Utility of Cannulated Prolactin to Exclude Stress Hyperprolactinemia in Patients with Persistent Mild Hyperprolactinemia

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ABSTRACT

BACKGROUND: Stress-induced hyperprolactinemia can be difficult to differentiate from true hyperprolactinemia and may result in patients having unnecessary investigations and imaging. We report the results of cannulated prolactin tests with serial prolactin measurements from an indwelling catheter to differentiate true from stress-induced hyperprolactinemia in patients with persistently mildly elevated prolactin levels in both referral and repeat samples.

METHODS: Data were collected for 42 patients who had a cannulated prolactin test between January 2017 and May 2018. After cannula insertion, prolactin was measured at 0, 60, and 120 minutes. Normalization is defined as a decline in prolactin to gender-defined normal ranges.

RESULTS: The mean age was 33.8 years (SD ± 9.9), and 37 (88%) were female. Menstrual irregularities were the main presenting symptom in 28.57% of the patients. Prolactin normalized in 12 (28.6%) patients of whom cannulated prolactin test was done. Repeat random prolactin levels were significantly higher in patients whose prolactin did not normalize during the cannulated prolactin test. MRI of the pituitary gland showed an abnormality in 23 out of 28 (82%) patients who did not normalize prolactin, a microadenoma in the majority of patients (18 patients).

CONCLUSION: The cannulated prolactin test was useful in excluding true hyperprolactinemia in 28.6% of patients with previously confirmed mildly elevated random prolactin on two occasions, thus avoiding over-diagnosis and unnecessary imaging.

KEYWORDS: Hyperprolactinemia, venipuncture stress, cannulated prolactin, prolactinoma

Introduction

Hyperprolactinemia is one of the most common endocrine disorders of the hypothalamic-pituitary axis. The prevalence of ever-treated hyperprolactinemia is approximately 20 per 100 000 male patients and approximately 90 per 100 000 female patients. In women aged 25 to 34 years, the annual incidence of hyperprolactinemia was reported to be 23.9 per 100 000 person years.1 However, hyperprolactinemia is present in 15% to 20% of women presenting with menstrual disturbances.2 The etiology of hyperprolactinemia can include physiological, pathological, and pharmacological causes. Physiological hyperprolactinemia can be seen in pregnancy, lactation, and physical exertion or during stress including venipuncture.3 The endocrine society guideline endorses a prolactin level >500 μg/L (10 600 mIU/L) as diagnostic for macroprolactinoma. It also points out that level >250 μg/L (5300 mIU/L) is indicative of presence of a prolactinoma. However, the guideline did mention that selected drugs including risperidone and metoclopramide, may cause prolactin elevation above 200 μg/L (4240 mIU/L) in patients without evidence of adenoma. In case of minimal prolactin elevation, non-prolactin secreting mass should first be considered even though prolactinoma can cause mild elevation.1 Prolactin values between the upper limit of normal and 2000 mIU/L may be due to psychoactive drugs, estrogen, disconnection hyperprolactinemia (caused by a non-functioning pituitary or non-pituitary tumor), infiltrative diseases of the hypothalamus (such as histiocytosis), primary hypothryoidism, renal failure or functional (idiopathic) causes, or microprolactinomas.4,5 Raised prolactin due to venipuncture is usually (but not always) <1000 mIU/L.6 Therefore, when initial prolactin values are above the normal laboratory range, but not high enough to clearly indicate a prolactinoma, sampling should be repeated on another day.4 In addition to that, the reported prevalence of macroprolactin in hyperprolactinemic patients ranges from 4% to 46% depending on the assay and referral population and needs to be excluded. The interference of macroprolactin has been overcome by the routine use of polyethylene glycol precipitation.7 The Endocrine Society guideline recommends a single measurement of serum prolactin above the upper limit of normal to confirm the diagnosis of hyperprolactinemia provided that the serum sample
was obtained without excessive venipuncture stress. This may lead to over-diagnosis and unnecessary imaging as it is impossible to know whether stress caused by venipuncture would have caused prolactin elevation. Therefore, the Endocrine Society guideline does emphasize that when in doubt, sampling can be repeated on a different day at 15 to 20 minutes intervals to account for possible prolactin pulsatility. A previous study repeated on a different day at 15 to 20 minutes intervals to find it mildly elevated random prolactin.

Methods
The study was conducted as an audit and was approved by the Imperial College Healthcare NHS Trust audit and governance committee. As it only included non-identifiable clinical audit data, no additional approval from a research ethics committee was required under the UK policy framework for Health and Social Care.

Audit registration number is ASM_HH_14. Consent was not needed for this study as only retrospective data were collected without any intervention.

Data were collected retrospectively for 42 patients who had a cannulated prolactin test between January 2017 and May 2018. Prolactin elevation was confirmed with repeat random prolactin testing, excluding the presence of macroprolactin (negative) and exclusion of secondary causes including medication review, thyroid, liver, and renal function tests if not done before, and a pregnancy test in females. In our practice, all hyperprolactinemic samples are screened for macroprolactin using PEG precipitation. Macroprolactinemia is defined as positive when the recovery rate is <40% of prolactin after PEG precipitation. Recovery of more than 50% is considered macroprolactin negative and recovery of 40% to 50% is considered equivocal.

Following clinical review, patients who had a persistently mildly raised prolactin who were macroprolactin negative and did not have a secondary cause were referred for a cannulated prolactin test.

The test was performed between 09:00 and 12:00 am in an ambulatory endocrine investigation unit at fasting state irrespective of the menstrual cycle phase in female patients. After cannula insertion, blood for prolactin measurements was drawn from the cannula at 0, 60 and 120 minutes. Patients were asked to rest between the sampling procedures. Normalization is defined as a prolactin drop to gender-specific normal reference ranges, which are 100 to 500 mU/L for females and 60 to 300 mU/L for males. Repeat random prolactin and cannulated prolactin test levels were analyzed in the same lab using the Abbott architect immunoassay analyzer (Abbott Diagnostics, Maidenhead, UK) with an intra- and inter-assay coefficient of variation (cv) of serum prolactin of 1.6% and 2.5%, respectively.

Statistical Analysis
Proportional outcomes were presented as numbers and percentages. Continuous values were expressed as mean with standard deviation (SD) or median with range if normally or non-normally distributed respectively. We performed Chi-Square and Fisher’s Exact tests for categorical variables, t tests for normally distributed variables and Mann–Whitney U test for non-normally distributed variables. A 2-tailed probability value of <5% was considered statistically significant. Statistical analysis was performed using statistical software (Stata13, Stata corp LP, Texas).

Results
In this cohort, the mean age of patients was 33.8 years (SD ± 9.9). The majority of patients were female (37 patients or 88%). There was a good correlation between the referral and the repeat random prolactin levels (Figure 1). The cannulated prolactin normalized in 12 (28.6%) patients, while it remained elevated in 30 (71.4%) patients. Repeat random prolactin was significantly higher in patients whose prolactin level did not normalize in a cannulated prolactin test (Table 1 and Figure 2). The main presenting symptoms were menstrual irregularities (amenorrhoea, oligomenorrhoea, prolonged cycle length) in 28.57% of the patients whereas 19% were asymptomatic (Figure 3). Overall, there was no significant difference in symptom distribution between patients whose prolactin normalized and those patients whose prolactin did not normalize during the cannulated prolactin test (P = .397). Pituitary MRI was available for 93% (28/30) patients who did not normalize their prolactin. There were pituitary abnormalities in 23 out of 28 (82%) and the majority of patients was found to have a pituitary microadenoma (18 patients) as shown in Figure 4. The median (range 5%-95%) size of the lesion was 6 (3-10) mm. Out of the patients who normalized prolactin during a cannulated prolactin test, 2 had normal pituitary MRI prior to the referral. Among the 5 male patients in the cohort, the median (range 5%-95%) repeat random prolactin was 526 (421-892) mU/L. Out of 4 male patients

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Figure 1. Correlation between referral and repeat random prolactin levels. Values for both genders are included. N = 37. P value < .0001.
Table 1. Characteristics of patients based on normalization status in cannulated prolactin test.

|                          | NORMALIZING IN CANNULATED PROLACTIN TEST (N=12) | NON-NORMALIZING IN CANNULATED PROLACTIN TEST (N=30) | P VALUE |
|--------------------------|-----------------------------------------------|-----------------------------------------------------|---------|
| Age (Years) Mean ± SD    | 33 ± 11                                       | 34 ± 10                                             | .67a    |
| Female:Male (N)          | 11:1                                          | 26:4                                                | .554b   |
| Referral Prolactin^ (mU/L) median (range 5%-95%) | 901 (454-1501) | 1227 (505-3257)         | .0847c  |
| Repeat random Prolactin* (milliunit/L) median (range 5%-95%) | 566 (421-995) | 1048 (526-2212)         | .0011c  |
| MRI performed (N)        | 2                                            | 28                                                  | <.05b   |
| MRI abnormality (N)      | 0                                            | 23                                                  | .152b   |

^Referral prolactin levels were available for 38 patients, 10 patients in normalizing group and 28 in non-normalizing group.
*Repeat random prolactin levels were available for 41 patients (11 patients in normalizing group and 30 non-normalizing group).
P Value derived from a T-Test, bFisher Exact Test & cMann–Whitney U test.

Normal Prolactin range: Females: 100 to 500 mU/L, Males: 60 to 300 mU/L.

who did not normalize prolactin during a cannulated prolactin test, 3 patients had no pituitary abnormality and 1 patient was found to have a partially empty sella without a distinct pituitary lesion. Among patients who normalized prolactin during a cannulated prolactin test, 7 patients had normal prolactin levels at time 0 of the test, while 3 and 2 patients’ prolactin normalized at times 60 and 120 minutes, respectively (Figure 5). In a univariate logistic regression analysis, a single repeat random prolactin sample was the only significant predictor for normalization in the cannulated prolactin test (P = 0.011).

Discussion

Prolactin is secreted in a pulsatile fashion by pituitary lactotroph cells. It has a circulating half-life of 20 to 50 minutes and is metabolized by the liver and kidney. It has been shown that it may also be produced by many extra pituitary cells. Prolactin plays an important role in lactation during pregnancy, but it is involved in other biological functions such as angiogenesis, immunoregulation, and osmoregulation. Prolactin exists in different forms in human serum with monomeric prolactin accounts for 80% to 95% of the total prolactin followed by dimeric prolactin that makes up <10%. Macroprolactin accounts for <1% of the total prolactin. The overall global prevalence estimate of macroprolactin is 18.9% (95% CI: 15.8%, 22.1%) which necessitates screening for before investigating for causes of elevated prolactin.

An initial report by Ferriani et al demonstrated that the stress induced by venipuncture has little influence on serum prolactin levels. However, due to the significant variability in the concept of stress, the difficulty of quantifying stress and the variation in individual sensitivity, doubts persisted regarding the need for rest before sample collection for the measurement of serum prolactin. Muneyyirci-Delale et al reported that serial blood samplings drawn at 0, 30, and 60 minutes from an indwelling catheter, as a patient rests in a quiet room, were sufficient to diagnose stress-induced hyperprolactinemia in 20 out of 70 (28.6%) women with mild to moderate elevations in serum prolactin between 20 and 100 ng/mL (426-2128 mIU/L) in 2 or more random blood samples. Ishay et al collected blood samples repeatedly under resting conditions from an indwelling brachial catheter every 30 minutes for a total of 6 samples in 3 women with suspected stress-related mild-to-moderate hyperprolactinemia. All 3 patients achieved normal prolactin levels 30 to 60 minutes after starting the test. Another study found that a 15-minute prolactin level following intravenous catheterization was not useful to diagnose stress-induced hyperprolactinemia, which would be expected given the half-life of prolactin.
2 time points, 0 and 120 minute, during a cannulated prolactin test. It is worth noting that in this study, the cannulated prolactin test was undertaken with previously 1 elevated prolactin level only. The baseline prolactin during the cannulated prolactin test was considered as the repeat prolactin. In a recent large retrospective study by Tsur et al included 757 patients, almost two thirds of patients normalized their prolactin level in cannulated prolactin test. In addition to the findings by Whyte et al and Tsur et al, we show that the cannulated prolactin test was useful in excluding true hyperprolactinaemia in 28.6% of patients with confirmed elevated prolactin levels on more than 1 occasion (on both referral and repeat random prolactin samples). Non-normalization during a cannulated prolactin test was associated with a significantly higher repeat random prolactin level and a higher trend in referral prolactin, but the latter was not statistically significant. Given the relatively small sample
size and the wide range of repeated prolactin levels in those who did not normalize during a cannulated prolactin test, we were not able to establish a prolactin threshold where pituitary MRI should be performed.

The presence or type of symptoms at the time of presentation was a poor predictor as to whether true hyperprolactinemia was present which is similar to the conclusions reached by Whyte et al. A possible explanation for this could be that the test was performed in patients with mild prolactin elevation who would be expected to have mild or non-specific symptoms only. Our patients were referred either from general practice or other specialties and some patients would have had their prolactin level checked without a clear indication. Overall, there was no statistically significant difference in symptom distribution, however, a higher number of patients with menstrual irregularities was present in those patients who did not normalize during a cannulated prolactin test (10 vs 2 who did normalize). It would be expected that patients with menstrual irregularities would seek medical attention more readily. In addition, the decision to investigate often depends on the primary physician’s threshold to carry out tests which would be lower in symptomatic patients.

It is also worthwhile emphasizing that in our practice, the decision to carry out a cannulated prolactin test is being made at the discretion of the attending physician as would be impractical to do cannulated prolactin tests in all patients with elevated prolactin levels. Since only patients who had a cannulated prolactin test were included in this retrospective study, there was no symptom comparison with those who were not subjected to a cannulated prolactin test.

In contrast to our study, Avari et al reported a 73% normalization during a cannulated prolactin test with 1 prolactin measurement after 60 minutes of rest. It is worth mentioning that the mean referral prolactin in this study was significantly lower (mean 796 mU/L) than in our and in Whyte’s et al study.

In the study by Whyte et al, pituitary MRI was performed in 85% (146/171) of patients with true hyperprolactinemia (defined by persistent hyperprolactinemia during a cannulated prolactin test) of which 65% (95/146) were abnormal. No further details of the MRI findings were provided. In our study, 93% (28/30) underwent pituitary MRI scanning of whom 82% (23/28) showed pituitary abnormalities.

In this study, only 5 male patients were included, 4 (80%) of whom did not normalize prolactin during a cannulated prolactin test. Out of those 4, only 1 patient had abnormal pituitary MRI. The majority of female patients who did not normalize prolactin during a cannulated prolactin test were found to have pituitary abnormalities. We do not have an explanation for this discrepancy, but the small number of male patients included in

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**Figure 5.** Prolactin levels at the 3 time points in cannulated prolactin test based on normalization status. Values for both genders are included. N=42.

One female patient with a pituitary microadenoma on MRI has a missing 120 minute point value. Normal reference range 100 to 500 mU/L for females and 60 to 300 mU/L for males.
stress such as an anxiety can cause mild hyperprolactinemia that might potentially also resolve during cannulated prolactin sampling. Also, our study lacks information about the levels of circulating estradiol and gonadotropins which may aid in differentiating true hyperprolactinemia

Conclusion
Reliance on 1 single random measurement of mild prolactin elevation leads to over-diagnosis of hyperprolactinaemia and inappropriate investigations. A cannulated prolactin test is a simple, but reliable method to rule out hyperprolactinaemia related to stressful venipuncture. It is of value in the context of mildly elevated prolactin levels, even on repeated occasions. Therefore, it could be considered as part of the investigations for mild hyperprolactinaemia with negative macroprolactin and absent secondary causes before exposing the patient to pituitary imaging.

Authors Contributions
RA & SZ shared first co-authorship. RA, SZ, FW, KM designed the study, prepared the manuscript and designed the figures and tables. RA, SZ conducted data collection. RA performed the statistical analysis. FW, KM were the project supervisor. RA, SZ, FW, KM reviewed and edited the manuscript. All authors have made a substantial, direct and intellectual contribution to the work and approved the manuscript prior to its submission.

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REFERENCES
1. Melmed S, Casanueva FF, Hoffman AR, et al. Diagnosis and treatment of hyperprolactinemia: an endocrine society clinical practice guideline. J Clin Endocrinol Metab. 2011;96:273-288.
2. Levine S, Muneyyirci-Delale O. Stress-induced hyperprolactinemia: pathophysiology and clinical approach. Obes Med. 2018;5:250-253.
3. Vilar L, Vilar CF, Lyra K, Da Conceição Freitas M, Pófals M. In the diagnostic evaluation of hyperprolactinemia. Neuroradiol. 2019;109:7-19.
4. Casanueva FF, Molitch ME, Schlechte JA, et al. Guidelines of the Pituitary Society for the diagnosis and management of pituitary tumors. Clin Endocrinol. 2006;65:265-273.
5. Thapa S, Bhussal K. Hyperprolactinemia. StatPearls Publishing. 2020. https://www.ncbi.nlm.nih.gov/books/NBK537331/
6. Saleem M, Martin H, Coates P. Prolactin biology and laboratory measurement: an update on physiology and current analytical issues. Clin Biochem Rev. 2018;39:3-16.
7. Samson SL, Hamrahian AH, Ezatz S. American association of clinical endocrinologists, american college of endocrinology disease state clinical review: clinical relevance of macroprolactin in the absence or presence of true hyperprolactinemia. Endocr Pract. 2015;21:1427-1435.
8. Whyte MB, Prazsodh S, Sirkuran L, et al. Importance of cannulated prolactin test in the definition of hyperprolactinemia. Pituitary. 2015;18:319-325.
9. Capozzi A, Scambia G, Pontecorvi A, Lello S. Hyperprolactinemia: pathophysiology and therapeutic approach. Gynecol Endocrinol. 2015;31:506-510.
10. Che Soh N, Yaacob N, Omar J, et al. Global prevalence of macroprolactinemia among patients with hyperprolactinemia: a systematic review and meta-analysis. Int J Environ Res Public Health. 2020;17:8199.
11. Ferriani RA, Silva de Sá MF. Effect of venipuncture stress on plasma prolactin levels. J Gynaecol Obstet. 1985;23:439-462.
12. Muneyyirci-Delale O, Goldstein D, Reyes FJ. Diagnosis of stress-related hyperprolactinemia. Evaluation of the hyperprolactinemia rest test. NY State J Med. 1989;89:205-208.
13. Ishay A, Luboshitzky R. Diagnosis of Hyperprolactinemia: determination of Prolactin Level at Rest. Harefuah. 1998;135:348-407.

14. Briet C, Saraval M, Lorie S, Topolinski-Duyme H, Fendri S, Desailloud R. The use of intravenous catheterisation with a rest period is useful for determination of plasma cortisol levels but not plasma prolactin levels. Ann Endocrinol. 2007;68:34-38.

15. Tsur A, Dreyfuss E, Ness-Abramof R, Pollack R, Cahn A. Role of cannulated prolactin test in evaluation of hyperprolactinemia – a retrospective study. Endocr Pract. 2020;26:1304-1311.

16. Avari P, Sharma S, Hui E, Qureshi A. Cannulated prolactin avoids over-diagnosis and unnecessary investigations in normoprolactinaemic patients. Presented at Society for Endocrinology BES 2017, Harrogate, UK. Endocr Abstr. 2017;50:P293.

17. Molitch ME. Nonfunctioning pituitary tumors and pituitary incidentalomas. Endocrinol Metab Clin North Am. 2008;37:151-171.

18. Pereira-Lima JF, Leães CG, Freitas Neto FM, Barbosa MV, da Silva ALM, Oliveira MDC. Hyperprolactinemia and body weight: prevalence of obesity and overweight in patients with hyperprolactinemia. Res J Endocrinol Metab. 2013;1:2.