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Association between endometriosis and heavy metals in a group of Sri Lankan women of reproductive age

by

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Thesis submitted to the University of Sri Jayewardenepura for the award of the Degree of Doctor of Philosophy in Physiology on "Association between endometriosis and heavy metals in a group of Sri Lankan women of reproductive age", on 12th December 2012.

I certify that the candidate has incorporated all corrections, amendments and additions recommended by the examiners.

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The work described in this thesis was carried out by me under the supervision of Prof. Sharaine Fernando of University of Sri Jayewardenepura, Prof. Hemantha Senanayake and Prof. Kamani Tennekoon of University of Colombo, Dr. Roshini Peiris-John of University of Auckland, New Zealand and Prof. Rajitha Wickramasinghe of University of Kelaniya and a report on this has not been submitted in whole or in part to any University or any other institution for another Degree / Diploma.

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We certify that the above statement made by the candidate is true and that this thesis is suitable for submission to the University for the purpose of evaluation.

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LIST OF ABBREVIATIONS

AKT - Threonine/serine kinases

AP-1 - Activating Protein-1

As - Arsenic

Au - Gold

Bcl-2 - B-Cell lymphoma/leukaemia-2

BLyS - B lymphocyte stimulator

Ca - Calcium

CAM - Chicken chorioallantoic membrane

Cd - Cadmium

$CdCl_2$ - Cadmium chloride

CEA - Central Environmental Authority

Co - Cobalt

Cr - Chromium

Cu - Copper

E_1 - Oestrone

E_2 - 17β-oestradiol

E_3 - Oestriol

ECM - Extracellular matrix

EGFR - Epidermal growth factor receptor

ER - Oestrogen receptor

ERα - Oestrogen receptor alpha

ERβ - Oestrogen receptor beta

ERE - Oestrogen response elements

ERK - Extra cellular signal-regulated kinases

Fe - Iron

FSH - Follicular stimulating hormone

GnRH - Gonadotropin releasing hormone

Hg - Mercury

HPO - Hypothalamo-pituitary-ovarian axis

IHC - Immonohistochemistry

IL - Interleukin

K - Potassium

mER - Membrane oestrogen receptor

MAPK - Mitogen-activated protein kinase

MMP - Matrix metalloproteinase

NK - Natural killer

Ni - Nickel

NF-κβ - Nuclear Factor-κβ

NSAID - Nonsteroidal antiinflammatory drug

OD - Optical density

L1 CAM - L1 cell adhesion molecule

LH - Luteinizing hormone

P - Phosphorus

Pb - Lead

PF - Peritoneal fluid

PMC - Peritoneal mesothelial cell

PR - Progesterone receptor

PTWI - Provisional tolerable weekly intake

S - Sulphur

SERM - Selective oestrogen receptor modulator

Sn - Tin

TFF 1 - Trefoil factor 1

TIMP - Tissue inhibitors of matrix metalloproteinase

TNFα - Tumour necrosis factor α

TSP-1 - Thrombospondin-1

TXRF - Total-reflection X-ray Fluorescence

WHO - World Health Organization

uPA - Urokinase plasminogen activator

UK - United Kingdom

US - United States

UV - Ultraviolet

VEGF - Vascular endothelial growth factor

Zn - Zinc

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ABSTRACT

Heavy metals such as cadmium, nickel and lead activate the oestrogen receptor (ER) to exert oestrogenic effects; thus implicated in the aetiology of endometriosis an oestrogen dependant disease. A purpoted role of heavy metals in the continuation of ectopic endometrial tissue could be hypothesized.

The objectives of this study were to compare the demographic, biological, exposure status of heavy metals, blood levels of heavy metals as well as the effects of heavy metals on endometrial stromal cell cultures between women with endometriosis and women with no evidence of the disease. In addition, presence of heavy metal in ectopic endometrial tissue was investigated.

In this case-control study women with endometriosis (n=150) were compared with age-matched controls (n=150) with no evidence of endometriosis, both confirmed by laparoscopy or laparotomy. A pre tested interviewer-administered questionnaire was used for data collection. In a sub population of women (n=50 in each group), venous blood samples and ectopic endometrial tissue samples were obtained and digested with supra pure 65% HNO_3. Blood and tissue samples were analyzed for heavy metals using Total-reflection X-ray Fluorescence and graphite furnace atomic absorption spectroscopy. From both groups (n=10 in each group) eutopic endometrial samples were obtained to isolate endometrial stromal cells (ESC). Primary ESC cultures were established in RPMI medium and the cultures in the third passage were treated with Cd, Pb and Ni at concentrations of 10^{-6} M, 10^{-9} M and 10^{-9} M respectively. At 24 h and 48 h

the relative cell proliferation was calculated using the Neubauer haemocytometer, while Progesterone receptor (PR) and ER expression were assessed by immunohistochemistry. SRB and MTT assays were performed following 48 hours of exposure of ESC cultures of both groups to Cd at concentrations of 10^{-8} M to 10^{-3} M.

Chi square tests and odds ratios (OR) (McNemar's) were used to determine associations between dichotomous variables. Conditional logistic regression analysis was done to adjust for potential confounding variables. Log transformed blood levels of metals were compared using paired t-test while blood and tissue metal levels were correlated using the spearman rank correlation. ANOVA was used to analyze relative cell proliferation, SRB and MTT results.

The risk of endometriosis was 2.084 times greater among those with an education above Advanced Level as compared to those having an education below Advanced Level (p=0.021) after adjusting for confounding factors. Endometriosis was commoner among women living close to a main road (OR=1.694; 95 % CI=1.037-2.767) (unadjusted) as compared to the respective reference group. Women with endometriosis had significantly higher (P=0·011) geometric mean (95% CI) blood nickel levels [2.65 (1.94-3.38) µg/L] as compared to women without endometriosis [0.80 (0.70-0.90) µg/L]. Blood levels [women with vs. without] of cadmium [0.84 (0.17-1.98) vs 0.85 (0.26-1.45) µg/L] and lead [11.09 (0.67-13.36) vs 6.90 (1.72-8.09) µg/L] were similar in the two groups (p=0·289 and p=0·123 respectively). The geometric mean (95% CI) levels of cadmium, nickel and lead detected in the ectopic endometrial tissue of women with endometriosis were 2.86 (2.13-3.60), 17.55 (13.15-21.94) and 25.78 (18.49-33.08) µg/Kg respectively with no significant correlation between blood and tissue levels of

cadmium, nickel or lead. Cd treatment increased the relative proliferation in ESC and at 48 h, cell proliferation was higher in cultures form women with endometriosis (p=0.02) than in women with no evidence of disease. Treatment with Cd reduced expression of ER and increased expression of PR in the ESC from women with endometriosis when compared to normal women. Relative cell proliferation and alterations in ER and PR levels were most prominent in ESC of women with endometriosis at 48 h. In the MTT assay, while significant differences were noted at 10^{-6} M of Cd there was no dose dependent response.

In this group of Sri Lankan women in the reproductive age, endometriosis is commoner among those who are more educated. Cadmium, nickel and lead were present in ectopic endometrial tissue and higher levels of nickel in blood were detected in women with endometriosis compared to women without endometriosis. Cadmium was capable of inducing oestrogenic effects in cultured endometrial stromal cells that were more prominent in women with endometriosis.