Published in final edited form as:
Cancer Epidemiol Biomarkers Prev. 2008 August ; 17(8): . doi:10.1158/1055-9965.EPI-08-0103.

Reproducibility of Serum Pituitary Hormones in Women

Alan A. Arslan[1,2,3,*], **Yian Gu**[1], **Anne Zeleniuch-Jacquotte**[1,3], **Karen L. Koenig**[1], **Mengling Liu**[1], **Lyudmila Velikokhatnaya**[4], **Roy E. Shore**[5], **Paolo Toniolo**[2,3], **Faina Linkov**[4], and **Anna E. Lokshin**[4]

[1]Department of Environmental Medicine, New York University School of Medicine, 650 First Avenue, New York, NY 10016, USA

[2]Department of Obstetrics and Gynecology, New York University School of Medicine, New York, 550 First Avenue NBV-9E2, New York, NY 10016, USA

[3]New York University Cancer Institute, New York University School of Medicine, New York, NY 10016, USA

[4]Department of Medicine, University of Pittsburgh Cancer Institute, University of Pittsburgh, 5117 Centre Avenue, Pittsburgh, PA 15213, USA

[5]Radiation Effects Research Foundation, Hiroshima, Japan

Abstract

Endogenous pituitary hormones are commonly used in clinical and epidemiological studies and some of them are thought to influence risk of several diseases in women. In most studies, endogenous levels of pituitary hormones are usually assessed at a single point in time, assuming that this single measurement represents the long-term biomarker status of the individual. Such an assumption is rarely tested and may not always be valid.

This study examined the reproducibility of the following pituitary hormones: adrenocorticotropic hormone (ACTH), growth hormone (GH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), thyroid-stimulating hormone (TSH), and prolactin (PRL) measured using the Luminex xMap™ method in serum of healthy premenopausal and postmenopausal women.

The study included 30 premenopausal women with three yearly samples and 35 postmenopausal women with two repeated yearly samples randomly selected from an existing prospective cohort. Analysis of intraclass correlation coefficients (ICCs) suggested higher reproducibility in postmenopausal women compared to premenopausal women for the following hormones: FSH (0.72 and 0.37, respectively), LH (0.83 and 0.44, respectively), and GH (0.60 and 0.35, respectively). The ICCs were relatively high and similar between postmenopausal and premenopausal women for ACTH (0.95 and 0.94, respectively), TSH (0.85 and 0.85, respectively), and PRL (0.72 and 0.69, respectively).

This study found that serum concentrations of FSH, LH, and GH are stable in postmenopausal women, and that ACTH, TSH, and PRL are stable in both premenopausal and postmenopausal women suggesting that a single measurement may reliably categorize average levels over at least a 2-year period.

[*]**Corresponding author**: Dr. Alan A. Arslan, Department of Obstetrics and Gynecology, New York University School of Medicine, 550 First Avenue NBV-9E2, New York, NY 10016, USA, Telephone: (212) 263 7792; Fax: (212) 263 8887; akhmea01@med.nyu.edu.

Keywords

Reliability; pituitary hormones; serum; intra-class correlation; prospective cohort

Introduction

The anterior lobe of the pituitary gland secretes several hormones playing critical roles in body growth (growth hormone, GH), function of adrenal glands (adrenocorticotropic hormone, ACTH), regulation of thyroid secretion (thyroid-stimulating hormone, TSH), secretion of breast milk (prolactin, PRL), and development and function of the reproductive system (follicle-stimulating hormone, FSH, and luteinizing hormone, LH).

Endogenous levels of pituitary hormones are commonly used in epidemiological studies and some of them are thought to influence risk of several diseases in women. For example, prolactin and GH have been shown to be associated with risk of breast cancer (1–3); gonadotropins (FSH, LH) are thought to play a role in ovarian cancer (4, 5) and ACTH and prolactin may play a role in osteoporosis (6).

The majority of the previous studies relied on a single measurement assuming that the intra-individual variability in hormone levels is smaller than the inter-individual variability. However, data on the intra-individual variability of pituitary hormones are limited. With large intra-individual variability, a single measurement may include a large degree of measurement error and, subsequently, observed associations and risk estimates could be substantially attenuated. Therefore, it is important to assess how well a single hormone measurement reflects longer term levels before conducting and evaluating studies of these associations.

The objective of the present study was to assess the reproducibility of a number of serum markers over a 2–3 year period measured using the Luminex xMap™ method in premenopausal and postmenopausal women. The markers analyzed for the current report include the following pituitary hormones: ACTH, GH, FSH, LH, TSH, and PRL.

Materials and Methods

Subjects

Participants were selected from women participating in the New York University Women's Health Study (NYUWHS) prospective cohort. Between March 1985 and June 1991, 14,274 women 35 to 65 years old were enrolled at a mammography screening center in New York. The cohort was restricted to women who in the preceding 6 months were neither pregnant nor treated with hormones (7, 8).

Blood Sampling

At the time of enrollment and at annual screening visits thereafter, subjects were asked to provide 30 mL of non-fasting peripheral venous blood, drawn using collection tubes without anticoagulant. Serum aliquots were stored at -80°C for future biochemical analyses. Fifty-one percent of the cohort members donated blood on more than one occasion, making a reproducibility study feasible.

Reproducibility Study Design

Subjects were selected at random among NYUWHS participants who fulfilled the criteria listed below: repeated blood donations (at least 2 yearly samples for postmenopausal women

and at least 3 yearly visits for premenopausal women); no diagnosis of any cancer (except non-melanoma skin cancer) or cardiovascular disease; no use of any exogenous sex hormones at the time of any of the selected blood donations. Subjects who had been included as cases or controls in any previous nested case-control study were not eligible. Women were classified as postmenopausal if they reported a) the absence of menstrual cycles in the previous 6 months; or b) a total bilateral oophorectomy; or c) a hysterectomy without total oophorectomy and their age was 52 years or older. Women were classified as premenopausal if they reported at least one menstrual cycle during the past 6 months prior to enrollment. In addition, for each premenopausal woman, the phase of menstrual cycle was calculated from the date of next menstruation, which was obtained from mail-back calendars distributed at the time of blood drawing. Based on the number of days before the first day of the next menstrual period, a subject was considered in luteal phase (0–11 days), ovulatory (12–16 days), or follicular (≥17 days) phase of the cycle at the time of blood donation (9). The annual serum samples of a given subject were collected usually 1-year apart during the same month, thereby limiting the potential effect of seasonal variation in hormone levels.

Among women meeting eligibility criteria, we randomly selected 35 postmenopausal women with two yearly samples and 30 premenopausal women with three yearly samples (with 2 of the samples taken during the same phase of cycle). For quality control, random duplicate samples of 5 premenopausal and 5 postmenopausal women were selected and analyzed on the same well-plate as the matching samples in order to assess intra-batch coefficients of variation (CVs). All samples were labeled to ensure blinding of the laboratory personnel.

Luminex Assay Specifications and Procedures

Serum one-milliliter aliquots which had not been previously thawed were packed in dry ice and sent to the laboratory, where they were stored at -80°C until they were assayed in a single batch. Hormones were analyzed using the xMap™ technology which combines the principle of a sandwich immunoassay with fluorescent-bead-based technology allowing multiplex analysis of up to 100 different analytes in a single microtiter well (10). Serum ACTH, GH, FSH, LH, TSH, and PRL were measured using Human Pituitary LINCO*plex* kits provided by Linco/Millipore Research (Billerica, MA). The xMap™ serum assays were done in 96-well microplate format according to the protocols. A filter-bottom, 96-well microplate (Millipore, Billerica, MA) was blocked for 10 minutes with PBS/bovine serum albumin. To generate a standard curve, 5-fold dilutions of appropriate standards were prepared in serum diluent. Standards and patient sera were pipetted at 50 μL per well and mixed with 50 μL of the bead mixture. The microplate was incubated for 1 hour at room temperature on a microtiter shaker. Wells were then washed twice with washing buffer using a vacuum manifold. Phycoerythrin (PE)-conjugated secondary antibody was added to the appropriate wells and the wells were incubated for 45 minutes in the dark with constant shaking. Wells were washed twice, assay buffer was added to each well, and samples were analyzed using the Bio-Plex suspension array system (Bio-Rad Laboratories, Hercules, CA). The samples were analyzed in a single measurement because in Luminex platform, reaction-to-reaction CV is measured based on analysis of 100 beads, each representing a separate reaction. Analysis of data was done using four-parameter-curve fitting (11). The inter-batch CVs were 7.4% for ACTH, 14.9% for GH, 4.9% for FSH, 6.7% for LH, 3.0% for TSH, and 8.2% for PRL. The intra-batch CVs were 10.8% for ACTH, 5.4% for GH, 7.2% for FSH, 6.3% for LH, 6.9% for TSH, and 7.0% for PRL.

Statistical Analysis

All analyses were performed on natural-logarithm-transformed values in order to reduce the positive skewness of the raw data. The temporal reproducibility was estimated by the

intraclass correlation coefficient (ICC), which is defined as the proportion of the total variability that is due to between-subject variability. The variance components were estimated with a random effects one-way analysis of variance model, using the SAS procedure MIXED. Exact 95% confidence intervals (CIs) for the ICCs were calculated as described by McGraw & Wong (12). All analyses were performed using SAS 9.1 (SAS Institute, Cary, NC).

Results

Table 1 presents geometric means (10–90%) of pituitary hormones by menopausal status. As expected, postmenopausal women had higher median levels of FSH and LH and lower levels of PRL and GH compared to premenopausal women. Levels of TSH and ACTH were comparable between premenopausal and postmenopausal women.

Geometric mean levels of pituitary hormones were fairly stable from visit to visit (Table 1). There were slight decreases in ACTH and LH levels in premenopausal women at year 3 visit compared to visits at year 1, but the differences were not statistically significant.

Analysis of ICCs (Table 2) suggested that reproducibility for FSH, LH, and GH differ by menopausal status. We observed high to moderate correlations across donations for these hormones in postmenopausal women (ICCs of 0.72, 0.83, and 0.60 for FSH, LH, and GH, respectively). In premenopausal women, ICCs for samples collected in all phases of the cycle had substantially lower temporal reproducibility (ICCs of 0.37, 0.44, and 0.35 for FSH, LH, and GH, respectively). However, restricting the analyses to repeated samples in the same phase of the menstrual cycle improved the reproducibility (ICCs of 0.62, 0.64, and 0.42 for FSH, LH, and GH, respectively). The ICCs were relatively high and very similar between postmenopausal and premenopausal women in all phases of cycle for ACTH (0.95 and 0.94, respectively), TSH (0.85 and 0.85, respectively), and PRL (0.72 and 0.69, respectively). Adjustment for body mass index, age, race/ethnicity, medication use, alcohol consumption, and smoking status at baseline did not change the ICCs substantially (data not shown).

Discussion

The importance of assessing the reliability of exposure measurement prior to planning the epidemiological studies is based on the fact that poor reliability may reduce the effective sample size (13), result in a loss of statistical power and a bias toward unity in relative risk estimates (14). The issue of reliability is even more important for cohort studies utilizing prospectively collected biological samples, where utilization of the valuable specimens for only reliable exposure measurements should be given priority.

The results of the study demonstrate that stability of pituitary hormone serum levels measured using the Luminex xMap™ method varies by menopausal status. In postmenopausal women, the levels of all six pituitary hormones studied (ACTH, GH, FSH, LH, TSH, and PRL) were very stable from visit to visit over 2–3 year period. In premenopausal women, three hormones (FSH, LH, and GH) demonstrated low reproducibility if the phase of menstrual cycle is not taken into account. However, measurements in the same phase of menstrual cycle yielded improved reproducibility of these hormones, whereas ACTH, TSH, and PRL demonstrated high reproducibility regardless of the phase of menstrual cycle.

To date, a limited number of studies had assessed the reproducibility of serum levels of pituitary hormones, with the exception of prolactin. Two studies have shown that prolactin is moderately to highly reproducible over at least a 2-year period in postmenopausal women

(9, 15). One study found low reliability of serum prolactin in postmenopausal women (16). In premenopausal women, studies have shown moderate reliability of prolactin with ICCs ranging from 0.40 (16) to 0.64 (17). The results of our study are consistent with the conclusion that a single measurement of prolactin is sufficient to characterize the serum prolactin level in both postmenopausal and premenopausal women.

The study results confirm the previous reports that a single measurement is sufficient to characterize the serum FSH level in postmenopausal women (18, 19) but not in premenopausal women (18) unless phase of menstrual cycle is taken into account. A similar conclusion can be made in regard to the reproducibility of LH in serum. We are not aware of previous studies that assessed the temporal reproducibility of TSH, GH, and ACTH. Our results suggest that TSH and ACTH have moderate to high reproducibility over a 2–3 year period in both premenopausal and postmenopausal women, whereas GH has lower reproducibility in premenopausal women.

Our study had several limitations. The study population included women only, so the results may not be generalized to males. The study assessed relatively short-term reliability using samples collected 2–3 years apart and did not investigate the effects of seasonal variability upon reproducibility. Studies of long-term reliability would be of great interest.

In conclusion, this study using Luminex xMAP™ method found that serum concentrations of FSH, LH, and GH are stable in postmenopausal women, and that ACTH, TSH, and PRL are stable in both premenopausal and postmenopausal women suggesting that a single measurement may reliably categorize average levels over at least a 2-year period.

Acknowledgments

Financial Support: This research was supported by the National Institutes of Health grants R01 CA98661 and R03 CA96428, and by Cancer Center grant CA16087 from the National Cancer Institute and center grant ES00260 from the NIEHS.

References

1. Hankinson SE, Willett WC, Michaud DS, et al. Plasma prolactin levels and subsequent risk of breast cancer in postmenopausal women. J Natl Cancer Inst. 1999; 91:629–634. [PubMed: 10203283]
2. Tworoger SS, Hankinson SE. Prolactin and breast cancer risk. Cancer Lett. 2006; 243:160–169. [PubMed: 16530327]
3. Wennbo H, Tornell J. The role of prolactin and growth hormone in breast cancer. Oncogene. 2000; 19:1072–1076. [PubMed: 10713692]
4. Cramer DW, Welch WR. Determinants of ovarian cancer risk. II. Inferences regarding pathogenesis. J Natl Cancer Inst. 1983; 71:717–721. [PubMed: 6578367]
5. Choi JH, Wong AS, Huang HF, Leung PC. Gonadotropins and ovarian cancer. Endocr Rev. 2007; 28:440–461. [PubMed: 17463396]
6. Matsuyama J, Eshima N, Fukunaga T, Hori S, Kobayashi H, Isono M. Various risks of osteoporosis in patients with pituitary adenomas. J Bone Miner Metab. 2003; 21:91–97. [PubMed: 12601573]
7. Toniolo PG, Pasternack BS, Shore RE, et al. Endogenous hormones and breast cancer: a prospective cohort study. Breast Cancer Res Treat. 1991; 18(Suppl 1):S23–S26. [PubMed: 1873553]
8. Toniolo PG, Levitz M, Zeleniuch-Jacquotte A, et al. A prospective study of endogenous estrogens and breast cancer in postmenopausal women. J Natl Cancer Inst. 1995; 87:190–197. [PubMed: 7707406]
9. Koenig KL, Toniolo P, Bruning PF, Bonfrer JM, Shore RE, Pasternack BS. Reliability of serum prolactin measurements in women. Cancer Epidemiol Biomarkers Prev. 1993; 2:411–414. [PubMed: 8220084]

10. Vignali DA. Multiplexed particle-based flow cytometric assays. J Immunol Methods. 2000; 243:243–255. [PubMed: 10986418]
11. Little JA. Comparison of curve fitting models for ligand binding assays. Chromatographia. 2004; 59:S177–S181.
12. McGraw KO, Wong SP. Forming inferences about some intraclass correlation coefficients. Psychol Methods. 1996; 1:30–46.
13. McKeown-Eyssen GE, Tibshirani R. Implications of measurement error in exposure for the sample sizes of case-control studies. Am J Epidemiol. 1994; 139:415–421. [PubMed: 8109576]
14. de Klerk NH, English DR, Armstrong BK. A review of the effects of random measurement error on relative risk estimates in epidemiological studies. Int J Epidemiol. 1989; 18:705–712. [PubMed: 2807678]
15. Hankinson SE, Manson JE, Spiegelman D, Willett WC, Longcope C, Speizer FE. Reproducibility of plasma hormone levels in postmenopausal women over a 2-3-year period. Cancer Epidemiol Biomarkers Prev. 1995; 4:649–654. [PubMed: 8547832]
16. Muti P, Trevisan M, Micheli A, et al. Reliability of serum hormones in premenopausal and postmenopausal women over a one-year period. Cancer Epidemiol Biomarkers Prev. 1996; 5:917–922. [PubMed: 8922301]
17. Missmer SA, Spiegelman D, Bertone-Johnson ER, Barbieri RL, Pollak MN, Hankinson SE. Reproducibility of plasma steroid hormones, prolactin, and insulin-like growth factor levels among premenopausal women over a 2- to 3-year period. Cancer Epidemiol Biomarkers Prev. 2006; 15:972–978. [PubMed: 16702379]
18. Arslan AA, Zeleniuch-Jacquotte A, Lukanova A, Rinaldi S, Kaaks R, Toniolo P. Reliability of follicle-stimulating hormone measurements in serum. Reprod Biol Endocrinol. 2003; 1:49. [PubMed: 12844363]
19. Falk RT, Dorgan JF, Kahle L, Potischman N, Longcope C. Assay reproducibility of hormone measurements in postmenopausal women. Cancer Epidemiol Biomarkers Prev. 1997; 6:429–432. [PubMed: 9184776]

Table 1

Geometric mean levels (10–90 percentile) of serum pituitary hormones at baseline and during repeated visits by menopausal status

Hormone	Premenopausal women n = 30			Postmenopausal women n= 35	
	Baseline visit	Year 2 visit	Year 3 visit	Baseline visit	Year 2 visit
FSH, mIU/mL	5.8 (2.8–12.4)	5.9 (2.7–11.6)	6.3 (2.2–14.8)	64.2 (30.0–111.4)	64.7 (28.9–110.9)
LH, mIU/mL	5.0 (2.0–14.9)	4.7 (1.8–11.5)	4.6 (1.4–10.0)	29.7 (17.9–44.9)	27.1 (16.6–46.0)
TSH, mIU/mL	2.2 (1.2–3.8)	2.0 (0.8–4.1)	2.2 (1.0–4.9)	2.0 (0.8–3.4)	1.9 (0.9–3.8)
PRL, ng/mL	14.4 (6.0–23.1)	13.6 (7.0–25.6)	13.8 (7.7–25.0)	10.7(5.6–18.7)	9.1 (4.5–16.8)
GH, ng/mL	0.9 (0.2–5.9)	1.1 (0.3–6.1)	0.9 (0.2–4.5)	0.5 (0.1–3.5)	0.4 (0.1–1.8)
ACTH, pg/mL	10.4 (<3–39.2)	10.3 (3.7–35.2)	8.4 (<3–34.9)	11.2 (5.1–29.6)	11.2 (4.3–8.2)

Table 2

ICCs (95% CIs) for repeated measures of pituitary hormones by menopausal status

Hormone	Premenopausal women (all phases of cycle) n = 30; 3 visits		Premenopausal women (same phase of cycle)* n = 30; 2 visits		Postmenopausal women n = 35; 2 visits	
	ICC	95% CI	ICC	95% CI	ICC	95% CI
FSH	0.37	0.15–0.60	0.62	0.35–0.80	0.72	0.52–0.85
LH	0.44	0.21–0.65	0.64	0.38–0.81	0.83	0.68–0.91
TSH	0.85	0.74–0.92	0.81	0.65–0.91	0.85	0.72–0.92
PRL	0.69	0.52–0.82	0.58	0.29–0.78	0.72	0.52–0.85
GH	0.35	0.13–0.58	0.42	0.08–0.67	0.60	0.34–0.78
ACTH	0.94	0.90–0.97	0.93	0.86–0.97	0.95	0.91–0.98

*Limited to 30 premenopausal women with 2 visits at the same phase of menstrual cycle.