Introduction

Progesterone (P) and estrogen (E) are steroid hormones needed for the intact physiology of the female reproductive system [1-5], and are key components in the complex regulation of normal female reproductive function. The major physiological roles of P and E are in uterus, ovary, mammary gland, and brain. There is also evidence that supports the role for P and E in modulation of normal female reproductive function. The major physiological roles of P and E are in uterus, ovary, mammary gland, and brain. Therefore, large studies have shown that unopposed P secretion may lead to the development of breast cancer and fibroids within the myometrium [8-11]. Nonetheless, P can also be a protective factor from the development of uterine cancer [12, 13]. Regarding E, it is well known that women with polycystic ovary syndrome (PCOS) have increased levels of this hormone [14], and it was demonstrated that women with PCOS have a three times higher risk to develop endometrial cancer [15].

The levels of these hormones can be surveyed through plasma or urine samples. However, the major limitation of plasma and urine hormone determination is rapid diurnal fluctuations of hormone concentration. Since hair grows at approximately 1 cm per month, scalp hormone determination offers an alternative tool to measure body hormone exposure over several weeks to months [16, 17], providing information on long-term hormone production. Over the past years, hair analyses for endogenous hormones, particularly cortisol, have been widely applied especially in fields of psychiatric and stress-related research [18]. To date, only few studies have shown that it is feasible to measure hair P concentration using ‘gas chromatography–mass spectrometry’ (GC–MS) [19]. However, while GC–MS showed good performance, it is time-consuming, costly, and not widely available [20].

The aim of this study was to determine the feasibility of measuring E and P scalp-hair concentration in pre- and post-menopausal women, using a widely used commercial immunoassay, which is in use to detect serum E and P in most laboratories.

Materials and Methods

This study is a prospective cohort study of 48 healthy women. Inclusion criteria were women under the age of 40 years with regular menstrual cycles, and postmenopausal women over the age of 50 years, having no menstrual cycles for at least five years. Exclusion criteria included pregnancy, lactation, recent use of glucocorticoid medications, recent oral contraception use, BMI, above 35 kg/m² and women having fertility problems including PCOS, unexplained infertility, or premature ovarian failure.

Authorization by The National Institute of Child Health and Development Institutional Review Board was obtained for the study (ClinicalTrials.gov identifier NCT02755038), and all women provided a written informed consent.

Summary

Background: The major limitation of blood hormone measurements is the rapid daily concentration fluctuations. However, hair hormone measurement may provide an information on chronic body hormone exposure. Objective: The aim of the current study is to determine concentration of estradiol (E) and progesterone (P) in scalp hair of pre and postmenopausal women, using the widely used commercial immunoassay, which is also in use to detect serum E and P in most laboratories. Materials and Methods: The study included women under age of 40 years with regular cycles, and postmenopausal women over the age of 50 years, with no cycles for at least five years. Following hair processing, concentration of E and P in hair was assayed by chemiluminescent enzyme immunoassay. Results: The study included 22 premenopausal women (mean age of 30±4.8 years), and 17 postmenopausal women, (mean age of 62 ± 8.1 years). Mean E and P concentrations were higher in premenopausal women compared to postmenopausal women; however, it did not reach statistical significance [8.6 (3.3-16.4) vs. 5.9 (4.3-8.7) pmol/gr-hair (p = 0.53)] and 0.006 ([0.004-0.011] vs. 0.005 (0.003-0.006) pmol/gr-hair (p = 0.10), respectively). Conclusion: Hair E and P concentrations can be quantified by immunoassay but it may not be the optimal method. Future studies should be conducted in order to determine the optimal technique in measurement of these hormones from scalp hair.

Key words: Estradiol; Progesterone; Hair; Immunoassay; Measurement.

Determination of estradiol and progesterone concentrations in human scalp hair

E. Kadour-Peero1,†, S. Khoury1,2, J. Awad1, L. Shpritz1, L. Chen-Konak2, C. Shechner2, L. Saiegh2

1Department of Obstetrics and Gynecology, Bnai -Zion Medical Center, Faculty of Medicine, Technion - Israel Institute of Technology, Haifa
2Department of Endocrinology, Bnai -Zion Medical Center, Haifa (Israel)

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Hair sampling and processing: for each subject, hair sample of approximately 100-150 strands and 2-3 cm long was collected using scissors from the posterior vertex, as close to the scalp as possible. Hair was grinded into powder using a mini-bead beater machine. Hair powder was incubated overnight in 1 ml of 50°C methanol while continuously and gently shaken. Then, methanol was removed from the hair and evaporated by nitrogen stream and the residue was reconstituted in 250 µl PBS buffer (pH 8.0) for hormone concentration determination.

Assays: E concentration was measured using solid phase competitive chemiluminescent enzyme immunoassay by an automated analyzer Immulite 2000. The method’s sensitivity was 55 pmol/l and intra- and inter-assay coefficients of variations were 7.8% and 6.4%, respectively. P concentration was also measured using solid phase competitive chemiluminescent enzyme immunoassay by an automated analyzer Immulite 2000. The method’s sensitivity was 0.3 nmol/L and intra- and inter-assay coefficients of variations were 21.7% and 17.4%, respectively.

SPSS version 21 was used for statistical analysis. Descriptive statistics were performed using mean, SD, median and interquartile range 25-75%. Normal distribution of the quantitative parameters was tested by Kolmogorov Smirnov test, and according to these results, parametric and non-parametric tests were used. Differences between groups (pre-menopausal vs. post-menopausal women) were tested by t-test, Mann Whitney U and Fisher exact tests, and p < 0.05 was considered as significant.

Results

This prospective study included 48 healthy women. Nine women were excluded from the study because of an insufficient hair samples or omitted data. Thirty-nine healthy adult volunteers were recruited for the study; 22 pre-menopausal women (mean age of 30 ± 4.8 years), and 17 post-menopausal women (mean age of 62 ± 8.1 years).

The demographic characteristics are presented in Table 1. The mean age of the pre-menopausal women was 30.1 years, compared to 62 years in the post-menopausal group. (p < 0.0001) Gravidity and parity were higher in the post-menopausal group compared to the pre-menopausal group. (3.5 vs. 1, p < 0.0001, and 3 vs. 1, p = 0.001, respectively) Post-menopausal women had a higher BMI (27.6 kg/m² ± 5.2 vs. 22.4 kg/m² ± 3.3, p = 0.001), and a higher rate of medical disorders than pre-menopausal women (75% vs. 18%, p = 0.008) (Table 1). There were no differences in the frequency of hair smoothing or coloring between the two groups [(27% in the pre-menopausal group vs. 12% in the post-menopausal group, p = 0.42) and (68% vs. in the pre-menopausal group vs. 88% in the post-menopausal group, p = 0.25), respectively]. Pre-menopausal women had a higher median hair E and P concentrations than pre-
menopausal women, however it did not reach statistical significance [8.6 (3.3-16.4) vs. 5.9 (4.3-8.7) pmol/gr hair (p = 0.53) and 0.006 (0.004-0.011) vs. 0.005 (0.003-0.006) pmol/gr hair (p = 0.10), respectively] (Figure 1).

Discussion

In the current study, the authors showed that it is possible to quantify E and P concentrations in scalp hair using a commercially available immunoassay, however it was not enough sensitive so show differences between groups. Consequently, immunoassay may not be the optimal method. This study represents the largest series addressing the feasibility to determine E and P hair concentrations, using the widely-used commercial immunoassay, which is also in use to determine serum E and P concentrations in most laboratories.

Although the mechanism of hormone incorporation into hair is not fully understood, measurement of hormone levels in scalp hair may be very promising technique. Until now, relatively small study populations were used, and therefore study replication is needed to evaluate the use of hair hormone as a marker of long-term endogenous hormone levels. Since Raul et al. detected for the first time the physiological concentration of cortisol and cortisone in human hair [20], hair analysis for cortisol has been introduced to the field of biopsychology and endocrinology. Hair cortisol has the potential to reflect long-term activity of the hypothalamo-pituitary-adrenal axis (HPA) and hair cortisol determination by immunoassay was shown to be a valid retrospective biomarker regarding changes for HPA activity related to chronic stress or to endogenous autonomous cortisol over secretion. [21–25] Regarding E and P, as premenopausal women have functioning ovaries, the present authors expected that their hair E and P concentrations would be significantly higher than in postmenopausal women. In the current study mean hair E and P concentrations were higher in premenopausal women compared to postmenopausal women; however, they did not reach statistical significance. One explanation of the non-significant difference between the two groups can be the small sample size or limited sensitivity of the used immunoassay. Although no correlation is known between E and P concentrations with weight, it cannot be ruled out that the difference in BMI values between the two groups caused some bias in the results. Despite the fact that the present authors did not find significant difference between hair E and P concentrations between premenopausal and postmenopausal women, the possibility cannot be ruled out that women harboring diseases influenced by abnormal E and P exposure such as PCOS, might have inappropriate levels of scalp hair hormones that could be determined using this available immunoassay.

The main limitation of the present study is the lack of plasma samples from women who volunteered in the study. Another limitation is the small sample size. Nevertheless, the present study represents the largest prospective series addressing the feasibility to determine E and P hair concentration, using the widely-used commercial immunoassay.

Conclusion

Hair E and P concentrations can be quantified by immunoassay; however, it is not be the optimal method. Future studies should be conducted in order to determine the optimal technique in measurement of these hormones in scalp hair and to evaluate its use as a biomarker of long-term endogenous hormone concentration.

References


Corresponding Author:
EINAV KADOUR-PEERO, M.D.
Department of Obstetrics and Gynecology,
Bnai-Zion Medical Center, Faculty of Medicine
Technion - Israel Institute of Technology
Haifa 30056 (Israel)
e-mail: einavkadour@gmail.com