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Increased risk of endometriosis by long term exposure to xenoestrogens: A case control study in Iranian women

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Abstract

Aims of the study: Endometriosis is a prevalent gynecologic disorder in young women at reproductive age but the underlying risk factors have not identified yet in Iran and other neighboring countries. Persistent exposure to environmental endocrine disrupting chemicals (EDCs) in particular dioxins, PAHs and polychlorinated biphenyls (PCBs) with xenoestrogenic potentials have been hypothesized in the etiopathogenesis of endometriosis .We aimed in the present study to investigate the joint effects of endogenous and exogenous sources of estrogens on the incidence of endometriosis in Iranian women who born and lived in Tehran .Methods: The age matched study population consisted of 34 women with endometriosis and 100 healthy women who underwent surgery from March 2009 to March 2012 . Results: Out of evaluated exogenous sources of estrogen exposure, living near xenoestrogen producing factories (p<0.001*, OR= 16.8, CI 95%5-56.8) in women with lower economical status (p= 0.001, OR=8.29 Cl95%, 3.37-20.37) was identified as the most important risk factor of endometriosis. The prevalence of this phenomenon was higher in women with histories of malignancy in their first degree family (P=0.014, OR=3.08, CI95% 1.23-8.53), infertility (p<0.001, OR=13.07, Cl95%, 5.14-33.23), hormonal dysregulations (p=0.003, OR=8.38, CI95%, 2.03-34.61) and bipolar disorders (p=0.046, OR=3.44, CI95%, 1.11-10.68). Conclusion: Incidence and development of endometriosis is dependent on long term exposure to environmental xenoestrogens especially in women with lower economical status which may affect the endogenous levels of estrogen in women with background factors.

Keywords: Endometriosis, Endocrine disruptors, xenoestrogen, environment, Polycyclic Aromatic Hydrocarbons

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Introduction

Endometriosis is a complex disease induced by genetic, epigenetic and environmental interactions (Kobayashi, 2014). It is a prevalent gynecologic disorder in industrialized regions which is characterized by ectopic growth of endometrial tissue without any clearly determined risk factor but its multifactorial origin has been considered in different studies (Cramer DW and Missmer SA 2002). One of the important risk factors of endometriosis is persistent exposure to environmental endocrine disrupting chemicals (EDCs) in particular dioxins and polychlorinated biphenyls (PCBs) which have been hypothesized to play a role in the disease etiopathogenesis (Porpora et al. 2006) but there is limited evidence (Moini et al. 2013) on the contribution of these chemicals to the incidence of endometriosis in Iran and neighboring countries.

Endometriosis mostly includes dysmenorrhea, dyspareunia, infertility and dysuria in young women (Kim et al. 2007) with increased risk of ovarian cancer (Nezhat et al. 2014). Although the etiologic factors of endometriosis are poorly described, the marked regulation of endometriosis susceptibility genes suggests epigenetic and genetic mutations as the basic mechanism of the micro-environmental changes (Kobayashi et al. 2014). Few modifiable risk factors like body size and figure have been identified earlier (Hediger, 2005) and later increased concentration of reactive oxygen species (ROS), nitric oxide (NO), lipid peroxidation (LPO), iron, lead, cadmium and reduced levels of total antioxidant capacity (TAC), superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), glutathione reductase (GR), vitamins A, C, E, copper, zinc and selenium was suggested (Singh et al. 2013). Some other recent studies imply the role of in utero exposures and dietary factors (Benagiano & Brosen, 2014) on the incidence of this phenomenon and the other ones discuss about the role of chronic immunosuppression in combination with hormonal regulation which may facilitate the aberrant growth of endometrial tissue within the peritoneum without any clear mechanism (Begum & Chowdhury, 2013). Now it is not clear whether the risk of endometriosis could be increased by endogenous levels of estrogens like early onset of menarche, late onset of menopause, nulliparity, late age of first pregnancy, lack of breastfeeding, taking oral contraceptive pill or hormone replacement therapy (Bidgoli, 2011) or the role of xenoestrogns are more considerable in this regard.

Other than mentioned endogenous estrogens resources, many compounds have now been found to have estrogenic activity which may increase the risk of exposure of human ovarian cells to environmental estrogens including pharmacological estrogens, phytoestrogens and xenoestrogens (Darbre, 2006). We aimed in this case control study to investigate any relationship between exogenous or endogenous sources of estrogens and development of endometriosis in Iranian women at reproductive age by recording reproductive and lifestyle factors in Tehran as a much polluted city worldwide.

Materials and Method

Population Study

Cases were selected from Buali and Rasool-Akram university hospitals from March 2009-2012 that were already operated by laparoscopy or hysterectomy. Following pathological diagnosis of endometriosis the questionnaires were completed by home interviews and elicited information on reproductive and other host and environmental factors. Participants of this study were not women who currently take hormonal contraceptives (had not had a hysterectomy), and were not currently pregnant or in breast feeding time. The study population comprised 134 women, including 34 endometriosis and 100 normal women who were Muslim, Iranian and lived in Tehran since their birth to the time of diagnosis.

Exclusion criteria

Diagnosis validation of the endometriosis was performed in the first step by two reference pathologists who reviewed all slides from selected cases. Exclusion criteria for cases and controls were menopausal evidence, recent evidence of pregnancy and lactation. Menopausal status at the time of recruitment was defined according to information on ovariectomy, hysterectomy and menstruation status (still menstruating, number of menses over the past 12 months) .Women were considered postmenopausal if they had undergone a bilateral ovariectomy or if their menses had stopped since 12 months or more

(unless due to hysterectomy). Women who were still menstruating by using exogenous hormones and women with no information on the number of menses over the past 12 months were also excluded from this study. The control group was matched with cases for age +/- 5 years as we described before (Bidgoli et al. 2011).

Endogenous estrogen assessment
The background of endogenous estrogen levels were assessed by recording delivery related factors (patient's age, mother's age, father's age, mother's weight and birth weight at delivery); menstruation related factors (age and weight at menarche and irregular menstruation), marriage related factors (age at first parity ,age at marriage, present marital status; pregnancy related factors (nulliparity, age at first full term pregnancy, age at second full term pregnancy, maximum weight gain at each pregnancy, weight changes after first delivery, weight changes after second delivery, duration of oral contraceptive use, type and dosage of OCP, age at first Oral Contraceptives Pills (OCP) consumption, other methods of child control (IUD, tubectomy, progestins, number of live births ,age at first breast feeding period ,frequency of breast feeding, duration of breast feeding and history of infertility, history of reproductive disorders covered, years of infertility (>2years without birth controlling methods), history of malignancy in first degree family, history of ovarian cyst and hyrsutism as general signs of hormonal dysregulations and any other background disease.

Exogenous estrogens
Sources of environmental estrogens were considered on the basis of the administration of pharmacological estrogens e.g. Oral Contraceptive Pills (OCP), Hormone Replacement Therapy (HRT) and use of ovulation stimulating drugs.

Other lifestyle factors
Personal history of endocrine disorders, background diseases ,pattern of physical activity, occupations, history of psychological disorders, insomnia, severe stressful conditions, anxiety, smoking (active vs. passive), alcohol consumption, using psychoactive drugs, use of oral supplements including calcium ,Vitamins ,Omega 3, radiation exposure, exact BMI and weight gain profile after 18 were recorded by pretested specific questionnaire.
Statistical methods

Values were expressed as percent per population or as the mean ± standard deviation (SD). To assess the association between expressions of markers and clinicopathological data, nonparametric chi square test was used. Relative risks and Odds ratios were calculated by Cochran's and Mantel Haenszel statistics using SPSS 21. Probability values of <0.05 and odds ratios >1 were considered significant.

Table 1: Comparison of demographic and background factors between cases and Controls

Characteristics	Endometriosis (n= 34)	Control (n= 100)	P- Value	OR	Cl 95%
Background factors					
Mother's age at delivery	26.25(5.01)	26.53(6.77)	0.81		NS
Father's age at delivery	32.16(6.79)	33.93(8.44)	0.238		NS
Metrological factors					
Birth weight >4kg 3-4kg <3kg	3(8.8%) 8(23.5%) 17(50%)	11(11%) 6(6%) 83(83%)	0.023*`		NS

Height Average weight before	62.09(10.21)	64.29(10.45)	0.294	NS	
BMI	23.33(4.70)	24.21(3.63)	0.329	NS	
Weight gain after 18					
No change	3(10.7%)	5(5%)	0.06	NS	
< 10 kg	8(28.5%)	16(16%)			
10-20 kg	9(32.1%)	38(38%)			
20-30 kg	6(21.4%)	26(2%)			
>30 kg	1(3.5%)	14(14%)			
Decrease	1(3.5%)	1(1%)			
Physical activity	13(38.2%)	60(60%)	0.031*	0.41	0.18-0.91
History of background diseases					
Anemia	4(11.7%)	25(25%)	0.14	0.40	0.12-1.24
Hormonal dysfunction	7(20.5%)	3(3%)	0.003*	8.38*	2.03-34.61
Thyroid dysfunction	2(5.8%)	12(12%)	0.51	0.45	0.97-2.16
Bipolar Disorders	7(20.5%)	7(7%)	0.046*	3.44*	1.11-10.68
Insomnia	10(29.4%)	40(40%)	0.31	1.6	0.69-3.70
Severe stress	19(55.8%)	59(59%)	0.84	1.13	0.51-2.49
History of malignant breast disease in first degree family yes no	1(2.9%) 33(97.0%)	12(12%) 88(88%)	0.183	4.50	0.563-35.97
History of other malignant disease in first degree family no yes	28(82.3%) 6(17.6%)	59(59%) 41(41%)	0.014	3.08*	1.23-8.53*

Results

1: Demographical and lifestyle factors:

As described in table 1, the demographical feature of cases and controls including metrological factors (weight, height, BMI, trends of weight changes after maturation) working at night times, economical status and history of background diseases were compared.

Cases had lower birth weights (p=0.023), lower levels of physical activity (p=0.031) and lower economical status (p<0.001) but history of hormonal dysregulations (p=0.003, OR=8.38, CI95% 2.03-34.61) and history of bipolar disorders (p=0.046, OR=3.44, CI95% 1.11-10.68) were considered as two new important background risk factors of this reproductive disorder in our population. The economic status of cases was significantly

lower than control (< 6000000 Rials, versus > 600 Rials in control group (p< 0.001, OR=8.29 Cl95%   3.37-20.37).

2: Reproductive factors
As table 2 describes, the reproductive history between cases and controls in two ages matched groups (31.6(7.64) vs 33.2(5.56), p=0.18) were compared to understand the elevated endogenous levels of estrogens in both groups. These variables were considered as below:

Table 2: Comparison of reproductive factors between cases and Controls

Characteristics	Endometriosis (n= 34)	Control (n= 100)	P- Value	OR	Cl 95%
Age	31.58(7.64)	33.2(5.56)	0.189	NS	
Delivery related factors					
Mother's age at delivery	26.25(5.01)	26.53(6.77)	0.81	NS	
Father's age at delivery	32.16(6.79)	33.93(8.44)	0.238	NS	
Birth weight >4kg 3-4kg <3kg	3(8.8%) 8(23.5%) 17(50%)	11(11%) 6(6%) 83(83%)	0.023*`	NS	
Menstruation related factors					
Age at menarche	12.94(1.57)	13.22(1.49)	0.367	NS	
Overweighed at menarche	3(11.11%)	18(18%)	0.833	NS	
Irregular menstruation Yes no	16(47.05%) 18(52.9%)	32(32%) 68(68%)	0.04*	2.49	1.08-5.77
Pregnancy related factors					
Pregnancy Yes no	13(38.2%) 21(61.7%)	89(89%) 11(11%)	<0.001*	13.07*	5.14-33.23
Child number No child 1 2-3	22(64.7%) 5(14.7%) 7(20.5%)	11(11%) 58(58%) 31(31.0%)	<0.001*	14.83*	5.78-38.04
Age at first full term pregnancy	24.84(5.87)	25.03(4.24)	0.913	NS	
History of abortion(yes)	2(5.7%)	(16%)16	0.159	3.04	0.663-14.00
Breastfeeding					
Lack of Breastfeeding	23(67.6%)	(11%)11	*001.>	*16.91	6.52-43.88

Breastfeeding < 2 year Breastfeeding > 2 years	3(8.5%) 8(23.5%)	(38%)38 (51%)51	0.518	0.50	0.12-2.20
History of Oral Contraceptives intake					
Not taking	18(51.4%)	51(51%)	0.687	NS	
Under 1 year	8(25%)	28(28%)			
1-3 years	5(15.6%)	9(9%)			
3-5 years	0(0%)	8(8%)			
5-10 years	0(0%)	3(3%)			
> 10 years	1(3.1%)	1(1%)			
Age at first OCP consumption	23.73(6.00)	(3.88)22.85	0.600	NS	

A: Parity related factor

Lack of parity were observed in 38.3% of cases whereas only 8% of controls remained unmarried without sexual intercourses (p=<0.001, OR=7.11, Cl95% 2.6-19.35) in this study.

B: Pregnancy related factors

Our results showed that pregnancy and live child birth could reduce the risk of endometriosis. In fact 61.7% of cases didn't have any history of pregnancy whereas the same feature was considered in 5% of control group (p<0.001, OR=13.07, Cl95% 5.14-33.23). Lack of breast feeding was another important issue. In cases 67.6% didn't have any history of breast feeding but the same situation was detected in 11% of control group (p<0.001, OR= 16.91, CI 95 %:6.52-43.88).

C: Menstruation related factors

History of irregular menstruation showed higher frequency in cases when compared with control group (p=0.04*, OR=2.49, Cl95% 1.08-5.77). In fact 47% of cases suffered from clear history of irregular menstruation against 26.2% in control group.

Occupation and lifestyle factors

Although most of women in both groups were house keeper and the rest didn't have any occupational exposure to chemicals, their living addresses showed that 41%(14) of patients lived near (<4km) PAHs producing factories for more than 5 years whereas the same feature were observed in 4% of controls

(p<0.001, OR=16.8, CI95% 5-56.8).Other factors like smoking and using plastic dishes didn't show any association with the incidence of endometriosis.

Discussion

Increased serum levels of some well-known xenoestrogens such as bisphenols with structural similarity to natural and synthetic estrogens (e.g. estradiol and dietilstilbestrol) showed some association with increased risk of endometriosis (Cobellis et al. 2009), but more studies are necessary to confirm this hypothesis regarding the possible association between exposure to xenoestrogens and increased risk of this abnormality. This study described and compared the role of exogenous and endogenous estrogens on the incidence of endometriosis and showed the significant role of long term (>5 yrs) exposure to PAHs via living addressee on the increased risk of this abnormality much more dominant than all other endogenous and exogenous resources of estrogen exposure (p< 0.001, OR=16.8, CI95% 5-56.8). Lower economical status (p= 0.001, OR=8.29 Cl95% 3.37-20.37) of these women and living in industrial towns around Tehran may predispose them to the incidence and development of Endometriosis especially in women who had sufficient background factors which were discovered in this study. The human endometrium is a cyclically regenerating organ under the effects of ovarian steroid hormones therefore any abnormal changes in this highly coordinated regulation of endometrial proliferation and differentiation may result in infertility and diseases such as

endometriosis and endometrial cancer (Willing et al. 2011). This study showed enough evidences regarding higher levels of infertility (p<0.001, OR=13.7, CI95% 5.14-33.23) and hormonal dysregulation (p=0.003, OR=8.38, CI 95% 2.03-34.61) in PAHs exposed women with endometriosis. As the differential role of endocrine disrupting chemicals especially environmental organochlorinated pollutants, dioxins and polychlorinated biphenyls (PCBs) is not exactly cleared in comparison to other suggested risk factors of present study, the contribution of each one on development of endometriosis (Porpora et al. 2013), should be assessed differentially in women with clinical and pathological features of endometriosis in Iranian population by using exposure biomarkers.

Although the role of exogenous estrogens are dominant in this phenomenon, several findings of present study support the important role of background factors especially bipolar disorders (p=0.046, OR=3.44, CI95% 1.11-10.68) on the incidence of endometriosis which is in accordance of two recent studies (Kumar et al. 2011), (Patri et al. 2009).

This was a preliminary small size case control study with many new and important clues for future works. Present results suggest that long term (>5years) exposure to PAHs by living near PAHs producing factories could significantly increase the risk of endometriosis in Iranian women .Higher prevalence of infertility , bipolar disorders, hormonal dysregulations and familiar history of malignancy emphasize also the necessity to work on the contribution of each chemicals on the incidence and development of mentioned disorders and to look for possible mechanisms of these background factors on the development of endometriosis in Iranian women.

Acknowledgements

Table 3: Comparison of environmental factors between cases and Controls

Characteristics	Endometriosis (n= 34)	Control (n= 100)	P- Value	OR	Cl 95%
Occupational Exposure Yes no	7(20.5%) 27(79.4%)	10(10%) 90(90%)	0.13	NS	
Living near PAHs producing factories	14(41%)	4(4%)	<0.001*	16.8*	5-56.8*
No smoker Passive smoker Active smoker	21(15.6%) 12(8.9%) 1(0.74%)	74(55.2%) 24(17.9%) 2(1.49%)	0.178	NS	
Using plastic dishes and bottles	7(20.5%)	4(4%)	0.006*	0.16	0.0-0.59

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