presence of nickel, cadmium and lead in ectopic endometrial tissue has been reported previously. While any association between blood levels of nickel and endometriosis is yet to be described in literature, conflicting reports are available with regards to cadmium and lead levels in blood and urine.

Findings: In fifty patients with endometriosis and fifty age-matched controls confirmed by laparoscopy or laparotomy, whole blood samples were collected and digested using supra pure 65% HNO3. Whole blood levels of nickel and lead were measured using Total Reflection X-ray Fluorescence (TXRF) while cadmium levels were evaluated using graphite furnace atomic absorption spectroscopy (GFAAS). Women with endometriosis had significantly higher (P=0.016) geometric mean (95% CI) whole blood nickel levels [2.6(1.9-3.3) μg/L] as compared to women without endometriosis [0.8 (0.7-0.9) μg/L]. Whole blood levels of cadmium and lead were similar between the two groups.

Conclusions: Although women with endometriosis in this study population had higher levels of nickel in whole blood compared to controls, whether nickel could be considered as an aetiological factor in endometriosis remains inconclusive in view of the smaller sample that was evaluated.

Keywords: Endometriosis, Metalloestrogens, Nickel
the hematogenous route (blood) as a possible source of metals in the ectopic endometrial tissue.

The present study was conducted with the objective of exploring further the association between endometriosis and whole blood levels of cadmium, lead and nickel in the same group of women with endometriosis who were previously studied. We compared the whole blood levels of the cadmium, lead and nickel in women having endometriosis (in whom these metals were detected in ectopic endometrial tissue -cases) with the blood levels of same metals in women who had no evidence of endometriosis (controls).

**Methods**

This case control study was conducted among women of the reproductive age group, at the Professorial Gynecology Unit of the National Hospital, Colombo, Sri Lanka. Patients awaiting elective laparotomy or laparoscopy for diagnostic and/or treatment purposes were included. Those who were diagnosed visually as having endometriosis subsequent to laparotomy or laparoscopy were selected as cases (n=50). Apart from the ectopic endometrial tissue sample that was collected as described in the previous study [11], simultaneous venous blood sample was collected from each case. Women matched for age in whom endometriosis had been excluded by laparoscopy or laparotomy were recruited as controls (n=50). Indications for laparoscopy or laparotomy in controls were subfertility, dysmenorrhea, chronic pelvic pain or detection of an ovarian mass in ultrasound scan.

A sample of venous blood was collected from all participants during intravenous cannulation at the time of induction of anesthesia into polypropylene tubes containing ethylene diamine tetraacetic acid (EDTA). Pre-operatively, informed written consent was obtained from all the participants. All the blood samples were stored in a −20°C freezer until analysis.

Whole blood samples were digested using a protocol described previously [14] with some modifications. Briefly, matched blood of cases and controls were allowed to reach the room temperature. Then whole blood samples were transferred to pre-treated, acid washed glass beakers. The wet weight of each sample was determined using a chemical balance. Each sample was prepared in duplicate. All the blood samples were digested using 65% supra pure Nitric acid (HNO₃, Merck, USA) while maintaining a uniform temperature. The final solution was made in 2mL of 5% HNO₃ that was prepared by diluting the 65% supra pure HNO₃ with double distilled deionized water.

The total-reflection X-ray fluorescence (TXRF) machine available at the Atomic Energy Authority (AEA) of Sri Lanka was utilized to measure metals apart from cadmium. TXRF is a multi element analysis technique [15] capable of detecting an array of elements at detection levels of picograms per liter (pg/L) [15]. An internal standard, Gallium, allows quantification of metals using the Axil software. However, the TXRF that was used had an inherent weakness in measuring cadmium since it had a Molybdenum x-ray tube. Therefore, we used atomic absorption spectroscopy (model GBC 933AA) together with a graphite furnace (model GBC GF 3000) available at the Institute of Fundamental Studies (IFS), Kandy, Sri Lanka to estimate cadmium levels as described elsewhere[16]. Both AEA and IFS [16] are national level apex institutions in Sri Lanka that have international certifications for trace element analysis where regular quality assurance programs are conducted.

Quality control and validation were performed using reference material supplied by the International Atomic Energy Authority (IAEA-A-13), Seronorm™ trace elements in whole blood levels 1 (MR 4210) and National Institute of Standards & Technology (NIST) Gaithersburg, USA water sample with trace elements (SRM 1643e). For 96% of the determinations, repeatability error did not exceed 10%. The detection limits for nickel, lead and cadmium in whole blood were as follows 0.05, 1.0, 0.01 μg/L. The precision for nickel, lead and cadmium in the range of the samples analyzed in this study was + 2, 3 and 6%, respectively.

SPSS version 13 for Windows was used for statistical analysis. Log transformation of metal levels was done and means were compared using t-test.

Ethical clearance was obtained from the Ethical Review Committees of the Faculty of Medical Sciences, University of Sri Jayewardenepura and the National Hospital of Sri Lanka.

**Results and discussion**

Mean (±SD) age in cases and controls were 33.0 (±5.4) and 32.7 (±5.4) years respectively. Cases and controls were similar in body mass index while none of the women who participated in the study were current smokers. Other demographic, biological and dietary characteristics of this group of women with endometriosis have been described previously [17].

Cadmium, lead and nickel were detected in whole blood of all the participants. The whole blood nickel levels in cases were significantly higher compared to controls. Cases had lower whole blood cadmium levels and higher

| Metalloestrogen | Cases (n=50) | Controls (n=50) | P value† |
|-----------------|-------------|----------------|---------|
| Nickel          | 2.6 (1.9-3.3)| 0.8 (0.7-0.9)  | 0.016   |
| Lead            | 11.0 (8.6-13.3)| 6.9 (5.7-8.0)  | 0.389   |
| Cadmium         | 0.7 (0.7-0.9)| 0.8 (0.6-1.0)  | 0.423   |

Data expressed as geometric mean (95% CI).
† t-test between blood levels of cases and controls.
the available scientific evidence for occupational and environmental exposure of women to metals including nickel, future research would prove to be invaluable in further exploring the association between nickel and endometriosis.

**Abbreviations**

AEA: Atomic Energy Authority; EDTA: Ethylene diamine tetracetic acid; IFS: Institute of Fundamental Studies; IAEA: International Atomic Energy Authority; NIST: National Institute of Standards & Technology; TXRF: Total-reflection X-ray fluorescence.

**Competing interests**

Authors declare that they have no competing interests.

**Authors’ contributions**

NS carried out the sample collection, analysis and drafted the manuscript. HS identified the study participants and carried out the surgical procedures. VW assisted in the TXRF analysis of the samples and interpreted the data. All authors read and approved the final manuscript.

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