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Clinical significance of invisible or partially visible luteinizing hormone

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It is well known that luteinizing hormone (LH), like many other glycoproteins, is heterogeneous and presents several circulating isoforms. Recently, new sensitive immunometric assays measuring intact LH were developed. These assays have been found to underestimate or to be incapable of recognizing LH in some patients. This study was undertaken to determine the prevalence of such cases and to define their clinical characteristics. We compared three LH assays using as capture antibodies either a monoclonal antibody that reacts exclusively with intact LH (ES 600 Boehringer, Stratus Baxter) or a monoclonal antibody against the β subunit of LH (IMX Abbott). In 17% of 90 patients tested, ES 600 measured >50% lower LH concentrations when compared with the IMX. Moreover, in two cases LH was not detectable by ES 600 or Stratus, whereas it was normal with the IMX. We found another five such cases and discuss here the clinical data and results of different hormone measurements in these seven cases of 'invisible LH'. Although bioactive LH (mouse Leydig cell assay) was normal, the existence of low or even undetectable LH was clinically confusing and led to expensive complementary investigations such as gonadotrophin-releasing hormone analogue tests and magnetic resonance imaging. The uses and limitations of these assays are illustrated by different clinical situations in which the results of the different assays have been misleading.

Key words: clinical aspects/immunoradiometric assay/invisible LH

Introduction

Luteinizing hormone (LH) is a glycoprotein hormone which consists of two non-covalently linked subunits. The α subunit is common to other pituitary glycoproteins such as follicle-stimulating hormone (FSH) and thyroid-stimulating hormone (TSH). The β subunit carries the specificities of the biological effects of LH. Both subunits are glycosylated at specific residues and the degree of glycosylation modulates the biological activity of LH. Glycosylation is heterogeneous and leads to many different circulating variants of LH (Jeffcoate, 1993). After isoelectric focusing, seven different isoforms of LH have been identified (Weise *et al.*, 1983). The more acidic isoforms have a longer half-life and display lower receptor binding and in-vitro bioactivity as compared with the more basic isohormones.

The specificity of an immunoassay is dependent upon the epitope recognized by the antibodies used in the assay. For the glycoprotein hormones, recognized epitopes are either amino acids or oligosaccharide structures. Classic radioimmunoassays for LH used polyclonal antibodies composed of immunoglobulins recognizing most if not all circulating forms of LH. Recently, new sensitive immunometric assays have been developed. These are based on two monoclonal antibodies recognizing different epitopes on the LH molecule (sandwich technique) These assays have improved sensitivity and specificity (Apter *et al.*, 1989; Jaakkola *et al.*, 1990). However, Pettersson and Söderholm, (1991) by comparing two of these LH assays, observed major discrepancies in LH concentrations in about one quarter of a randomly selected population. Moreover, in five cases LH was not recognized at all by certain antibodies.

The aim of this study is to assess the prevalence of 'partially visible' and 'invisible' LH in our population and to discuss the clinical implications of these findings.

Materials and methods

A total of 90 consecutive patients needing an LH determination between August and December 1993 were included in the prevalence study. These included 17 males consulting for impotence or sterility, 26 women consulting for menopausal complaints, 21 women with infertility, 14 women with spaniomenorrhoea (including polycystic ovary syndrome), nine women with suspected hypothalamic amenorrhoea and three with galactorrhoea. Serum samples were obtained and analysed by two different types of assay. Assay 1 was an immunoenzymometric assay (IEMA) using a monoclonal antibody recognizing an epitope on the αβ dimer of intact LH as the capture antibody and a monoclonal anti-β subunit as the detection antibody (ES

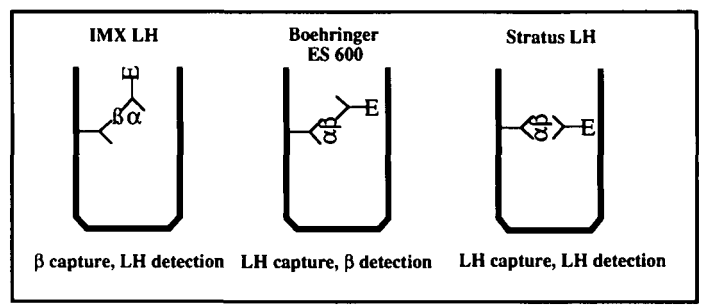

Fig. 1. Schematic representation of luteinizing hormone (LH) assay constructs.

600; Boehringer, Mannheim, Germany; Figure 1). Assay 2 was a microparticle enzyme immunoassay (MEIA) using a monoclonal anti-β subunit as the capture antibody and a polyclonal anti-human LH as the detection antibody (IMX; Abbott, Chamonix, Switzerland; Figure 1). The detection limit of both assays was 0.5 mIU/ml.

Sera from seven patients with LH values undetectable by ES 600 and Stratus were obtained from the practice of two private endocrinologists and from our own patients. These sera remained frozen (−20°C) for up to 2 years before being re-assayed. The concentration of LH in these samples was measured by the two assays described above and with an IEMA using two monoclonal antibodies directed against intact LH as capture and detection antibodies (Stratus; Baxter, Düdingen, Switzerland; detection limit 0.5 mIU/ml; Figure 1). FSH and oestradiol were also measured in these samples by Stratus (Baxter) and Vidas (Biomérieux, Genève, Switzerland) respectively. Finally, in five out of seven samples bioactive LH was also determined by the mouse Leydig assay as described by Dufau *et al.* (1974).

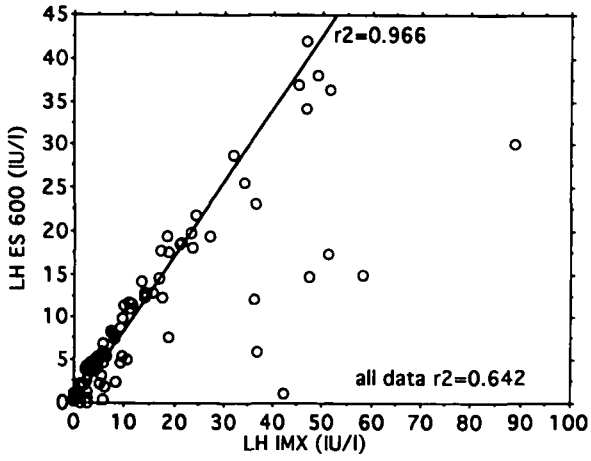

Fig. 2. Correlation between luteinizing hormone (LH) values determined by IMX and ES 600. Correlation coefficient $r^2 = 0.642$ for all data, and 0.966 when 18 pairs of values in which LH as measured by ES600 was 50% lower than that found in IMX.

Results

LH values obtained by ES 600 and IMX assays for the 90 consecutive samples are shown in Figure 2. The correlation coefficient ($r^2 = 0.642$) was significantly different from zero ($P < 0.0001$). By eliminating 18 pairs of values for which the concentration of LH as measured by ES 600 was 50% lower than the corresponding value found with IMX, the correlation coefficient increased to 0.966. Among these 18 samples, two had undetectable LH on the ES 600 assay whereas readings of 2.4 and 1.3 IU/l were obtained with the IMX assay. Thus 'partially visible LH' (values 50% lower with ES 600 than with IMX) were observed in 17.8% (16/90) of the samples and 'invisible LH' (values undetectable with ES 600 assay but detectable with IMX assay) were observed in 2.2% (2/90) of our samples. Similar values with both assays were observed for 80% of the samples.

Table I. Hormone measurements in the seven cases with 'invisible LH'

Patient age and sex	History and complaints	LH measurements (IU/l)				FSH (IU/l)	Oestradiol (pmol/l)
		IMX	Stratus	ES 600	Bioactive		
Case 1: 48, F	RP, hot flushes	59	<1	<1	41	59	60
		↓	↓				
		234	<1	ND	ND	ND	ND
Case 2: 44, F	RP, hot flushes	47	<1	<1	85	57	80
		7	<1	<1	26		
		↓	↓		↓		
		18	<1	ND	58	ND	ND
Daughter, 23		10	3.5	ND	ND	ND	ND
Son, 20		5	2.6	ND	ND	ND	ND
Case 3: 40, F	RP, pre-menstrual	ND	<1	<1	ND	8.4	100
	syndrome	24.6	<1	<1	66	9.3	1351
Case 4: 32, F	PCO, post-pill	ND	<1	<1	ND	6.2	112
	amenorrhoea	18.2	<1	<1	ND	10	303
	Mother, 68	37	7	6	ND	65	ND
Case 5: 41, F	RP, primary	ND	<1	ND	ND	7	772
	infertility	15.2[a]	<1	<1	ND	ND	2739
		26[a]	<1	<1	ND	ND	3697
		157.3[a]	<1	<1	ND	ND	3877
							Testosterone (nmol/l)
Case 6: 51, M	impotence	4	<1	ND	25	4.6	8.4
		↓	↓		↓		
		25	<1	ND	32	ND	ND
Case 7: 37, M	infertility	ND	<1	<1	ND	19	10.4
		8.5	3.8	ND	41	ND	ND

RP = regular periods; PCO = polycystic ovary disease; ND = not determined; F = female; M = male; ↓ = 30 min after GnRH.
[a]After clomid.

The two samples with 'invisible LH' plus another five samples from other subjects were analysed in greater detail. The samples came from five women and two men. Four of the five women (cases 1, 2, 3 and 5, Table I) had an uncomplicated history with regular cycles; one had a spaniomenorrhoea due to polycystic ovary syndrome. They consulted for hot flushes (n = 2), premenstrual syndrome (n = 1), post-pill amenorrhoea (n = 1) or primary infertility (n = 1). The two men consulted for impotence or infertility (Table I). In all seven cases LH concentrations measured by Stratus and/or ES 600 were undetectable, whereas the concentrations of LH as measured by IMX or bioassay where either normal or increased (Table I). In two women and one man (cases 1, 2 and 6), 100 μg of gonadotrophin-releasing hormone (GnRH) induced an increase in bioactive- and IMX-measured LH, whereas LH measured in the same samples by Stratus remained undetectable. In one patient (case 5, Table I), clomiphene citrate was given to induce ovulation and LH measured daily on three consecutive days; serum LH concentrations were undetectable with Stratus or ES 600 but were measurable with IMX. LH measurements were also performed in the children of patient 2 and in the mother of patient 4. In these relatives, Stratus gave lower LH values than IMX.

Discussion

The lack of recognition or partial recognition of circulating LH has been known for some time. During GnRH agonist or antagonist therapy, several authors (Bhasin and Swerdeloff, 1986; Lahlou *et al.*, 1987; Bischof and Herrmann, 1988) observed discrepancies between different LH immunoassays. One month after GnRH treatment, LH concentrations as measured by polyclonal radioimmunoassays were not different from pre-treatment values, whereas LH concentrations measured by radioimmunometric assays or by bioassays were very low or even undetectable. This was explained by the fact that circulating free α subunits recognized by polyclonal antibodies are significantly increased by GnRH treatment, whereas β subunits specifically recognized by most monoclonal antibodies are decreased to undetectable amounts (Meldrum *et al.*, 1984). It was therefore considered that under those circumstances, polyclonal radioimmunoassays tend to overestimate LH.

Among the 90 patients in whom we measured LH by two different assays, 17.8% of them had ES 600 LH values (only intact LH) which were 50% smaller than IMX LH values (only β subunit). Restricted reactivity against monoclonal antibodies that react exclusively with intact LH was reported for the first time by Pettersson and Söderholm (1991) in 25% of 244 samples tested with 11 different antibodies. In a later study from the same group (Pettersson *et al.*, 1991) a similar observation was described in 19 out of 83 patients (22.9%). Gervasi *et al.* (1991) also observed important discrepancies between three commercially available LH kits in ~20% of 155 samples. Our present observations confirm and extend these previous reports.

These cases of 'partially visible LH' are frequent (17–25%). From a clinical point of view underestimation of LH can impair the detection of the ovulatory LH peak (e.g. case 5) or artificially decrease the LH/FSH ratio leading to non-recognition of polycystic ovarian disorder (PCOD) (e.g. case 4). 'Invisible LH' is much less frequent (~2%) and is clinically speaking more easily recognized.

In our seven patients with 'invisible LH', the two assays using monoclonal antibodies and recognizing intact LH as capture antibodies (Stratus and ES 600) did not recognize LH in the serum samples, whereas the assay using a capture antibody to the β subunit (IMX) measured normal or increased concentrations of LH. Although gonadotrophins were measured for clinical reasons, these patients with 'invisible LH' did not seem to have a specific clinical profile.

Low LH can be observed in certain cases of secondary amenorrhoea, such as anorexia nervosa, human chorionic gonadotrophin (HCG) producing tumours, hypogonadotrophic hypogonadism, or pregnancy. In these cases, however, FSH is also low. None of our patients fell into any of these categories since their FSH were either normal or increased. Because certain cases of pituitary adenomas have been described with low LH and normal FSH concentrations (Daneshdoost *et al.*, 1991), 'invisible LH' as observed in our study can be confusing and lead to unnecessary and expensive investigations such as magnetic resonance imaging of the pituitary, as we performed in patients 2 and 7.

The inability to detect LH by monoclonal antibodies raised against intact LH does not seem to be due to the non-recognition of a particular isoform of LH (Gervasi *et al.*, 1991).

Imse *et al.* (1992), using a bioassay and five different immunoassays, studied LH pulsatility in the serum of women suffering from PCOD. They observed that all pulses registered by the bioassay were detectable by a conventional radioimmunoassay (polyclonal antibodies) but some pulses were not seen by certain monoclonal antibodies. These authors suggested that conformational changes, occurring during post-translational processing or exocytosis, modify the molecular structure of LH in such a way that a particular epitope cannot be recognized by highly specific monoclonal antibodies. Similarly, gel filtration studies in one case of 'invisible LH' have shown the presence of an LH species of lower molecular weight than intact LH but heavier than its free β subunit. The authors concluded that 'invisible LH' was due to the existence of a fragmented form of LH where at least part of the β subunit was lost (Pettersson *et al.*, 1992). Since similar observations were made with HCG leading to the discovery of nicked β HCG (Puisieux *et al.*, 1990; Bischof *et al.*, 1994), we postulate that the fragmented forms of LH could be nicked β LH.

Among our patients with invisible LH, the two children of patient 2 and the mother of patient 4 had 'partially visible LH', suggesting a genetic origin of these variant forms of LH. In the patient studied by Pettersson *et al.* (1992), the mother had undetectable LH and the concentrations found in the father and three sisters were only 'partially visible'. As pointed out by the authors, these observations suggest an autosomal dominant inheritance of this (these) LH variant(s).

In conclusion we consider that the development of new immunoassays for LH, based on monoclonal antibodies that recognize specifically the intact LH and not its free β subunit, have led to the discovery of some genetic variants of LH, which are either partially recognized (17–25% of cases) or not recognized at all (2% of cases). This report illustrates the potential

clinical problems associated with 'invisible LH'. Discrepancies between LH values on the one hand and FSH and sexual steroid values on the other will frequently allow undetectable LH to be questioned. However, in cases of 'partially visible LH' it is not possible to guess which are the underestimated values. Therefore we would recommend that either a polyclonal assay is used directly (although this can overestimate LH in certain circumstances) or that an assay which captures LH through its β subunit is used.

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