

Urinary Estrogen Levels in Women on Contraceptives in Enugu, South-East Nigeria

Ignatius C. Maduka, Francis E. Ezeonu¹, Emeka E. Neboh², Elvis N. Shu³, Ebele J. Ikekpeazu⁴

Departments of Chemical Pathology, University of Nigeria Teaching Hospital, PMB. 01129, Enugu State, ¹Applied Biochemistry, Nnamdi Azikiwe University, PMB. 5025 Awka., Anambra State, ²Chemical Pathology, College of Medicine, Enugu State University of Science and Technology, G.R.A., ³Pharmacology and Therapeutics, ⁴Medical Biochemistry, College of Medicine, University of Nigeria Enugu Campus, Enugu State, Nigeria

ABSTRACT

Background: Substantial evidence supports a causal relationship between the risk of human breast cancer and levels of endogenous estrogens. **Aim:** To evaluate the urinary estrogen of women on contraceptives and also compare the levels in two different classes of contraceptives; hence, the possible predisposition of such women to the risk of breast cancer. **Setting and Design:** Urinary estrogen level was evaluated in 84 women attending family planning clinic in University of Nigeria Teaching Hospital Enugu, Nigeria, who have been on contraceptive device for 10 years or less (≤ 10 years). They were aged between 21 and 50 years and were divided into three groups (21-30 years, 31-40 years, and >40 years). The control group consisted of 30 age-matched apparently-healthy women who were not on any contraceptive device. **Materials and Methods:** Estrogen was analyzed using Ecologenia[®] Estrogen (E1/E2/E3) microplate enzyme-linked immunosorbent assay (ELISA) kit, Batch No. T2GR4, from Japan Envirochemicals Ltd, Japan. **Statistical Analysis Used:** Significant differences between means were determined by two-tailed Student's *t*-test using graph pad prism computer software program. **Result:** There was a statistically significant increase ($P=0.0462$), in the mean urinary estrogen level of women on contraceptives when compared with the control. The highest amount of estrogen was excreted by the women in the 21-30 years age group. When the contraceptive devices were divided into two classes of intra-uterine device and oral/injectables, there was no statistical difference ($P=0.8112$) in the mean urinary estrogen output of the women. **Conclusion:** The synthetic estrogen content of contraceptive device most probably contributed to the level excreted in the urine. The increased estrogen output observed in women on contraceptive device was not dependent on the class of contraceptive device used.

Keywords: Contraceptives, intra-uterine device, oral contraceptive, urinary estrogen, women

Introduction

Estrogens, androgens, and progestins are produced in both genders, but in different amounts.^[1] Steroid hormones are lipophilic, fat-soluble molecules, which are mainly excreted as water-soluble glucuronates or sulfate conjugates. Under environmental conditions, these conjugates are quickly hydrolyzed, leading to the free hormones or their metabolites.^[2] Since it was marketed over 40 years ago, the combined oral contraceptive (COC) has proved to be a popular, highly effective method of hormonal contraception.^[3] Levels of serum and urinary estrogens are higher in breast cancer cases compared with controls,^[4] but clinical studies linking the

ratio of estrogen metabolites to breast cancer risk are not entirely consistent.^[5] One mechanism that has been proposed to explain the role of estrogen in breast cancer is that different pathways in the metabolism of estrogen may differently affect risk.^[6] Substantial evidence supports a causal relationship between the risk of human breast cancer and levels of endogenous estrogens.^[7] Increased risk has been reported in women with high serum and urinary estrogen levels,^[8] as well as those exposed to increased estrogen concentrations over time as a result of postmenopausal obesity, early onset of menstruation, and late menopause.^[9] Variations in hormone concentrations across the menstrual cycle are known to have a potentially clinically significant impact on the metabolism of exogenous chemicals such as drugs,^[10] in addition to that of endogenous substances such as the estrogens. A growing appreciation of the potential physiological effect of these

Access this article online

Quick Response Code:



Website:
www.jfmpc.com

DOI:
10.4103/2249-4863.94450

Address for correspondence: Mr. Emeka E. Neboh, Department of Chemical Pathology, College of Medicine, Enugu State University of Science and Technology, G.R.A., Enugu State, Nigeria.
E-mail: emmyneboh@yahoo.com

metabolites has developed, leading investigators to evaluate their contribution to variation in breast cancer frequency,^[11] bone mineral density levels,^[12] and oxidized LDL cholesterol concentration, an important contributor to the atherogenic process.^[13] With respect to gender, different organisms excrete various amounts of sex steroids, depending on parameters such as age, state of health, diet, or pregnancy.^[1] The primary objective of the present study is to estimate the level of urinary estrogen release in women on contraceptives, whereas the secondary objective is to compare the urinary estrogen of women on the two classes of contraceptive devices; the intra-uterine device (IUD) and oral/injectable. The information obtained from the study might reveal the possible predisposition of this group of women to breast cancer.

Materials and Methods

Selection of study subjects

The subjects for the study consist of 84 women attending family planning clinic at the University of Nigerian Teaching Hospital (UNTH), Enugu, Nigeria. They were aged between 21 and 50 years and have been on contraceptive device for 10 years or less. For comparative studies, 30 age-matched apparently-healthy women who are not on any contraceptive device were used as control subjects. All the subjects were selected via simple random sampling technique, once they met the requirements for inclusion in the study. Written informed consent was obtained from each of the subjects and a well-explained questionnaire was issued to each of them. Approval was also given by the ethical committee of the institution before the commencement of the study. Only those adults who returned their questionnaire were enlisted for the study. Forty-seven (47) of the test subjects were on oral/injectable contraceptives, whereas 37 were on IUD. The women on contraceptives were grouped according to their ages into three groups of 21-30, 31-40, and >40 years respectively for the study which took place between March and July, 2009, and women who had been on contraceptives were excluded from the control group of the study whereas all the recruited subjects had no condition of ill health at the time of the study, as this may affect the study outcome.

Sample collection

The subjects were asked to collect about 10 ml spot urine samples directly into clean glass tubes. The urine samples were immediately preserved with two drops of concentrated hydrochloric acid (HCL), stored in a refrigerator, and analyzed within 48 h.

Pre-treatment of urinary samples

The refrigerated samples were allowed to come to room temperature. All the urine samples were first diluted 1 in 20 with distilled water prior to analysis. Methanol was then added to the well mixed and diluted urine samples to form a methanol concentration of 10% (v/v).

Urinary sample analysis

Estrogen was analyzed using the Ecologenia[®] Estrogen (E1/E2/E3) microplate ELISA kit (Envirochemicals Ltd, Japan),

Batch No. T2GR4.^[14] Samples were analyzed in duplicate and the average was used for calculation.

Analysis of results

Results were expressed as mean±standard deviation (mean±SD). Significant differences between means were determined by two-tailed Student's *t*-test using graph pad prism computer software program.

Result

Table 1 shows the statistics of the urinary estrogen level (µg/l) in women of different age groups. It also shows the mean±SD estrogen levels of the women in the 21-30, 31-40, and >40 year age groups. From the table, the highest and the lowest urinary estrogen levels were observed in women in the 21-30 and 31-40 year age groups, respectively.

Urinary estrogen levels (µg/l) of the women on contraceptives was significantly increased ($P=0.0482$) compared to those not on any form of contraceptive (controls) [Table 2].

Considering the secondary objective, the comparison between the women on the two classes of contraceptives; the IUD and oral/injectable showed no statistical difference ($P=0.8112$) in their mean urinary estrogen level [Table 3].

Table 1: Urinary Estrogen levels (µg/l) in women of different age groups, on contraceptive

	Age range (years)		
	21-30 (n=26)	31-40 (n=36)	>40 (n=22)
Minimum	10.00	2.000	2.000
Maximum	40.50	38.50	45.00
Mean	29.53	23.69	24.79
Median	31.50	26.75	25.00
Standard deviation	4.80	6.04	8.03
Standard error	2.89	3.02	6.07
Lower 95% CI	23.09	17.25	23.93
Upper 95% CI	35.97	30.13	39.64

Table 2: Urinary estrogen (µg/l) in women on contraceptive device and control

	Control (n=30)	Contraceptive device (n=84)
Mean	20.87	29.32
Standard deviation	14.68	5.06
<i>P</i> value	$P=0.0482$	

Table 3: Test of difference in mean of urinary estrogen (µg/l) between two different classes of contraceptive devices

	Contraceptive device	
	IUD (n=37)	Oral/Injectable (n=47)
Mean	22.75	23.99
Standard deviation	14.02	12.08
<i>P</i> value	$P=0.8112$	

Discussion

A total of 84 women on contraceptives and 30 women not on contraceptives were involved in the study and the outcome showed a statistically significant increase ($P=0.0462$) in mean urinary estrogen output of the women on contraceptive device compared to the control subjects. The synthetic estrogen content of some contraceptive devices could have contributed to the level excreted in the urine. When the contraceptive devices were divided into two classes of IUD and oral/injectables, there was no statistical difference ($P=0.8112$) in the mean urinary estrogen output of the women. This implies that the increased estrogen output observed in women on contraceptive device is not dependent on the class of contraceptive device used. The actual chemical compositions of the different classes of contraceptive devices used by the respondents were not available. However, most of them were estrogen-based devices.

Lifestyle practices, including dietary and beverage consumption, can influence estrogen metabolism as reflected in the urinary excretion of 2-hydroxyestrone (2-OHE1) and 16 α -hydroxyestrone (16 α -OHE1);^[15] however, this might not have been part of the reason for the significant difference observed in the urinary estrogen excretion in the women on contraceptives the study compared to the controls, since both groups of subjects were selected from the same part of the country where beverage consumption common. Understanding the contribution of lifestyle factors to estrogen catabolism is important because 16 α -hydroxyestrogens appear to retain substantial estrogenic activity through covalent binding to the estrogen receptor^[16] and histone proteins.^[17]

Increased risk of breast cancer has been reported in women with high serum and urinary estrogen levels,^[8] as well as those exposed to increased estrogen concentrations over time as a result of postmenopausal obesity, early onset of menstruation, and late menopause;^[9] hence, the women in the present study, with a significant increase in the urinary estrogen level should be cautious about the use of these groups of contraceptives, which most likely may predisposed them to breast cancer risk. Also, the study subjects would do well to increase their consumption of cruciferous vegetables, as this might enhance their estrogen metabolism. The roles of dietary lignans, normally found in the diet from whole grain and fruits and vegetable sources, and of indole-3 carbinol (I3C),^[18] most often sourced dietarily from cruciferous vegetables, have received increasing attention in the modulation of breast cancer risk.

Evidence for their metabolic effects on estrogen elimination pathways is emerging, in both epidemiological studies, and in clinical trials using either source foods or derived supplements.^[18] The highest concentration of urinary estrogen released by the 21-30 years age group in the present study might be linked to early onset of menstruation which was pointed out by Henderson *et al.*,^[6] as one of the factors which might expose women to increased estrogen concentrations over time and as a result,

increased risk of breast cancer. The presence of noticeable amount of environmental estrogens have been previously observed in tap water in Enugu municipality by Maduka *et al.*^[19] Although the concentration was not significant compared to other potable water sources such as well, river, and rain water. This said amount of environmental estrogen obviously did not affect the present study since both the control and test subjects are all exposed to the same potable water sources for their domestic water consumption and the test subjects still had significant increase in the urinary estrogen output compared to controls.

Conclusion

The synthetic estrogen content of contraceptive device could most probably have contributed to the level excreted in the urine, which was not dependent on the class of contraceptive device used. Adequate enlightenment should be made available for the women during their visits to the family planning clinics where these contraceptive devices are prescribed for them in order to enable them to make informed decisions about contraception and the impending risks involved. Also, contraceptives should be prescribed with caution since they appear to increase the endogenous estrogen levels which might predispose to breast cancer as shown by this study.

Study limitations

The major limitation of the study can be seen in the difference between the sample size of the test and control subjects. This was as a result of lack of willingness on the part of some subjects who should have been recruited as control subjects. Selection bias may play a role, if the overall response rate is relatively low (f50%), and the willingness to participate in a study may have been influenced by cultural beliefs and degree of acculturation.^[5]

Acknowledgement

The Authors sincerely appreciate the family planning clinic at the UNTH, Enugu, Nigeria, for allowing their patients to participate in this study. Without them, the study would not have been a success. Also to the women that made up the control subjects, we say, thank you for your participation.

References

1. Lintelmann J, Katayama N, Kurihara L, Shore L, Wenzel A. Endocrine Disruption in the Environment (IUPAC Technical Report). Pure App Chem 2003;75:631-81.
2. Ternes TA, Kreckel P, Miller J. Behavior and occurrence of estrogens in municipal sewage treatment plants. II. Aerobic batch experiments with activated sludge. Sci Total Environ 1999;225:91-9.
3. Baird DT, Glasier AF. Science, medicine and the future: Contraception. Br Med J 1999;319:969-72.
4. Key TJ, Pike MC. The role of oestrogens and progestagens in the epidemiology and prevention of breast cancer. Eur J Cancer Clin Oncol 1988;24:29-43.

5. Falk RT, Fears TR, Xu X, Hoover RN, Pike MC, Wu AH, *et al.* Urinary Estrogen Metabolites and their ratio among Asian American Women. *Cancer Epidemiol Biomarkers Prev* 2005;14:221-6.
6. Kabat GC, Chang CJ, Sparano JA, Sepkovic DW, Hu X, Khalil A, *et al.* Urinary Estrogen Metabolites and Breast Cancer: A case control study. *Cancer Epidemiol Biomarkers Prev* 1997;6:505-9.
7. Colditz GA. Relationship between estrogen levels, use of hormone replacement therapy, and breast cancer. *J Natl Cancer Inst* 1998;90:814-23.
8. Toniolo PG, Levitz M, Zeleniuch-Jacquotte A, Banerjee S, Koenig KL, Shore RE, *et al.* A prospective study of endogenous estrogens and breast cancer in postmenopausal women. *J Natl Cancer Inst* 1995;87:190-7.
9. Henderson BE, Ross R, Bernstein L. Estrogens as a cause of human cancer: The Richard and Hinda Rosenthal Foundation Award Lecture. *Cancer Res* 1988;48:246-53.
10. Harris RZ, Benet LZ, Schwartz JB. Gender effects in pharmacokinetics and pharmacodynamics. *Drugs* 1995;50:222-39.
11. Wellejus A, Olsen A, Tjonneland A, Thomsen BL, Overvad K, Loft S. Urinary hydroxyestrogens and breast cancer risk among postmenopausal women: A prospective study. *Cancer Epidemiol Biomarkers Prev* 2005;14:2137-42.
12. Lim SK, Won YJ, Lee JH, Kwon SH, Lee EJ, Kim KR, *et al.* Altered hydroxylation of estrogen in patients with postmenopausal osteopenia. *J Clin Endocrinol Metab* 1997;82:1001-6.
13. Seeger H, Mueck AO, Lippert TH. Effect of estradiol metabolites on the susceptibility of low density lipoprotein to oxidation. *Life Sci* 1997;61:865-8.
14. Japan Envirochemicals. Bisphenol-A and estrogen (E1/E2/E3) ELISA kits user's guide; Japan Envirochemicals Ltd, Tokyo. 105-0023, 2007. p. 1-10.
15. Bradlow HL, Hershcopf RE, Fishman JF. Oestradiol 16 alpha-hydroxylase: A risk marker for breast cancer. *Cancer Surv* 1986;5:573-83.
16. Swaneck GE, Fishman J. Covalent binding of the endogenous estrogen 16 α -hydroxyestrone to estradiol receptor in human breast cancer cells: Characterization and intranuclear localization. *Proc Natl Acad Sci USA* 1988;85:7831-5.
17. Yu SC, Fishman J. Interaction of histones with estrogens. Covalent adduct formation with 16 α -hydroxyestrone. *Biochem* 1985;24:8017-21.
18. Higdon JV, Delage B, Williams DE, Dashwood RH. Cruciferous vegetables and human cancer risk: Epidemiological evidence and mechanistic base. *Pharmacol Res* 2007;55:224-36.
19. Maduka IC, Ezeonu FE, Neboh EE, Shu SN, Ikekpeazu EJ. BPA and Environmental estrogen in potable water sources in Enugu municipality, South-East, Nigeria. *Bull Environ Contam Toxicol* 2010;85:534-7.

How to cite this article: Maduka IC, Ezeonu FE, Neboh EE, Shu EN, Ikekpeazu EJ. Urinary estrogen levels in women on contraceptives in Enugu, South-East Nigeria. *J Fam Med Primary Care* 2012;1:39-42.

Source of Support: Nil. **Conflict of Interest:** None declared.

Staying in touch with the journal

1) Table of Contents (TOC) email alert

Receive an email alert containing the TOC when a new complete issue of the journal is made available online. To register for TOC alerts go to www.jfmpc.com/signup.asp.

2) RSS feeds

Really Simple Syndication (RSS) helps you to get alerts on new publication right on your desktop without going to the journal's website. You need a software (e.g. RSSReader, Feed Demon, FeedReader, My Yahoo!, NewsGator and NewzCrawler) to get advantage of this tool. RSS feeds can also be read through FireFox or Microsoft Outlook 2007. Once any of these small (and mostly free) software is installed, add www.jfmpc.com/rssfeed.asp as one of the feeds.