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Original Article

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Role of endocrine disrupting chemicals on the tissue levels of AhR and sex steroid receptors in breast tumours

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Abstract

Breast cancer affects Iranian women at least one decade younger than their counterparts in other countries and the incidence of breast fibroadenoma is growing in the last two decades in Tehran. This study aimed to compare the AhR levels in premenopausal breast cancer and breast fibroadnemo with appropriate normal groups. Possible associations of AhR with lifestyle and reproductive risk factors and other fundamental genes of breast cancer and reproductive disorders were the other major goals of present study. To conduct the comparisons all possible reproductive, environmental and lifestyle risk factors of mentioned diseases were recorded in 100 breast cancer, 100 breast fibroadenoma and compared with 400 women in normal group from 2009 to 2011. AhR overexpression in epithelial cells of premenopausal patients emphasized the susceptibility of these cells to environmental induced reproductive disorders. The AhR overexpression was contributed to ER-/PgR- immunophenotype in malignant tissues. Weight gain (after 18 and after pregnancy), long term (>5yrs) OCP consumption, smoking, severe stress ,history of ovarian cysts, hormonal deregulations, living near PAHs producing sources, were correlated with increased risk of breast cancer and reproductive disorders and were correlated with elevated tissue levels of AhR. It seems that increased risk of breast cancer and other reproductive tumours in Tehran may be the result of exposure to environmental endocrine disruptors. Long term exposure to environmental estrogens can increase the tissue levels of AhR and deregulate the expression pattern of sex steroid receptors and other genes in target tissues.

Keywords: Endocrine Disrupting chemicals, Steroids, Breast cancer , Tumors

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Introduction

Although the possibility of breast cancer incidence increases with the age (Bray et al. 2004), this malignancy could occur in young women before their menopause (Zhang et al. 2010). In Iran, the breast cancer problem seems more serious because it affects Iranian women at least one decade younger than their counterparts in developed countries (Zhang et al. 2010, Kolahdoozan et al. 2010) and the underlying risk factors of early breast cancer could be related to hormonal and environmental factors (Bidgoli et al. 2010). Risk of breast cancer could be increased by increased exposure to endogenous estrogens from early onset of menarche to late onset of menopause, nulliparity, late age of first pregnancy, and lack of breastfeeding, taking oral contraceptive pill or hormone replacement therapy (Van der Heiden et al. 2009). Other than physiological steroidal estrogens, many compounds have now been found to have some estrogenic activities which may increase the risk of exposure of human breast cells to environmental estrogens including pharmacological estrogens, plant estrogens (phytoestrogens) and manmade estrogen-mimicking chemicals (xeno-estrogens) (Pasqualini et al. 1997; Darbre, 2006).

Huge numbers of epidemiologic evidences suggest the role of premenopausal exposure to exogenous sex steroid hormonal resources in the development of female breast cancer. These steroid hormones have been classified as group 1 human carcinogen by international agency for research on cancer (IARC) (Cogliano et al. 2005) moreover the role of polycystic ovary syndrome has been suggested (Soran et al. 2005) in the occurrence of early breast cancer, which may be contributed to the altered levels of estrogens and other sex steroid hormones in young patients. Endocrine disrupting chemicals (EDCs) are natural or synthetic agents that interfere with normal functions of human endocrine systems. Many EDCs with estrogenic activities, i.e., xenoestrogens, are resistant to biodegradation, due to their structural stability, and persist in the environment (Dickerson et al. 2007). There is increasing concern about EDCs that are able to mimic hormones and interact with hormone transport proteins. As EDCs potentially disrupt hormone metabolic pathways, they can mimic functions of endogenous hormones and, in some cases, completely block the functions. A substantial number of environmental pollutants, such as polychlorinated biphenyls, dioxins, polycyclic aromatic hydrocarbons (PAHs), phthalates, bisphenol A, pesticides, alkylphenols and heavy metals (arsenic, cadmium, lead, mercury), have been shown to disrupt endocrine functions and may cause breast cancer (Balabanič et al. 2011).

The aryl hydrocarbon receptor (AhR) mediates the toxicity of EDCs with xenoestrogenic activities (Van der Heiden et al. 2009). Roles of AhR in the incidence of benign and malignant breast tumors (Bidgoli SA et al. 2010), reproductive disorders and infertility have been described recently by us (Bidgoli SA et al. 2011). AhR overexpression has been found in estrogen receptor (ER) -negative human breast tumors (Bidgoli SA et al. 2010) and its overexpression is positively correlated with the expression of other genes (Vogel et al. 2011). Lower levels of ER alpha and progesterone receptor were suggested in breast cancer tissues of premenopausal patients compared to the levels in those of postmenopausal patients (Bidgoli SA et al. 2010, Vogel et al. 2011). This is consistent with the hypothesis that long term exposure to endogenous or exogenous estrogenic resources down regulates the tissue levels of ER and progesterone receptor.

Given the lack of available data, current concerns about the possible involvement of EDCs in the increase in the incidence of breast cancer, reproductive benign has tumors remain hypothetical. Long term exposure to environmental estrogens can increase the tissue levels of AhR in young women and deregulate the expression of sex steroid receptors and other genes.

This study reviews our recent studies which

aimed to demonstrate the roles of long term exposure to xenoestrogens in early incidence of breast cancer in Iran. In addition, we will review the relationships among reproductive factors, adiposity and endogenous levels of estrogens in breast cancer and breast fibroadenoma according to our recent findings.

Materials and Methods

Population study:

A case-control study was carried out among 100 newly diagnosed breast cancer patients, 100 breast fibroadenoma, 137 uterine fibroadenoma and 137 young women with endometriosis and compared with 400 women in normal group from 2009 to 2011 in Tehran. Cancers and all other female reproductive tumors and disorders were identified from both self-reports registration and pathological reports. Exclusion criteria for cases and controls were menopausal evidence, sign of pregnancy and recent lactation by self reports. Menopausal status at the time of recruitment was defined according to information on ovariectomy, hysterectomy and number of menses over the past 12 months. 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 excluded from this study. The control group was matched with cases for age ± 5 years.

Identification of reproductive variables:

Demographical variables were obtained from specific questionnaire items: A Delivery related factors including mother's and grandmother's age, father and grandfather's age, mother's weight and birth weight at delivery; B, Menstruation related factors including age, weight and height at menstruation, irregular menstruation, amenorrhea and dysmenorrhoea; C, Marriage related factors including marital status, age at marriage, age at first intercourse and frequency of intercourses per week.

Identification of hormonal disorders:

Information on hormone use was obtained from specific questionnaire items. They covered questions on ever and current use of oral contraceptive pills (OCPs), the brand names, age at start and total duration of the use. Other methods of contraception were recorded including use of intrauterine device, tubectomy and use of progestins. Pregnancy related factors including number of full-term pregnancies, age and maximum weight gain at each pregnancy, months of breast feeding at each delivery, history of abortion induction were recorded. History of infertility covered questions on years of infertility, i.e., more than 2years without birth controlling methods, use of ovulation stimulating drugs, hormone therapy or history of in vitro fertilization. Patients were asked their gynecological disorders including ovarian cysts, uterine firoadenoma, irregular menstruation, hyrsutism and other disorders.

Identification of background factors:

Exact weight and height of cases and controls as well as their weight changes from menstruation to maturation, from pregnancy to breast feeding until present were recorded by pretested questionnaires. Body mass index (BMI) was calculated and compared between cases and controls.

Identification of environmental resources of AhR ligands:

Exact living and working addresses of cases and controls were recorded and matched with the map of factories generating PAHs and dioxins. A complete list of factories that release toxicants with hormone-like effects was made before starting the study. The women who lived within 4 km from the pollutant factories were considered as high risk people.

Other lifestyle factors:

Personal history of endocrine disorders, pattern

of physical activity, occupations, smoking (active versus passive), alcohol consumption, using any drug, radiation exposure, weight gain after age 18 were recorded by pretested specific questionnaire. Dietary factors of exposure will be discussed separately.

Immunohistochemical studies:

Out of each category of patients, 30 parrafinized blocks were selected for pathological and immunohistochemical studies. As previously described (Bidgoli SA et al. 2011, dewaxed and rehydrated tissue sections were subjected to antigen retrieval using microwave oven and boiling citrate buffer (pH = 6.0). Endogenous peroxidase activity and nonspecific binding sites were blocked by incubating sections with 0.3% hydrogen peroxide in methanol for 30 min. and 3% bovine serum albumin for 60 min, successively. Sections were then incubated 30 min at room temperature with AhR mouse monoclonal antibody (clone PRT9, abcam) that recognizes the cytoplasmic expression of human AhR in breast tissues, ER alpha (Clone 105, Dakocytomation), PgR (Clone 636, Dakocytomation) that recognize the nuclear and expression of these steroidal receptors in breast tissues. The results were visualized using the envision system (Dakocytomation) based on the manufacturer's instruction with necessary modifications. Sections were also counterstained with Meyer's haematoxyline. The ideal staining conditions were established in our preliminary experiments. Method of scoring has been recently described (Bidgoli SA et al. 2011).

Statistical Methods:

Values were expressed as percent per population or as the mean ± standard deviation. To assess associations between expression of AhR, ER, PR and clinicopathological data, the nonparametric chi square test was used. Relative risks and odds ratios were calculated by the Cochran-Mantel-Haenszel statistics using SPSS 16 and the odds ratios were reported for this case control retrospective study. When the odds ratio in cases was >1, if the probability values was <0.05 and when the 5% confidence interval of the true odds ratio was greater than 1, then we interpreted it as significant risk factor. When the odds ratio in controls was <1, if the probability values was <0.05 and when the 5% confidence interval of the true odds ratio was less than 1, then we interpreted it as protective factor significant .

Results

AhR levels in breast cancer patients:

AhR overexpression was identified in 42.9% of epithelial cells of breast cancer patients whereas it was found in 9.5% of adjacent stromal cells of malignant tumors (Bidgoli SA, 2010).

AhR levels in breast fibroadenoma:

AhR overexpression was recorded in 82.7% of epithelial cells of fibroadenomas whereas it was found in 57.1% of their adjacent stromal cells (Bidgoli SA et al. 2010).

Comparison of AhR levels between malignant and benign breast tumors:

Benign epithelial cells showed higher levels of AhR when compared with malignant cells (82.8% vs. 57.1%) (p = 0.01). Benign stromal cells showed also higher expression levels of AhR when compared with malignant cells (55.2% vs. 9.5%) (p = 0.001).Co-expression of stromal and epithelial AhR was detected in fibroadenoma cells (p = 0.006) that means 88% of stromal cells expressed the AhR in the cytoplasmic level. AhR (+) tumors showed AhR expression in their corresponding epithelial cells but the same pattern was not detectable in malignant cells (Bidgoli SA et al. 2010).

Role of menopausal status on AhR levels:

Significant difference was recorded in tumoral

AhR levels of pre and post menopausal breast cancer patients (86% vs. 43% p = 0.023, OR=3.84 (CI 95% 0.176-12.54)). No significant difference was recorded between epithelial levels of AhR in premenopausal breast cancer patients and breast fibroadenoma (86% vs. 82.6%) (Bidgoli SA et al. 2010). Surprisingly, 70% of tissues showed strong staining of AhR and 16 % showed moderate staining of this receptor. The expression levels of AhR were not associated with clinicopathological parameters (Bidgoli SA et al. 2012).

PAHs exposure and premenopausal breast cancer:

Exposure to PAHs and dioxins were examined by recording the living and working addresses. Out of 50 cases 24% were exposed to EDCs whereas the same situation were detected in 5% of controls (p=0.001, OR =4.80). Passive or active exposure to cigarette smokes were weakly associated with increased risk of premenopausal breast cancer (p= 0.005, OR =1.43). Linear regression analysis showed that the expression levels of AhR were significantly higher (p=0.006) in patients living close to factories generating PAHs than those living far from the factories (Bidgoli SA et al. 2012).

Association between AhR and other critical breast cancer:

Glycodeline (Gd):

Significant association between tumoral Gd and AhR expression in premenopausal breast cancer patients (p=0.036) was observed. Four possible immunophenotypes were calculated in pre and post-menopausal breast cancer patients. The immunophenotype (AhR+/ Gd+) was more common in young/premenopausal breast cancer patients (35.5% vs. 20%, p=0.035) (Bidgoli SA et al. 2012).

K-ras

Significant association between tumoral K-ras and AhR expression in breast cancer patients (p=0.036) was observed .The immunophenotype (AhR+/K-ras+) was more common in young/premenopausal breast cancer patients (Bidgoli SA et al. 2010).

p53

Benign tumors didn't express p53 in different cells but malignant tumors expressed it in 36.6% of malignant tumors. The expression pattern of p53 was not different between pre- and post-menopausal breast cancer patients (p=0.213) but higher risk of p53 expression was detectable in post-menopausal cases (OR=2.2, 95% CI 0.56–8.817) .No significant association was detectable between p53 and AhR in pre- and post-menopausal patients (Bidgoli SA et al. 2010).

Discussion

There are over one million automobiles, hundreds of PAHs producing factories, chemical industries , and over 12 million people living in the city of Tehran and the incidence of cancer especially breast cancer in critically growing in this city. This study has revealed the role of environmental PAHs exposure as the most important risk factor in premenopausal breast cancer in Tehran.

AhR overexpression was detected in 87% of epithelial cells of young breast cancer patients and 82.7% of epithelial cells of breast fibroadenomas. The AhR binds with high affinity to PAHs, but also binds with lower affinity to structurally diverse exogenous and endogenous chemicals. In fact AhR agonists induce ER alpha dependent transactivation. These compounds also induce binding of AhR and ER alpha to the CYP1A1 and pS2 gene promoters, which is consistent with their activities as both selective AhR modulators and selective ER modulators (Liu S et al 2006). Human epidemiological studies and experimental animal data have strongly suggested that xenobiotics with

estrogenic properties may increase the incidence of breast cancer and we have confirmed this hypothesis by our recent studies.

In recent 15 years, many studies have reported positive correlations between levels of xenoestrogenic compounds in blood or peritumoral adipose tissues and breast cancer risk (Salehi et al. 2008). Overexpression of activated AhR in premenopausal breast cancer patients in this study may emphasize the possible role of xenoestrogenic compounds as AhR ligands in this phenomenon. The role of nutritional sources of PAHs, obesity and xenostrogens will be discussed in our next studies.

Exposure to endocrine disruptors which are AhR ligands induce abnormal genital tract development and decreases fertility in rodents (Hassa A,2003). Dioxin, TCDD and similar organic toxicants might play direct mechanistic roles in the etiopathogenesis of endometriosis and female infertility by increasing Gd production but the importance of this association in breast cancer was demonstrated for the first time in this research. Current study demonstrated a significant association between tumoral levels of Gd and AhR (p=0.002) in breast cancer which confirms the preliminary hypothesis about the role of TCDD exposure on Gd biosynthesis and secretion in TCDD-treated endometrial epithelial cells (Mandavia C et al. 2015).

The higher prevalence of AhR+/K-ras+immunophenotype in pre-menopausal breast cancer patients in this study suggest the possible role of AhR on up-regulation of K-ras and other oncognes which should be considered in future studies. Down regulation of p53 in AhR+breast cancer patients promotes the hypothesis about the role of AhR activation on tumor suppressor genes deactivation in breast cancer whereas the significant association between tumoral AhR and K-ras promotes the hypothesis about the role of AhR activation on proto-oncogene activation in breast cancer (Arbabi Bidgoli, 2010) .It seems that early incidence of breast cancer could be the result of interactions between hormonal and environmental factors. Further studies are necessary to find the epidemiological risk factors of early and high incidence of breast cancer, their interaction with hormonal factors and AhR levels in other reproductive benign and malignant tumors.

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