Comparison between Serum Prolactin Levels Determined by VIDAS and RIA Techniques

Rayah S. Baban *PhD*, **Yahya Y.Farid** *PhD*.

Abstract

Background: Human prolactin can be determined in human serum or plasma quantitatively by many techniques such as ELISA, RIA, and ELFA (miniVIDAS).

Objectives: To estimate the strength of association of total serum prolactin and free serum prolactin values measured by two different methods [RIA and VIDAS]. And to predict the prolactin value measured by RIA corresponding to a given value by VIDAS.

***Methods*:** Two technical methods VIDAS and RIA were used in determination of prolactin level in sera of twenty five women with uterine fibroid conducted at two laboratories admitted at Al-Khademyia Teaching Hospital during the period January 2008 to April 2009. Total and free serum prolactin was measured by both VIDAS and RIA using their assay kits. Statistical methods of correlation and regression were used to compare between the two methods.

Results: The study revealed a highly significant positive correlations between VIDAS and RIA total and free serum prolactin, r=0.999, R^2 = 0.998, P<0.001, r=0.998, R^2 = 0.997, P< 0.001 respectively. High linear regression equations were found between VIDAS and RIA total and free serum prolactin, y= 0.358x+ 0.57, R^2 = 0.998 and y= 0.355x+0.49, R^2 = 0.997 respectively. The recovery percentages of prolactin (R %) in two methods were approximately equal to each other, VIDAS R%=50.52±0.89% and RIA R%= 50.38±1.57% respectively.

***Conclusion*:** A highly significant positive correlation was found between RIA and VIDAS for both total and free serum prolactin. And high linear regression equations were found between the two methods to predict RIA values corresponding to a given VIDAS value.

Keywords: VIDAS prolactin, RIA prolactin, PEG precipitation, macroprolactinemia, uterine fibroid.

IRAQI J MED SCI, 2009; VOL.7 (4): 20-26

Introduction

Prolactin is synthesized as a pre-hormone with a molecular weight of 26 kDa: when the pre-prolactin is cleaved, the resulting polypeptide has a molecular weight of 23 kDa. This monomeric form, biologically active, accounts for approximately 85-95% of the total PRL present in normal individuals [(1)], but other circulating species are identifiable on gel filtration chromatography (GFC): the "big" PRL (50-70 kDa), a dimeric/ trimeric form variably glycosylated, and the "big big" PRL or "macroprolactin" (150- 170 kDa), mainly a complex between monomeric PRL and an anti-PRL immunoglobulin G (IgG) [(2-4)], in some cases a covalently or non-covalently bound aggregate of monomeric PRL molecules with increased glycosylation [(5,6)]. In normal population the big PRL accounts for less than 10% of circulating PRL, whereas the macroprolactin represents a small (less than 1%) amount of total PRL[(7)].

In particular cases (mainly in sera from hyperprolactinemic subjects) the relative proportions of circulating forms can be quite different: macroprolactin can represent even the 90% of circulating PRL [(8)]. Macroprolactin is cleared more slowly than monomeric

[1]Dept. Chemistry and Biochemistry, College of Medicine, Al-Nahrain University.
Address Correspondence to: Dr. Rayah S. Baban .
E- mail: r_baban@hotmail.com
Received: 16th August 2009, Accepted: 18th October 2009.

PRL and hence accumulates in the serum. It is generally believed that macroprolactin has a low or absent biological activity in vivo, because it cannot cross the endothelium and reach the cell surface receptors [9- 11] .

The reference method for demonstration and quantification of high molecular mass forms of PRL is the GFC, but it is time-consuming and expensive for routine use. An alternative technique, more usually employed, is the polyethylene glycol (PEG) precipitation.

The PEG is able to precipitate large molecular mass proteins; in this way, after the precipitation of macroprolactin by PEG, the supernatant should contain prevalently the monomeric PRL form.

Afterwards, the PRL levels are measured, in serum and in supernatant, with the immunometric assay used in the laboratory. The results of PEG test, interpreted on the basis of the percentage of PRL recovery (R %), allow to detect the macroprolactin interference and, furthermore, to obtain an estimate of monomeric, biologically active, PRL [12].

Prolactin can be determined in human serum or plasma quantitatively by many techniques such as ELISA, RIA, and ELFA (miniVIDAS). The assay principle of VIDAS prolactin is an automated quantitative test for use on the VIDAS instruments, for the enzyme immunoassay using ELFA technique (Enzyme linked fluorescent Assay) [VIDAS Prolactin Kit, REF 30 410] while the assay principle of RIA prolactin is based on the competition between unlabelled hPRL and fixed quantity of [^{125} I] labeled hPRL for a limited number of binding sites on a specific antibody [^{125} I] RIA KIT (REF: RK-553).

Subjects and Methods

The study was conducted during the period from January 2008 to April 2009 on twenty five women diagnosed with uterine fibroids in Obstetrics and Gynecology department at Al-Khademyia Teaching Hospital. They had different clinical presentations like amenorrhea, menstrual disturbance, and galactorrhea. All of them were sent for MRI to exclude the presence of pituitary adenoma and abdominal ultrasound to confirm the diagnosis of uterine fibroid.

Ten milliliters of blood were aspirated from each patient in order to estimate the level of prolactin in their sera prior to the operation [myomectomy or hysterectomy]. Blood sample was left to clot at room temperature and then separated by centrifuging at 800 xg for 10 minutes. Each patient serum was divided into two parts and sent to two different laboratories to evaluate the total serum prolactin and free prolactin after precipitating with Polyethylene glycol (PEG 8000) by miniVIDAS and RIA techniques. The VIDAS Prolactin was determined by using ELFA technique (Enzyme Linked Fluorescent Assay) (VIDAS Prolactin Kit, REF 30 410), while the RIA Prolactin was determined using [^{125} I] RIA KIT) (REF: RK-553).

The PEG 8000 precipitation test was performed according to the method proposed by Fahie-Wilson and Soule [13]. Two hundred micro liters of a 25% PEG 8000 solution was added, at room temperature, to equal volume of serum and was centrifuged (after thorough vortex mixing) at 1800 x g for 30 min at 20 °C. Prolactin was measured, without delay; in the supernatant obtained after PEG precipitation using the miniVIDAS and RIA, after correction for dilution, were compared with those obtained from untreated serum.

The results of PEG test were expressed as PRL recovery. In agreement with literature data [7, 11, 13, 14], we assumed an R% value <40% as indicative of presence of substantial amounts of macroprolactin in serum (macroprolactinemic subjects); on the contrary, a R% value >60% was considered indicative of substantial absence of macroprolactin (true hyperprolactinemic subjects).

Results

Table 1 shows the mean±SD for total serum prolactin and free serum prolactin measured by two methods. The mean±SD of total serum prolactin measured by VIDAS technique was found to be higher than that of the same sample measured by RIA technique (389.14±160.85 ng/ml, 138.86±58.76 ng/ml respectively). And also after precipitating with PEG 8000, VIDAS free serum prolactin mean±SD was higher in same sample measured by RIA technique (192.55±80.04 ng/ml, 68.34±28.80 ng/ml) respectively.

A highly significant positive linear correlation was found between VIDAS and RIA total serum prolactin (r=0.999, R^2 = 0.998, P<0.001). And after treating samples with PEG 8000, the VIDAS and RIA free serum prolactin measured also revealed a highly significant positive linear correlation (r=0.998, R^2 = 0.997, P< 0.001) (Table 2).

In order to confirm these correlations, a highly significant linear correlations was found between VIDAS and RIA total serum prolactin with linear regression equations (y= 0.358x+ 0.57, R^2 = 0.998) (Figure 1) as well as between VIDAS and RIA free serum prolactin when measured by the same two techniques (y= 0.355x+0.49, R^2 = 0.997) as shown in (Figure 2).

The mean values of recovery prolactin percentage (R %) were approximately the same in both VIDAS and RIA techniques for all patients (50.52±0.89, 50.38±1.57) respectively (Table 3). It means that all samples do not contain macroprolactin and both techniques give the same results approximately.

Table 1: A descriptive analysis for total serum prolactin(Total S.PRL) and free serum prolactin (Free S.PRL) measured by VIDAS and RIA techniques. (n=25)

Serum prolactin		*mean±SD (ng/ml)*	*Range(ng/ml)*
VIDAs	***Total S.PRL***	389.14±160.85	101.34-589.43
	Free S.PRL	192.55±80.04	51.76-295.99
RIA	***Total S.PRL***	138.86±58.76	33.26-213.83
	Free S.PRL	68.34±28.80	17.52-107.1

Note: mean±SD is mean ± standard deviation.

Table 2: Correlations for both total serum prolactin and free serum prolactin measured by VIDAS and RIA techniques.

serum prolactin	*r*	*P*	*t*	*95% Confidence Interval*		R^2
				Lower	*Upper*	
Total S.PRL(ng/ml)*	0.999	<0.001	119.218	0.359	0.372	0.998
Free S.PRL(ng/ml) ➢	0.999	<0.001	88.639	0.351	0.368	0.997

❖ Correlation between total serum prolactin measured by VIDAS and RIA technique.

➢ Correlation between free serum prolactin measured by VIDAS and RIA technique.

Table 3: Recovery prolactin percentages measured by VIDAS and RIA techniques (n=25).

Recovery prolactin percentages	*mean±SD*	*Range*	*P value*
VIDAS (R%) prolactin	50.52±0.89	48.92-52.62	Not
RIA (R%) prolactin	50.38±1.57	46.61-53.05	Not

Note: R%= Recovery prolactin percentage

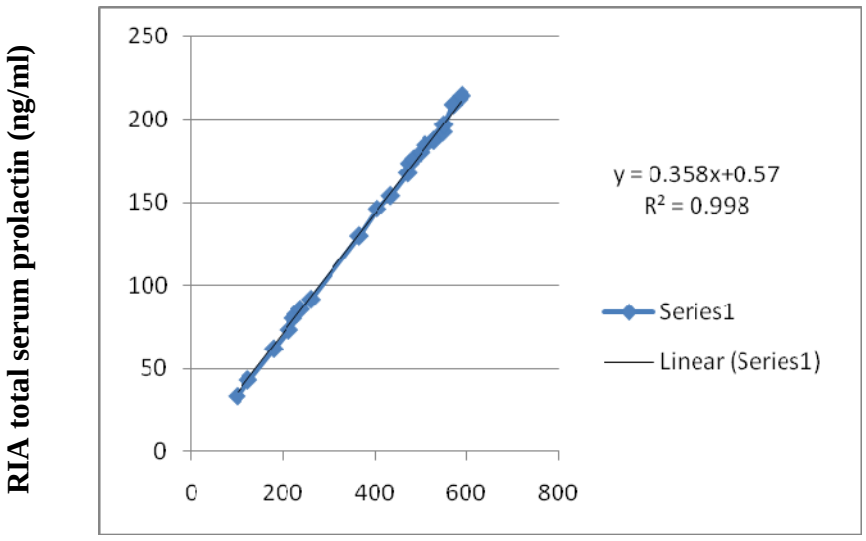

Figure 1: Correlation with linear regression line between VIDAS and RIA total serum prolactin levels.

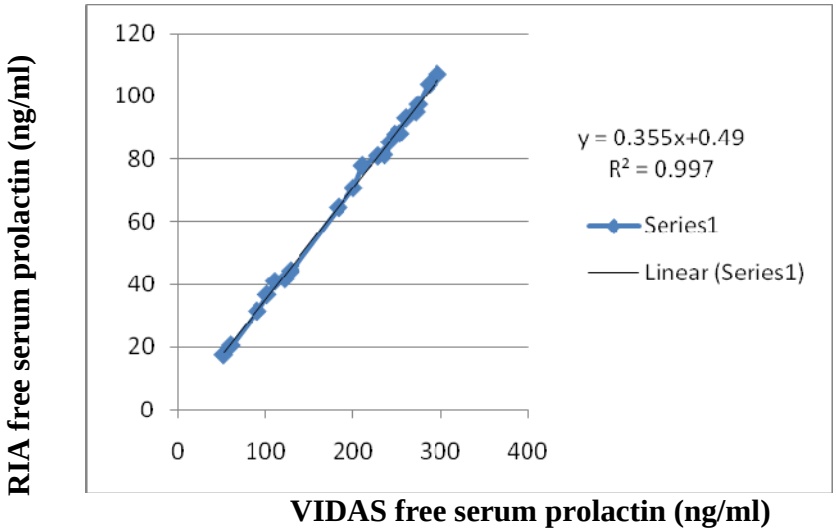

Figure 2: Correlation with linear regression line between VIDAS and RIA free serum prolactin levels.

Discussion

Hyperprolactinemia is a common problem encountered in reproductive disorders [15]. The understanding that prolactin hypersecretion not only causes galactorrhea and amenorrhea but also gonadal dysfunction and infertility led to the wider use of prolactin estimations [16].

All methods for measuring prolactin show some cross-reactivity with macroprolactin, and the extent to which it contributes to the measured prolactin concentration depends on the choice of reagent antibody. Because the presence of macroprolactin does not appear to contribute to the hyperprolactin**e**mic syndrome in the majority of patients, it is important to raise awareness of the problem in clinical chemistry laboratories and with physicians [17].

Confirmation of the presence of macroprolactin can be made by gel filtration chromatography, but this is time-consuming, costly, and beyond the scope of most clinical laboratories.

Laboratories in the United Kingdom have been using mainly PEG precipitation techniques to identify macroprolactinemia[17]. As highlighted in these twenty-five clinical cases, macroprolactin needs to be identified early in a patient's work-up to avoid unnecessary, costly, and invasive procedures.

All patients in this study were with elevated prolactin level in their sera (table-1) although they were all non pituitary adenoma patients and they were all with uterine fibroid (ectopic or extra pituitary secretion). Nowak *et al.* 1993 have investigated the actions of several hormones known to stimulate PRL secretion by the pituitary gland or decidua on PRL secretion by leiomyoma-derived smooth muscle cells (SMC) in monolayer culture. They conclude that leiomyomas express PRL mRNA in vivo and that leiomyoma-derived SMC in culture continue to express the PRL mRNA and secrete PRL in the absence of ovarian steroids. PRL secretion by SMC in culture appears to be modulated primarily by changes in cell density [18]. Szécsi *et al* 2006

investigated via HPLC-RIA determinations of intratissular concentrations of eleven main steroid hormones. The data verify that the determination of intratissular steroid concentrations by HPLC-RIA methods may identify even the most peculiar hormone sources and the hormone profiles facilitate studying pathophysiology of ectopic endocrine tumors [19].

PEG precipitating method was used to determine the prolactin R% and to identify the prolactin profile present in their serum. PEG pretreatment yielded results that correlated best and are recommended as the first-choice alternative to GFC (gel filtration chromatography) [20].

Two techniques in this study were used to predict which one is faster in clinical diagnosis. VIDAS prolactin assay needed only 40 minutes to generate results while RIA prolactin assay needs 120 minutes and 2 days to generate the results. So time consuming is less in VIDAS than RIA technique. Although both VIDAS and RIA techniques gives almost the same linear regression equations (figure-1 and 2) and significant positive correlations (table-2) when samples underwent assay before and after PEG 8000 treatment.

As mentioned in VIDAS prolactin kit leaflet, when VIDAS compared with other test methods, a correlation was established between VIDAS PRL kit and enzyme immunoassay (x), r=0.984, and y VIDAS PRL= 1.10 x + 5.81 (Biomérieux, VIDAS PRL, 07325J-GB-2004/09).

Batra *et al* 1989 made a comparison between HPLC and RIA methods in measuring the AZT (Zidovudine) level after oral administration. The results of the two methods did not correlate statistically (correlation coefficient = 0.79) . The zero, one and two hours post administration of Zidovudine serum levels were also compared. The correlation coefficients were 0.20, 0.75, and 0.68 respectively. They concluded that results obtained by the RIA method did not correlate well with the HPLC method. The variation was not consistent. The RIA method values were consistently higher for the one hour and two hours post ingestion levels [21].

Barlow *et al* 1986 used an enzyme-linked immunosorbent assay (ELISA) to evaluate serum alpha-fetoprotein in the antenatal screening for fetal open neural tube defects. They concluded that The ELISA method was simple, required about one quarter less operator time than the RIA and enabled results to be generated in one day rather than the two days required by RIA. The ELISA method is a suitable alternative to RIA for routine use in screening for fetal neural tube defects [22].

References

1. Sapin R, Gasser F, Grucker D. Free prolactin determinations in hyperprolactinemic men with suspicion of macroprolactinemia. Clin Chim Acta 2002; 316(1-2):33-41.

2. Cavaco B, Leite V, Santos MA, *et al*. Some forms of big big prolactin behave as a complex of monomeric prolactin with an immunoglobulin G in patients with macroprolactinemia or prolactinoma. J Clin Endocrinol Metab 1995; 80(8): 2342-6.

3. De Schepper J, Schiettecatte J, Velkeniers B, *et al*. Clinical and biological characterization of macroprolactinemia with and without prolactin-IgG complexes. Eur J Endocrinol 2003; 149(3):201-7.

4. Hattori N, Ikekubo K, Nakaya Y, *et al*. Immunoglobulin G Subclasses and Prolactin (PRL) Isoforms in Macroprolactinemia Due to Anti-PRL Autoantibodies. J Clin Endocrinol Metab 2005; 90(5): 3036-44.

5. Fahie-Wilson MN, Soule SG. Macroprolactinaemia: contribution to hyperprolactinaemia in a district general hospital and evaluation of a screening test based on

precipitation with polyethylene glycol. Ann Clin Biochem 1997; 34:252-8.
6. Fahie-Wilson MN, Ahlquist JA. Hyperprolactinaemia due to macroprolactins: some progress but still a problem. Clin Endocrinol 2003; 58(6):683-5.
7. Olukoga AO, Kane JW. Macroprolactinaemia: validation and application of the polyethylene glycol precipitation test and clinical characterization of the condition. Clin Endocrinol 1999; 51(1):119-26.
8. Smith TP, Suliman AM, Fahie-Wilson MN, *et al*. Gross variability in the detection of prolactin in sera containing big big prolactin (macroprolactin) by commercial immunoassays. J Clin Endocrinol Metab, 2002; 87(12):5410-5.
9. Bonhoff A, Vuille JC, Gomez F, *et al*. Identification of macroprolactin in a patient with asymptomatic hyperprolactinemia as a stable PRL-IgG complex. Exp Clin Endocrinol Diabetes 1995; 103(4): 252-5.
10. Leite V, Cosby H, Sobrinho LG, *et al*. Characterization of big, big prolactin in patients with hyperprolactinaemia. Clin Endocrinol 1992; 37(4):365-72.
11. Schlechte JA. The macroprolactin problem. J Clin Endocrinol Metab 2002; 87(12):5408-9.
12. Germano L, Mormile A, Filtri L, *et al* .Evaluation of polyethylene glycol precipitation as screening test for acroprolactinemia using Architect immunoanalyser . LigandAssay 2006; 11 (2).
13. Fahie-Wilson MN, Soule SG. Macroprolactinaemia: contribution to hyperprolactinaemia in a district general hospital and evaluation of a screening test based on precipitation with polyethylene glycol. Ann Clin Biochem 1997; 34:252-8.
14. Leslie H, Courtney CH, Bell PM, *et al*. Laboratory and clinical experience in 55 patients with macroprolactinemia identified by a simple polyethylene glycol precipitation method. J Clin Endocrinol Metab 2001; 86(6):2743-6.
15. Choudhary SD, Goswami A. Hyperprolactinemia and reproductive disorders - a profile from north east. J Assoc Physicians India 1995; 43:617-8.
16. Avasthi K, Kaur J, Gupta S, *et al.* J Obstet Gynecol India Vol. 56, No. January/February 2006 Pg 68-71.
17. Rhys J, Ian F.W. McDowell, Maurice F*et al.* Macroprolactin Reactivities in Prolactin Assays: An Issue for Clinical Laboratories and Equipment Manufacturers Clinical Chemistry . 2000; 46: 884-885.
18. Nowak RA, Rein MS, Heffner LJ, *et al*. Production of prolactin by smooth muscle cells cultured from human uterine fibroid tumors.J Clin Endocrinol Metab. 1993 May; 76(5):1308-13.
19. Batra K, DeSouza M, Stephy E, *et al*. Comparison of radioimmunoassay (RIA) and high performance liquid chromatography (HPLC) methods for serum zidovudine (AZT) level measurements. Int Conf AIDS. 1989 Jun 4-9; 5: 279.
20. Lucille K. Joseph McKenna, Michael N, *et a l*. Specificity and Clinical Utility of Methods for the Detection of Macroprolactin. Clinical Chemistry 52: 1366-1372, 2006.
21. M. Szécsi , I. Tóth, J. Gardi, *et al*. HPLC-RIA analysis of the ectopic cortisol production in a cancerous pancreas tumor. Journal of Biochemical and Biophysical Methods. 2006; November, Volume 69, 1-2, 30, Pages 51-55.

22. Barlow RD, Thompson SG, Cuckle HS, *et al*. Comparison of an ELISA with a RIA method for serum alpha-fetoprotein determination in screening for fetal neural tube defects. Ann Clin Biochem. 1986 May; 23 (Pt 3):334-9