Luteal-phase inhibin A and follicular-phase inhibin B levels are not characteristic of patients with an elevated LH-to-FSH ratio

Erik E. Hauzman · Péter Fancsovits · Ákos Murber ·
Thomas Rabe · Thomas Strowitzki · Zoltán Papp ·
János Urbancsek

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Abstract *Purpose:* To test whether serum inhibin levels are related to differences in gonadotropin concentrations between patients with an elevated LH-to-FSH ratio (ELF patients) and controls.

Methods: 32 ELF patients were matched with controls by age, body mass index (BMI), and cycle length.

Results: No statistically significant difference was found in follicular-phase inhibin B levels or midluteal inhibin A levels between cases and controls. Significant negative correlation was observed between follicular-phase inhibin B concentrations and BMI in ELF patients but not among controls. LH and FSH were positively related to inhibin B levels in ELF patients. Midluteal inhibin A correlated with sex hormone-binding globulin in controls but not in ELF patients.

Conclusions: Neither follicular-phase inhibin B levels nor midluteal inhibin A levels are characteristic of patients with an elevated LH-to-FSH ratio. Opposite correlations with LH and BMI suggest dysregulation of inhibin secretion rather than dimeric inhibins having a central role to the endocrinological imbalance observed in polycystic ovary syndrome.

Keywords FSH · Inhibin A · Inhibin B · LH · Polycystic ovary syndrome

Introduction

Chronic anovulation is a major cause of infertility, with approximately 80% of anovulatory patients presenting with normal FSH concentrations [1]. The most common cause of normogonadotropic normoestrogenic ovulatory disorders (constituting the so-called WHO 2 group) is polycystic ovary syndrome (PCOS), characterized by chronic anovulation, hyperandrogenism [2], and often by hyperinsulinemia [3].

Inhibins are gonadally derived heterodimeric glycoprotein hormones, which are built up from an α and one of two partially homologous β subunits, β_A or β_B , giving rise to the two isoforms inhibin A and inhibin B. Inhibin B levels increase from the early follicular phase to reach a peak coinciding with the onset of the midfollicular phase decline in FSH levels and then decrease during the luteal phase apart from a periovulatory peak [4]. This suggests inhibin B secretion by the developing small antral follicles. In fact, it was demonstrated in regularly cycling women that inhibin B is the predominant form of inhibin in the follicular fluid throughout the follicular phase of the cycle [5]. By contrast, inhibin A concentrations are low in the early follicular phase, show a small midfollicular peak and, after a rapid rise after ovulation, reach maximal levels during the midluteal phase. This profile is suggestive of secretion by the dominant follicle [6]. Indeed, inhibin A is detectable in follicles as small as 6.5 mm and its concentration in follicular fluid increases linearly with follicle size [7].

It has been postulated that inhibins might stimulate LH-induced androgen biosynthesis in theca cells in vitro [8, 9] and thus contribute to the pathogenesis of PCOS. It was also suggested that inhibin B might be responsible

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E. E. Hauzman (✉) · P. Fancsovits · Á. Murber · Z. Papp ·
J. Urbancsek

Department of Obstetrics and Gynecology, Semmelweis
University, Faculty of Medicine, P.O. Box 104, H-1442,
Budapest, Hungary
e-mail: humanic@noi1.sote.hu

T. Rabe · T. Strowitzki
Division of Gynecological Endocrinology, Department of
Obstetrics and Gynecology, Ruprecht-Karls University,
Heidelberg, Germany

for an elevated LH/FSH ratio observed in most patients with PCOS, since it selectively suppresses the secretion of FSH from the pituitary [10]. Furthermore, inhibin B concentrations were supposed to mirror the extent of ovarian dysfunction since inhibin B is mainly produced by small follicles, which are to be found in increased numbers in PCOS patients [11]. Decreased inhibin concentrations during the midluteal phase may be at least in part responsible for impaired corpus luteum function often present in PCOS. However, to the best of our knowledge, only total immunoreactive inhibin levels have been compared in the second half of the menstrual cycle between PCOS patients and normal controls [12].

Recent studies have reported an inverse correlation between inhibin B levels and body mass index (BMI) in PCOS, suggesting a role of some factor responsible for decreased inhibin B levels observed in obese PCOS patients [1, 13, 14]. More recently, it was shown that the negative effect of obesity on inhibin B levels is not specific to PCOS [15], and it was demonstrated by an interventional study that insulin is involved in the negative regulation of inhibin B secretion [16], which is in line with reports on the resumption of follicular growth and ovulation that occur with the use of insulin-lowering agents [17, 18].

Contradictory results have been published in the literature regarding serum and follicular fluid levels of inhibins in PCOS. Some authors reported raised concentrations of total immunoreactive inhibins [19], α -inhibin [9, 13] or inhibin B [20, 21, 22], supporting the above hypotheses, while others observed normal levels of total immunoreactive inhibin [12] or inhibin B [1, 10, 13, 23] in the early follicular phase.

The goal of our retrospective study was to test whether there is a difference in early follicular phase serum inhibin B levels between PCOS patients and controls that might be responsible for the differences in serum gonadotropin levels and to see if inhibin B concentrations are related to other endocrine parameters. Since PCOS patients can be characterized by elevated LH levels in ~60% of cases [24] and an elevated LH-to-FSH ratio can be observed in up to 95% of women with PCOS [25], we used this simple criterion to select the cases from our patient collective. We also performed midluteal measurements of serum inhibin A concentrations to test whether the reported difference in total immunoreactive inhibin levels in the midluteal phase can be specifically demonstrated on the dominant inhibin isoform of the luteal phase.

Materials and methods

Patients

In a case-control design, 32 patients with a follicular-phase LH-to-FSH ratio ≥ 2 (henceforth referred to as patients with

an elevated LH-to-FSH ratio or ELF patients) were matched with patients with LH-to-FSH ratio ≤ 1 . These controls were selected from patients attending our division of assisted reproduction with tubal and/or male factor infertility. No patients with clinical or laboratory signs of PCOS were included as controls. Matching was performed by age, BMI, and cycle length.

The study was approved by the institutional review board of the First Department of Obstetrics and Gynecology, Semmelweis University Faculty of Medicine, Budapest, Hungary.

Hormone assays

Venous blood samples were taken in the early follicular phase of a spontaneous cycle (day 3 to 5) as part of the routine infertility work-up and assayed for serum FSH, LH, estradiol (E_2), testosterone, and sex hormone-binding globulin (SHBG). In case of oligomenorrhea or amenorrhea, sampling was performed on day 3 to 5 of withdrawal bleeding induced by norethisterone (5 mg twice daily) administered orally for 5 days. Samples were stored at -20°C until assaying for inhibin B. A second serum sample, collected on the 7th day after the LH peak of the same cycle (or on day 20 in absence of an LH peak) and stored frozen, was assayed for inhibin A.

Serum FSH, LH, and E_2 were measured by using the AxSYM microparticle enzyme immunoassay (Abbott, Abbott Park, IL), while testosterone was assayed by a radioimmunoassay (Institute of Isotopes Co., Budapest, Hungary). Levels of SHBG were determined by using a time-resolved solid-phase fluoroimmunoassay (Wallac Oy, Turku, Finland). Intra- and interassay coefficients of variation were, respectively: FSH, 3.8%, 3.5%; LH, 4.5%, 4.3%; E_2 , 10.9%, 10.6%; SHBG, 1.6%, 10.1%.

Dimeric inhibin A was measured in duplicate using a solid-phase sandwich ELISA (Oxford Bio-Innovation, Oxford, UK), which uses two monoclonal antibodies, as described elsewhere [26]. Both intraassay and interassay coefficients of variation were $<20\%$, and the sensitivity of the assay was 3.9 pg/mL.

Inhibin B measurements were also performed in duplicate using the same ELISA system (Oxford Bio-Innovation). Both intra- and interassay coefficients of variation were $<20\%$, and the assay sensitivity was 15 pg/mL.

Statistical analysis

Because metric data did not follow Gaussian distribution, continuous variables were analyzed by Wilcoxon's matched pairs signed rank sum test, and Spearman's rank correlation (r_s) was used to examine correlations between inhibin

Table 1 Clinical and endocrine characteristics of patients with an elevated LH-to-FSH ratio (ELF patients) and controls

Note. Data are presented as medians and interquartile (25th–75th percentile) ranges.

^aWilcoxon's matched pairs signed rank sum test.

^bResults for pairs where ELF patients were hyperandrogenemic (serum testosterone >2.1 nmol/L; $n = 13$ in both groups).

	ELF patients ($n = 32$)	Controls ($n = 32$)	p value ^a
Age (years)	28 (25–33)	28 (25–33)	0.24
BMI (kg/m^2)	22.2 (19.8–25.1)	22.4 (20.6–24.4)	0.51
Average cycle length (days)	32 (29–41)	30 (29–32)	0.18
FSH (IU/L)	5.4 (3.5–6.7)	6.6 (5.5–8.2)	<0.001
LH (IU/L)	14.6 (10.0–18.8)	4.5 (3.4–6.0)	<0.001
LH/FSH	2.9 (2.3–3.3)	0.7 (0.6–0.8)	<0.001
E ₂ (pg/mL)	51.6 (36.6–71.0)	40.5 (35.8–53.4)	0.11
SHBG (nmol/L)	50.3 (28.0–61.3)	53.7 (37.8–72.5)	0.36
Testosterone (nmol/L)	1.8 (0.9–2.4)	1.4 (1.1–2.5)	0.88
Inhibin B (follicular phase) (pg/mL)	123.5 (68.3–178.0)	119.1 (68.1–141.9)	0.52
Inhibin A (luteal phase) (pg/mL)	15.6 (5.2–32.4)	19.7 (12.3–30.9)	0.45
Testosterone (nmol/L) ^b	2.9 (2.3–4.8)	1.3 (0.9–1.7)	0.008
Inhibin B (follicular phase) (pg/mL) ^b	123.1 (81.6–177.4)	92.6 (67.8–141.2)	0.29
Inhibin A (luteal phase) (pg/mL) ^b	12.5 (5.9–24.8)	22.0 (14.1–28.4)	0.60

levels and other variables. Data are presented as medians and interquartile (25th–75th percentile) ranges. Analyses were performed using the Statistica 6.1 (StatSoft, Inc., Tulsa, OK, USA) software system. A p value <0.05 was considered to indicate statistical significance.

Results

Sixty-four subjects (32 patients with an elevated LH-to-FSH ratio and 32 controls) were included in this study. Clinical and endocrine characteristics of the two groups are presented and compared in Table 1. As expected from the matching criteria, the two groups were comparable in terms of age, BMI, and cycle length, and serum LH and LH-to-FSH ratio were significantly higher in the ELF group, whereas serum FSH was significantly lower. Although follicular-phase serum inhibin B levels were somewhat higher in the group with elevated LH-to-FSH ratios, this difference was far from statistical significance ($p = 0.52$). Similarly, inhibin A concentrations in the midluteal phase were slightly lower in the ELF group but again, the hypothesis of no difference could not be rejected ($p = 0.45$). No statistically significant difference was found in other variables.

We observed similar, non-significant differences in follicular inhibin B and midluteal inhibin A levels when restricting our analyses to pairs with hyperandrogenemic ELF patients (serum testosterone >2.1 nmol/L, i.e., upper limit of reference range for healthy women of reproductive age, as established by our endocrinological laboratory; $n = 13$). In this subgroup, as expected, serum testosterone was significantly higher in PCOS patients [2.9 (2.3–4.8) nmol/L] than in controls [1.3 (0.9–1.7) nmol/L] ($p = 0.008$).

A significant negative correlation was observed between follicular-phase inhibin B levels and BMI in the ELF group but not among controls. Furthermore, serum FSH and LH concentrations were significantly positively related to in-

hibin B levels in PCOS patients (Fig. 1). Midluteal inhibin A levels correlated with SHBG concentrations only in controls ($r_s = 0.61$; $p < 0.001$) but not in PCOS patients ($r_s = 0.12$; $p = 0.54$). No other significant correlations could be demonstrated between inhibin A and any of the parameters investigated.

Discussion

Our data indicate that neither serum inhibin B levels in the early follicular phase nor inhibin A concentrations in the midluteal phase differ between patients characterized by an elevated LH-to-FSH ratio and controls.

Ovaries of PCOS patients contain an increased number of small antral follicles, which represent the major site of inhibin B production. It was previously shown that, similar to ovaries of healthy women, inhibin B is the predominant form of inhibin in the follicular fluid of polycystic ovaries and each individual follicle in PCOS produces a similar amount of inhibin B to that secreted by small antral follicles in normally cycling women [10]. Taken together with the fact that we found similar serum concentrations of inhibin B in the early follicular phase in ELF patients and controls, which is in line with the results of previous reports [1, 13], this suggests certain mechanisms within the follicles that concentrate and sequester hormones in the follicular fluid. The idea of individual hormone-dependent transport mechanisms is further corroborated by the finding that the ratio between follicular fluid and serum levels is different for E₂, inhibin A, and inhibin B [7].

Since serum FSH concentrations are normal in PCOS patients and granulosa cells respond in a normal fashion to stimulation in vitro with FSH [27], local ovarian products might be involved in the follicular arrest characteristic of PCOS. In a recent report by Welt et al. [28], decreased concentrations of inhibin A and B were found in follicular fluid

Fig. 1 Correlations between follicular-phase serum inhibin B and BMI (A), serum FSH (B), and serum LH levels (C) in patients with an elevated LH-to-FSH ratio (ELF patients; closed circles and continuous line) and controls (open squares and dotted line)

samples from PCOS patients as compared to concentrations in size-matched follicles obtained from healthy volunteers. This is in contrast to the findings mentioned above [10], and was attributed by the authors to the fact that follicular fluid in the previous study was collected from ovarian samples removed from women undergoing laparotomy, which was performed under general anesthesia, whereas they collected follicular fluid samples by transvaginal aspiration without the use of any anesthetics. The observation that both

inhibin A and inhibin B were deficient in follicular fluid of PCOS patients also argued against the role of FSH as an etiological factor since FSH regulates inhibin A and B production differently [29]. Instead, the role of LH in deficient production of inhibin subunits through premature luteinization and inhibition of proliferation seems plausible. Decreased secretion of inhibin A and B by single follicles is consistent with our results and those of other studies demonstrating similar serum inhibin A and B concentrations

Fig. 1 Continued

despite an increased total number of follicles in PCOS [1, 13, 15, 16, 28].

Inhibin B levels have been shown to be inversely related to BMI [13, 15, 16] and a positive correlation has been demonstrated with serum LH levels [15, 16]. While the former is not specific to PCOS, the positive correlation between serum LH and inhibin B concentrations could only be demonstrated in this group of patients. As postulated by Cortet-Rudelli *et al.* [15], the positive effect of LH and the negative impact of obesity might be explained in the context of a model which is similar to the classical ‘two-cell’ theory of ovarian steroid synthesis, originally proposed by Erickson [30]. As opposed to normal women, where inhibin B production by granulosa cells in the early follicular phase is under the control of FSH but not LH, theca interstitial cells around small antral follicles in PCOS patients might respond with an increased α -subunit expression to elevated LH levels, supplying granulosa cells with an increased amount of this subunit, which would result in an elevated secretion of inhibin B by granulosa cells. Furthermore, premature sensitivity of granulosa cells from small antral follicles to LH has been reported in PCOS [31], thus LH might also stimulate inhibin B production directly in granulosa cells. In contrast to these findings and theories, several studies demonstrated reduced expression of the α -subunit in PCOS granulosa cells, which, possibly regulated by elevated LH levels, might be responsible for decreased inhibin A and B concentrations in follicular fluid [32, 33]. Furthermore, an acute stimulatory effect of LH could not be mimicked by administering a single dose of hCG to PCOS patients [16].

Although the mechanism of the negative effect of obesity on inhibin B production remains unclear, it seems that it acts through some putative factor on the FSH-induced inhibin β_B -subunit expression by granulosa cells. Therefore, the cumulative effect of elevated LH levels and a high BMI might be an unaltered inhibin B concentration in obese PCOS patients, which would obscure differences in levels of inhibin B as compared to controls. Even though the positive correlation between inhibin B and LH levels as well as the inverse relationship between inhibin B levels and BMI observed in our ELF patients is in good agreement with the results of previous studies mentioned earlier, the fact that we matched our patients by BMI renders it unlikely that the lack of significant difference in inhibin B levels between ELF patients and controls would be a result of the opposite effect of obesity and serum LH.

Recent observations demonstrated strong correlations between serum levels of pro- α_C (a monomeric inhibin precursor) and androgens [13] in PCOS patients with no consistent relationship between inhibin B and androgen levels [1, 13, 15]. We could not demonstrate a significant correlation between serum inhibin B and testosterone levels either, nor was there any significant relationship between inhibin A and testosterone concentrations. It therefore seems that increased androgen production in theca interstitial cells, as proposed by earlier in vitro studies [8, 34], is not an effect of inhibin A or B in vivo.

Our results also argue against the role of inhibin B in the elevation of the LH-to-FSH ratio, which is characteristic

of most patients with PCOS [25]. If FSH secretion were suppressed in PCOS as a result of prematurely increased inhibin B production, levels of these two hormones should be inversely related. By contrast, in line with other investigators [1], we observed a significant positive correlation between serum inhibin B and FSH concentrations in ELF patients, which does not support this theory.

In summary, our data suggest that neither inhibin B levels in the early follicular phase nor inhibin A levels in the mid-luteal phase are characteristic of patients with an elevated LH-to-FSH ratio. Opposite correlations with LH and BMI demonstrated in this group of patients support the results of other investigators suggesting a dysregulation of inhibin secretion in PCOS rather than dimeric inhibins having a central role to the endocrinological imbalance observed in this syndrome.

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