

RADIOIMMUNOASSAY OF PROLACTIN

H. M. Gwee
D. Chua
P.P.B. Yeo
J.S. Cheah
P. Lim

**University Department of Medicine
Singapore General Hospital
Singapore 0316.**

H.M. Gwee, MBBS, M.MED. (INT. MED.), FRACP
Senior Lecturer

D. Chua, B. Sc. (Hon.) (Canada), M.Sc. (Canada)
Clinical Biochemist

P.P.B. Yeo, MBBS, MRCP, FRACP
Senior Lecturer

J.S. Cheah, MD, MBBS, FRACP
Professor

P. Lim, MA (Camb), MB Bchir (Camb),
MD (Camb), FRCP (Lond), FRACP, Am
Professor

INTRODUCTION

Although prolactin is phylogenetically one of the oldest hormones known to man it was only in 1970 that investigators were able to confirm that the primate pituitary secretes both prolactin and growth hormone as distinct molecular species. Hwang et al (1971)¹ were the first investigators to introduce a sensitive and specific radioimmunoassay for prolactin. This led to a wealth of information about the control of prolactin secretion and also its clinical significance. This paper describes our initial experience of radioimmunoassay of prolactin.

MATERIALS AND METHOD

The RIA of human PRL was carried out using the modified technique of Gwee and Mashiter (1978).² Human prolactin VLS 1 was used as standard, human prolactin VLS 3 for iodination and rabbit anti VLS 3 as anti-serum. All reagents were generously donated by the National Pituitary Agency NIAMDD, USA. PRL was labelled with ^{125}I (IMS 30) using the lactoperoxidase technique. The assay was simple and required 7 days for completion.

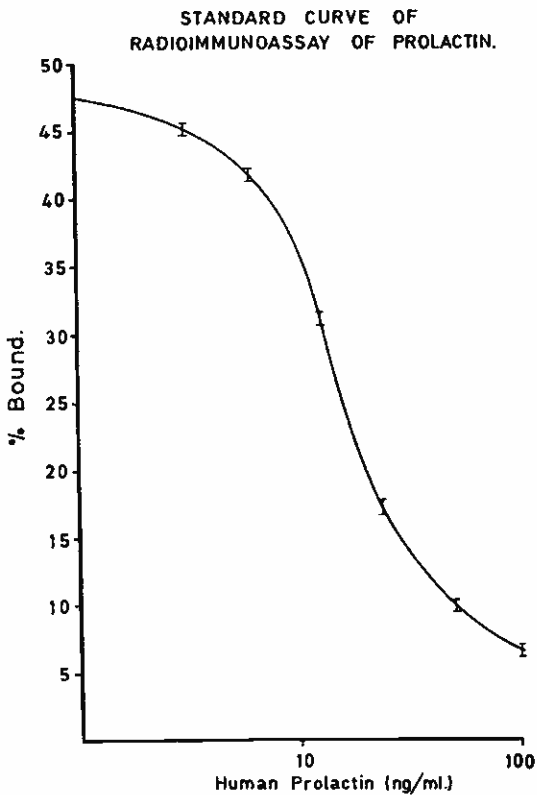
In 7 normal males and 5 normal females, two serum prolactin samples were measured, one immediately after venepuncture and another 1 hour later through an indwelling butterfly venous catheter. Random serum PRL was taken in 23 normal males, 47 normal females, 8 acromegalics, 5 patients with galactorrhoea, 2 patients with pituitary tumour and 2 males with impotence.

In a further 16 normal males and 26 females blood was collected through an indwelling catheter before, 20 and 60 mins after the intravenous administration of 200 μg of thyrotrophin releasing hormone. (TRH).

RESULTS

A typical standard curve for RIA of PRL is shown in Figure 1. The lower limit of detection was 2 ng/ml and measurable range < 2 ng/ml to 100 ng/ml. Values for the intra and interassay variation for a concentration of prolactin normally found in serum (14 ng/ml) were, respectively 8% ($n = 6$) and 12% ($n = 8$).

Figure 1



Mean serum prolactin for 47 normal women was 12.3 ng/ml (range 2.2 to 22.3 ng/ml) and 8.6 ng/ml (range 1.8 to 15.4 ng/ml) for 23 normal males. (Table 1).

TABLE 1
SERUM PROLACTIN IN 72 NORMAL SUBJECTS

SUBJECT	PRL in ng/ml		
	Mean	SD	Range
Males (23)	8.6	3.4	1.8 to 15.4
Females (47)	12.3	5.0	2.2 to 22.3

The effect of venepuncture was studied in 7 normal males and 5 normal females. Table 2 shows that the mean serum prolactin levels at insertion of needle was 5.3 ng/ml for males and 15.3 ng/ml for females. After an hour's rest prolactin levels fell to 4.2 ng/ml in males (ns) and 10.2 ng/ml in females ($p < 0.05$) (Table 2).

TABLE 2
STRESS OF VENEPUNCTURE AND SERUM PROLACTIN

SUBJECT	PRL in ng/ml	
	Venepuncture	1 hr later
Normal males (7)	5.3	4.2 ⁺
Normal females (5)	15.3	10.2 [*]

⁺ $p > 0.05$ ns

^{*} $p < 0.05$

In 27 normal females the mean basal prolactin was (12.5 ng/ml range 8.9 to 16.1 ng/ml); following 200 μ g of intravenous thyrotrohin releasing hormone (TRH) prolactin levels rose to a mean of 56.7 ng/ml (range 38 to 75.4 ng/ml) after 20 minutes before decreasing to 31 ng/ml (range 19.7 to 41.3 ng/ml) at 60 minutes. In 16 normal males prolactin levels were 10.1 ng/ml (range 7.8 to 12.4 ng/ml) at 0 minute and 33.2 ng/ml range (23.7 to 42.7 ng/ml) at 20 minutes and 17 ng/ml range (12.2 to 21.8 ng/ml) at 60 minutes respectively after TRH. (Table 3 and Figure 2).

TABLE 3
THYROTROPHIN RELEASING HORMONE AND SERUM PROLACTIN

Time Mins	Se PRL ng/ml	
	16 males Mean \pm 1 SD	27 females Mean \pm 1 SD
0	10.1 \pm 2.3	12.5 \pm 3.6
20	33.2 \pm 9.5	56.7 \pm 18.7
60	17.0 \pm 4.8	30.9 \pm 11.3

Figure 2

THYROTROPHIN (TRH) TEST AND SERUM PROLACTIN (PRL)

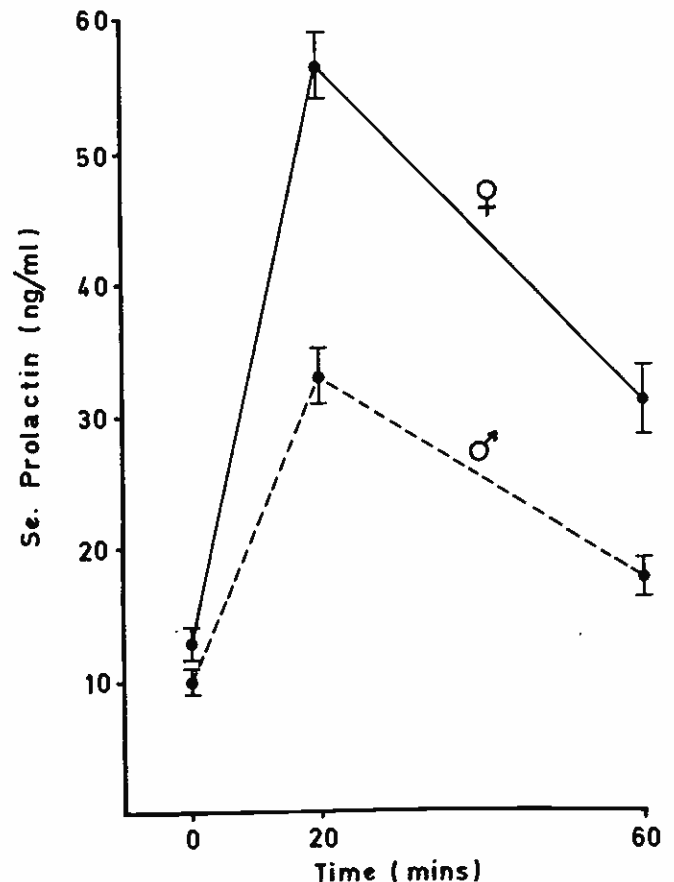


Table 4 summarises the clinical findings and serum prolactin in 5 female patients presenting with galactorrhoea. The range of serum prolactin varied from 2 ng/ml to 150 ng/ml. Only three of five patients had elevated levels.

TABLE 4
SERUM PROLACTIN IN GALACTORRHOEA

PATIENT	AGE/SEX	PITUITARY FOSSA	BASAL PRL ng/ml
CBG	37/F	Normal	132
JEC	34/F	Normal	2
PSK	28/F	Normal	150
TCW	32/F	Normal	65
YGK	19/F	Normal	6.4

Prolactin levels were normal in 6 out of 8 acromegalic patients. In one female patient with diabetes insipidus due to pituitary microadenoma (confirmed at operation) serum prolactin was only 21 ng/ml. A 72 year old Chinese man with a huge pituitary tumour had a random serum prolactin of 35 ng/ml. In 2 males presenting with decreased libido and impotence prolactin level was 5 ng and 20 ng respectively. (Table 5).

TABLE 5

PATIENT	AGE/SEX	DIAGNOSIS	BASAL PRL ng/ml
LT	50/M	Acromegaly	30.5
NBK	13/F	Gigantism	25.0
TAK	28/M	Acromegaly	10.5
KAS	29/M	Acromegaly	20.5
YST	58/M	Acromegaly	6.6
CYL	53/F	Acromegaly	13.5
OTC	25/M	Acromegaly	6.2
TAK	34/M	Acromegaly	8.6
NAH	56/F	Diabetes Insipidus	21.0
YLH	72/M	Pituitary Tumour	35.0
MAW	39/M	Impotence	20.0
CSL	34/M	Impotence	5.0

DISCUSSION

The clinical importance of prolactin radioimmunoassay is well established and their wide availability has been aided by the generous distribution of reagents by the National Institute of Health, USA.

There are many factors that influence the regulation of PRL secretion in man (Hwang et al).¹ There are also many methods available in the RIA of PRL and results may vary considerably (Cotes et al 1978)³.

In our series of 47 normal females and 23 normal males the mean serum prolactin was 12.3 ng/ml (range 2.2 to 22.3 ng/ml) and 8.6 ng/ml (range 1.8 to 15.4 ng/ml) respectively. This compares favourably with most authorities who consider resting values of less than 20 ng/ml normal for males and less than 25 ng/ml normal for females (Kleinberg et al, 1977).⁴ Higher values in females reflect the higher oestrogen environment in women since prolactin secretion is increased in the presence of oestrogens (Guyda and Friesen, 1973,⁵ Yen and Siler, 1974⁶).

Prolactin levels are elevated in conditions of stress in animals (Neill 1970).⁷ In man general anaesthesia, endoscopy and intravenous oxytocin can all cause elevation of PRL. The effect of venopuncture was studied in 7 normal males and 5 females. Table 2 shows that mean serum prolactin levels was higher at time of venepuncture than levels taken 1 hour after rest in both males and females. The difference was not significant in males but significant in females. This emphasises the importance of resting patients before serum prolactin is estimated. Single random estimations of serum prolactin have therefore to be interpreted with caution if values are just slightly elevated.

Thyrotrophin releasing hormone (TRH) is the first hypothalamic hormone to be synthesised and made commercially available. It is a tripeptide that acts directly on the pituitary to release thyrotrophin and prolactin. In our study mean serum prolactin rose from a basal value of 12.5 ng/ml to a peak of 56.7 ng/ml in 26 normal females. The corresponding values for 16 normal males was 10.1 ng/ml to 33.2 ng/ml. (Table 3, Figure 2). In both males and females the peak serum prolactin was more than twice basal values indicating a normal response (Neelon 1978)⁸ Subnormal prolactin response to TRH imply pituitary disease except in patients with hyperthyroidism which induces a functional impairment of response. Synder in 1973 suggested that the loss of prolactin secretory response to TRH is probably the most sensitive indicator of deranged hypothalamic and pituitary function. The TRH test may therefore become an important clinical manoeuvre in the investigation of hypothalamic and pituitary disorders.

In five patients with galactorrhoea serum prolactin was elevated in three patients. One had a serum prolactin of only 2 ng/ml. This finding is not unexpected because although most patients with galactorrhoea had elevated serum prolactin this was not an invariable finding (Edwards 1971)¹⁰

Two acromegalics (26%) had raised serum prolactin which confirms the finding of Hwang (1971)¹ and Friesen (1972)¹¹. Elevated serum prolactin in acromegaly could arise from either the pituitary tumour or from normal pituitary tissue disconnected from the hypothalamus.

In one female patient with diabetes insipidus due to a pituitary microadenoma serum prolactin was only 21 ng/ml. A 72 year old Chinese man with a large pituitary tumour and bilateral optic atrophy had a random serum prolactin of 35 ng/ml. These results suggests that they do not have prolactinomas because prolactin secreting tumours frequently produce serum prolactins above 200 ng/ml (Kleinberg 1977)⁴

Although hyperprolactinaemia does not seem to provide the explanation of most cases of idiopathic

impotency with normal testosterone serum prolactin evaluation is nevertheless important to exclude the presence of a prolactinoma (Buvat 1978)¹². In 2 males presenting with decreased libido and impotence, hyperprolactinaemia is unlikely to be the etiological factor since serum prolactin was normal in one and slightly elevated in the other.

The introduction of RIA for PRL has considerably advanced our knowledge of prolactin in health and disease. This study illustrates the usefulness of RIA of prolactin in the investigation of galactorrhoea impotency and hypothalamic and pituitary disorders.

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