Consequence of exposure to Endocrine disruptors chemicals on female fertility

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ABSTRACT

Objective: To evaluate the internal concentration of endocrine disruptor chemicals in sub-fertile women and their influence on suitable indicators such as nuclear receptors.
Study Design: A comparative analytical study.
Place and Duration: From 1st Jan 2019 to 1st June 2019 in the Gynecology department at Jinnah Medical College & Hospitals Karachi.
Methodology: 48 sub-fertile women aged 18–40 years were chosen for research. Whereas, women with in same age group, who had spontaneous pregnancy last year were considered as control group. 20ml of blood sample was taken from participants to measure the level of endocrine chemical disruptors. The detected enzymes responsible for endocrine disorders are extracted using the fluid-liquid separation technique and measured using high-performance fluid chromatography. Further, Nuclear receptor genes are investigated for regulation through PCR.
Results: The mean age of the research group for the control group was 36.0 ±4.7 years and 32.6 ± 5.2 years. Immunological infertility (8, 16.6%), tubal infertility (9, 18.7%), unexplained infertility (15, 31.2%), thyroid dysfunction (6, 12.5%), polycystic ovarian syndrome (2, 4.1%), and endometriosis (8, 16.6%) were the main causes of sub-fertility. The concentrations of Bisphenol were significantly higher in infertile patients than fertile patients (70.8% vs 30.7% percent, $p<0.01$). There was no substantial difference between groups in the levels of perfluoro octane sulphonate, perfluorooctanoic acid, mono-ethyl hexyl phthalate, and di-(2-Ethylhexyl) phthalate ($p<0.05$). Sub fertile women had a significantly higher mean expression than fertile people in alpha and beta receptor, androgen receptor, and pregnant X receptor ($p>0.05$). The mean levels of Peroxisome proliferator-activated gamma and Aryl hydrocarbon receptor between the two groups had no significant difference ($p<0.05$).
Conclusion: In conclusion, our results have further validated the evidence of the role of endocrine disrupting chemicals exposure in causing infertility among women and highlights their influence on nuclear receptors, hence indicating them as an effective biomarker of the disruptions caused by endocrine disrupting chemicals.
Keywords: Endocrine disruptors, Sub-fertility, Chemical exposure, Nuclear Receptors, Sub fertile women

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INTRODUCTION

Subfertility is characterized as failure to become pregnant after maintaining unprotected intercourse for at least 12 months. Among the developed states, every one in seven couples have been estimated to be infertile due to female subfertility while this prevalence can be as high as one in four couples in developing countries[1]. Successive researches have been increasingly identifying risk factors that pose threat to the reproductive health of women including occupational factors and lifestyle factors.

Among many risk factors that disrupt normal endocrine functioning among women, toxicological studies suggest the increasing role of endocrine disordering chemicals (EDC) in female subfertility. Endocrine signaling is found to be critical in the functioning of the reproductive system and embryo development along with other functions such as energy production and storage, growth and development, electrolyte balance, and neuronal activities[2]. Disruption in these signaling results in diverse disorders including subfertility.

According to the US Environmental Protection Agency (EPA), EDC is defined as the externally produced compounds that impede the formation, excretion, metabolism, and functionality of endocrine hormones; thus disturbing their homeostasis[3]. US EPA has further classified EDC's that are capable of altering estrogenic and androgenic activity into five classes: drugs, phytoestrogens, pesticides, plasticizers, and industrial materials[4,5]. Studies have shown the increasing role of EDC in impeding the development of the ovary by interfering with reproductive mechanisms including meiosis and germ cell physiological development[6]. Similarly, relatively greater exposure to EDCs was found in diagnosed cases endometriosis as compared to control group. However, these studies couldn't establish the contribution of EDCs in the pathogenesis of endometriosis[7,8]. Among these EDC's, bisphenol A (BPA), perfluoro-butane sulfonate (PFOS), di-(2-Ethylhexyl) phthalate (DEHP), Mono-ethylhexyl phthalate (MEHP), and perfluorooctanoic acid (PFOA) are assumed to be potential EDC's that disrupt women reproductive cycle[9].

Pakistan is an industrial country with a high pollution level. Therefore, High levels of EDCs are predicted in our environment. The rationale of this study is to explore the contribution of EDCs in causing infertility in women so that preventive treatment of high-risk women can be introduced accordingly. Therefore, the objective of the study is to evaluate the internal concentration of endocrine disruptor chemicals in sub-fertile women and their influence on suitable indicators such as nuclear receptors.

METHODOLOGY

This comparative analytical study was conducted at the gynecology ward of Jinnah Medical College & Hospital for 6 months from 1st January 2019 to 1st June 2019. A total of 48 women, aged between 18-40 years, who were confirmed for subfertility, were included in the study through random sampling technique after getting informed consent. Subfertility was characterized as the inability to get pregnant following unprotected sexual contact for at least 12 months[1]. Whereas, women within the same age group who had spontaneous pregnancy in the last year and who left breastfeeding for at least six months were considered as the control group. The patients were administered a questionnaire to ascertain their life habits, including food, alcohol and drug use, smokes, homes, and occupations to determine the presence of confounding factors. Women with a history of smoking, malabsorption, any underlying comorbidity, or those affected by male infertility alone were excluded from the study to remove the bias. Sub fertile women then underwent clinical and laboratory examination to ascertain their cause of sub fertility and to classify endometriosis, a prevalent disorder, as a cause of subfertility from other possible causes.

The blood, being in chemical equilibrium with organs and tissues, is a well-established indicator of environmental contaminants that affect the organisms. Therefore, following the survey, a 20ml fasting sample of venous blood was collected from the participants on empty stomach. Glass vials were used to prevent endocrine-disordering chemicals released from plastic products. The collected sample was divided into two aliquots, 5ml of heparin mixed whole blood and another 5ml was separated for separation of serum through centrifugation. The serum and an all-blood sample were sent to the Department of Environmental Science in a well-protected environment; whereas, the entire remaining heparinized liquor was quickly forwarded to the Department of Veterinary Public Health and Food Safety Laboratory, where it was processed for Nuclear Receptors level determination within 72 minutes. Nuclear receptors are defined as the major targets of EDC for triggering the adverse mechanisms[10].

The analyses of EDC's were carried out through a liquid-liquid separation protocol and were quantified through high-performance liquid chromatography (HPLC) with electrospray ionization tandem mass spectrometry. To avoid risk of laboratory contamination and analytical error blanks were run with each batch and measuring instruments were calibrated properly. Expression of NRs was analyzed in mononuclear cells suspended in the whole blood. Peripheral mononuclear cells were removed from whole blood through centrifugation. These cells were treated for the extraction of RNA which was later transcribed into DNA. This DNA was then utilized for evaluation of NRs genes expression through real-time polymerase chain reaction (PCR) by using specially designed primers.

Data Analysis: An SPSS version 23.0 was used to analyze the results. The continuous variables were produced as mean with standard deviation (SD) and percentage values. The significance level between the two studied groups was found through the student's t-test and chi-square test. A significance value of or less than 0.05 was considered significant.

RESULTS

A total of 48 Sub fertile women were included in the study. The mean age of the research group and control group was 36.0 ±4.7 years and 32.6 ±5.2 years, respectively. Through standard laboratory and clinical evaluation, following causes of infertility were found: immunological infertility (8, 16.6%), tubal infertility (9, 18.7%), unexplained infertility (15, 31.2%), thyroid dysfunction (6, 12.5%), polycystic ovarian syndrome (2, 4.1%), and endometriosis (8, 16.6%). Endometriosis was further classified and 5 women were at stage I–II and 3 women were at stage III-IV.

Table I presents the percentage of the samples that reported EDCs higher than LOD in the form of a comparison between fertile and sub fertile women. The total number of participants with measurable levels of Bisphenol A in infertile patients was significantly more than fertile ones (70.8% vs 30.7% percent, $p<0.01$). However, there were no major differences ($p<0.05$) in the detectable levels of Perfluoro-acid, Perfluorooctanoic acid, MEHP, and Bis (2-Ethylhexyl) phthalate group concentrations between the two studied groups (Table-I).

Levels of estrogen receptor alpha (ERa) and beta (Erb), androgen receptor (AR), pregnant X receptor (PXR), aryl hydrocarbon receptor (AhR), and peroxisome proliferator-activated receptor-gamma (PPARg) were measured to evaluate associated NRs.

Although the measured NRs were detectable in both the groups, in the sub fertile population mean values of delectable Era, PXR, Erb and AR were significantly more than fertile patients. The AhR and Peroxisome proliferator-activated receptor genes had a very low mean expression, and there were no major differences between the two classes (Table-II)

Table-I: Categorical Classification of Participants having EDC Serum Level > LOD (N=61)

		Subfertile patients		
Endocrine disruptor	**Fertile patients (n=13)**	**All Sub fertile Patients (n=48)**	**Patients with Endometriosis n (%)**	**Other causes of Subfertility, n (%)**
BPA (n/%)	4 (30.7)	34 (70.8)*	6 (75.1)	29 (73.5)
PFOS (n/%)	0 (0)	16 (33.3)	5 (63.5)	10 (25.0)
PFOA (n/%)	1 (7.6)	7 (14.5)	1 (12.7)	5 (12.6)
MEHP (n/%)	8 (61.5)	32 (66.6)	7 (87.7)	28 (70.1)
DEHP (n/%)	0 (0)	0 (0)	0 (0)	0 (0)

*Statistical difference between fertile and infertile groups ($p<0.05$)

[BPA (bisphenol A), PFOS (perfluorobutane sulfonate), DEHP (di-(2-Ethylhexyl) phthalate), MEHP (mono-Ethylhexyl phthalate), and PFOA (perfluorooctanoic acid)]

Table-II: Expression of Nuclear Receptors in Fertile and Sub-fertile Groups (N=61)

Receptors	**Sub-fertile patients (N= 48) Mean (SD)**	**Fertile patients (n=13) Mean (SD)**	**P=value**
Era	0.27 (±0.39)	0.01 (±0.04)	P<0.05
ERb	0.22 (±0.35)	0.018 (±0.040)	P<0.05
AR	0.24 (±0.39)	0.017 (±0.05)	P<0.05
AHR	0.07 (±0.18)	0.005 (±0.008)	NS
PER	0.22 (±0.35)	0.017 (±0.036)	P<0.05
PPARg	0.0003 (±0.0007)	0.0002 (±0.0008)	NS

[Era (estrogen receptor alpha) and Erb (estrogen receptor beta), AR (androgen receptor), PXR (pregnane X receptor), AhR (aryl hydrocarbon receptor), and PPARg (peroxisome proliferator-activated receptor-gamma)]

DISCUSSION

The present study aimed at the analysis of non-bio accumulating EDC in the serum to get an indication about internal levels of biologically active compounds after being exposed to variable environmental sources for long period and its influence on fertility. We also measured nuclear receptor expressions considering them a significant indicator of biological effect.

The study reported that a detectable level of MEHP, PFOS, BPA, and PFOA in a higher number of infertile women than fertile women. However, a significant difference could only be established for BPA concentration. It has also been found that the higher percentage of women affected by endometriosis had serum level > than LOD for BPA, MEPH, and PFOS when compared with other sub-fertile women without endometriosis. Widespread exposure to BPA was observed in sub-fertile women.

BPA is a basic constituent of polycarbonate plastics and other food-containing materials which contaminate the edibles by migrating through the polymers[11]. BPA act as xenoestrogen that binds to estrogen receptors and triggers alteration in the expression of estrogen-responsive gene expression[11], however, it may also bind with androgen receptors. Our study has provided evidence of the relationship between female reproductive malfunction and BPA. Recent studies have reported the role of BPA in the alteration of the steroid genesis pathway in ovaries[12], oocyte quality[13], estradiol release to gonadotropin stimulation, and uterine implantation[14]. Collis et al[15] demonstrated a detectable concentration of BPA in the serum of more than 50% of women diagnosed with endometriosis while it was not found in patients without endometriosis.

PFOA and PFOS are characterized as novel EDCs that are utilized as the surfactant in multiple industries[16]. Epidemiological and toxicological studies have demonstrated the association between PFOS exposure and sub-fertility[17]. In an already published study, infertile women had a higher concentration of PFOS than fertile women[18].

In our study, MEHP was largely detected in fertile women along with infertile women while DEHP was not detected in any of the enrolled participants. The presence of MEHP could be accounted to the occurrence of phthalates in variety of daily use products (cosmetics, food packages, and medical instruments)[19]. Some of the previous researches have reported association of phthalates with delayed ovulation or anovulation, reduced serum progesterone, reduced production of estradiol, raised serum FSH levels and smaller preovulatory follicles[20]. Itoh et al. conducted a study on Japanese women and found increased urinary secretion of MEHP in women affected with endometriosis than fertile women[21]. Ferguson et al. reported a positive association between phthalates exposure and inflammatory markers and negative association with bilirubin, an endogenous antioxidant. It was noted that phthalate exposure caused endometriosis which invoked inflammatory response[22].

We also found an increased level of multiple nuclear receptors, representative of associated EDC targets, in infertile women. Interestingly, the expression levels of NRs were not found to be the direct representative of EDC action. However, persistently increased expression of PXR, an NR related to estrogen or androgen and metabolism of endogenous compounds, is worth noticing. The modulated effect of receptors might predict the response to the overall effect of various risk factors of subfertility. The results of our study shows promising compliance with a previous study that aimed to identify the effect of environment on the reproductive health of both men and women and found NRs as an effective biomarker of endocrine disruption[23]. Previous studies showed an increased expression of PPARg in women with endometriosis than women with other factors of infertility. PPARg expresses in the endometrium and ovarian cells as a regulator of folliculogenesis[24]. It has been found modulating the mechanism

of proteases involved in angiogenesis and tissue remodeling that act as a significant step in the pathogenesis of endometriosis[23]. Apart from it, it also triggers an inflammatory response leading to the development of inflammatory lesions in the endometrium.

The study is limited in regards to study design and sample size. Therefore, future studies are expected to be conducted on larger sample size and for longer duration.

CONCLUSION

In conclusion, our results have further validated the evidence of the role of EDC exposure in causing infertility among women and highlights their influence on nuclear receptors, hence indicating them as an effective biomarker of the disruptions caused by endocrine disrupting chemicals.

AUTHOR'S CONTRIBUTION

Ali N: Conceived idea, Designed Methodology, Data analysis, Manuscript writing, Data analysis, Data collection, Literature review

Ghafoor S: Data analysis, Manuscript writing, Final critical review of manuscript

Zareen S: Data collection, Data compilation, Literature review

Saleh F: Manuscript drafting, Data compilation, Data analysis

Qureshi ZUN: Data collection, Data compilation, Literature review

Sajid A: Manuscript drafting, Data compilation, Data analysis

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