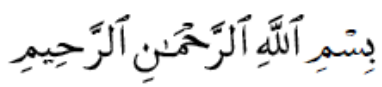
ACKNOWLEDGEMENT



In the name of Allah, the Most Compassionate, the Most Merciful. Salutation be upon His Messenger Muhammad S.A.W., His family and His companion. With help and success granted by Allah, I have finished and completed my dissertation on time. I would like to take this opportunity to express my deepest gratitude to my supervisor Dr Julia Omar from Department of Chemical Pathology Universiti Sains Malaysia, and my co-supervisors Dr Wan Mohd Izani Wan Mohamed from Department of Internal Medicine, Hospital Universiti Sains Malaysia.

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LIST OF SYMBOLS AND ABBREVIATIONS

+	:	plus
±	:	plus minus
=	:	Equal to
%	:	Per cent
<	:	Less than
>	:	More than
°C	:	Degree celcius
bp	:	Base pair
CI	:	Confidence interval
CT	:	Computed tomography
df	:	Degree of freedom
FSH	:	Follicle-stimulating hormone
GFC	:	Gel filtration chromatography
GH	:	Growth hormone
GnRH	:	Gonadotropin-releasing hormone
HPLC	:	High performance liquid chromatography
HyperPRL	:	Hyperprolactinemia

HUSM	:	Hospital Universiti Sains Malaysia
Ig	:	Immunoglobulin
IH	:	Idiopathic hyperprolactinemia
IQR	:	Interquartile range
IU/L	:	International Unit per liter
kDa	:	kilo Dalton
LH	:	Luteinizing hormone
m^2	:	Meter square
mg	:	miligram
ml	:	mililiter
μL	:	microliter
MRI	:	Magnetic resonance imaging
mIU/L	:	mili International Unit per Liter
PA	:	Protein A
PG	:	Protein G
PEG	:	Polyethelene glycol
pmol/L	:	picomol per liter
PRL	:	Prolactin

SD	:	Standard deviation
UF	:	Ultrafiltration
UK	:	United Kingdom
US	:	United State
w/v		Mass/volume

ABSTRAK

Makroprolaktinemia di kalangan pesakit hiperprolaktinemia (HyperPRL) di Hospital Universiti Sains Malaysia (HUSM)

Latarbelakang kajian : HyperPRL adalah gangguan hipotalamus- pituitari yang paling kerap dihadapi dalam endokrinologi. Makroprolaktinemia merupakan penyebab yang tidak merbahaya bagi hyperPRL. Makroprolaktin adalah bentuk tidak aktif PRL yang terdiri daripada prolaktin (PRL) monomer dan antibodi Immunoglobulin G. Penularan makroprolaktinemia semakin meningkat dalam amalan endokrinologi. Adalah penting untuk membezakan di antara makroprolaktinemia dan hyperPRL kerana makroprolaktinemia tidak memerlukan rawatan. Selain itu, gejala klinikal tidak dapat membezakan antara kedua - dua keadaan.

Tujuan: Untuk mengenalpasti prevalens makroprolaktinemia dan ciri-ciri klinikal yang ketara berkaitan dengan makroprolaktinemia di kalangan pesakit hyperPRL di HUSM.

Rekabentuk kajian: Satu kajian hirisan lintang telah dijalankan pada tahun 2013 yang melibatkan pesakit yang didiagnosis sebagai hyperPRL di HUSM 2011 to 2013. Serum daripada pesakit ini diukur untuk PRL dengan menggunakan cobas e411 (prinsip sandwich) dan serum yang sama dirawat dengan polietilena glikol (PEG) 8000 untuk membezakan hyperPRL benar dan makroprolaktinemia. Pemulihan PRL kurang dari 40% menunjukkan kehadiran makroprolaktin.

Keputusan kajian: Sejumlah 133 pesakit hyperPRL , 120 (90 %) wanita dan 13 (9.8%) lelaki berusia di antara 18 hingga 68 tahun dengan min (SD) umur 34.37 tahun (11.75) telah dimasukkan dalam kajian ini. Sembilan pesakit (semua perempuan) didapati

mempunyai makroprolaktinemia [kelaziman = 6.8 % (95 % CI: 2.4 %, 11.1 %)]. Tiada hubungan yang signifikan di antara tanda-tanda klinikal dan diagnosis makroprolaktinemia dalam kajian ini.

Kesimpulan: Penularan makroprolaktinemia dikesan dengan menggunakan PEG 8000 di kalangan pesakit yang didiagnosis sebagai hyperPRL adalah rendah. Saringan untuk makroprolaktin menggunakan PEG 8000 menunjukkan bahawa kebanyakan pesakit yang datang dengan hyperPRL di HUSM adalah hyperPRL benar. Oleh itu, tanda-tanda klinikal tidak dapat membezakan antara hyperPRL dan makroprolaktinemia.

ABSTRACT

Macroprolactinemia among Hyperprolactinemia (HyperPRL) Patients in Hospital Universiti Sains Malaysia (HUSM)

Background: HyperPRL is the most common hypothalamic-pituitary disorder encountered in clinical endocrinology. Macroprolactinemia is a known benign cause of hyperPRL. Macroprolactin is a non-bioactive form of PRL, composed of monomeric PRL and Immunoglobulin G antibodies. The prevalence of macroprolactinemia is increasing in endocrinology practice. It is important to differentiate between macroprolactinemia and hyperPRL as macroprolactinemia does not require any treatment. However the clinical symptoms are could not differentiate between these two conditions.

Aim: To determine the prevalence of macroprolactinemia and significant clinical features associated with macroprolactinemia among hyperPRL patient in HUSM.

Design: A cross sectional study was conducted in 2013 involving patients diagnosed as hyperPRL in HUSM from 2011 to 2013. Serum from these patient were measured for PRL using cobas e411 (sandwich principle) and the same serum were treated with polyethylene glycol (PEG) 8000 to differentiate true hyperPRL and macroprolactinemia. PRL recovery of less than 40% indicates of presence macroprolactin.

Results: A total of 133 hyperPRL patients, 120 (90%) female and 13 (9.8%) male aged between 18 to 68 years old with mean (SD) age of 34.37 (11.75) years old were included in this study. Nine patients (all female) were found to have

macroprolactinemia [prevalence=6.8% (95% CI: 2.4%, 11.1%)]. There were no significant association between clinical symptoms and diagnosis of macroprolactinemia in this study.

Conclusion: The prevalence of macroprolactinemia detected using PEG 8000 among patients diagnosed as hyperPRL was low. Screening for macroprolactin using PEG 8000 showed that majority of patients presented with hyperPRL in HUSM were true hyperPRL. Clinical symptoms alone therefore, could not distinguish between hyperPRL and macroprolactinemia.

CHAPTER 1: INTRODUCTION

Prolactin (PRL) is a hormone secreted by specialized cell within the adenohypophysis. The primary role of PRL is to stimulate and sustain lactation during postpartum period. PRL has many other effects including maintenance of immune system and in ovarian steroidogenesis. PRL also known as lactogen, lactotropin, luteotropin, mammotropin or galactopoietic, lactation, lactogenic or luteotropic hormone (Tietz, 2006).

PRL is synthesized as a prehormone with a molecular weight of 26 kDa. PRL contain 199 amino acids and has three intramolecular disulphide bridges. When the prePRL is cleaved, the resulting polypeptide has a molecular weight of 23 kDa (60 to 90%). This monomeric form accounts for the majority of total PRL. Serum also contains a 48-56 kDa (15 to 30%) form that is termed as big PRL and another isoform with a molecular weight of more than l00 kDa (zero to10%), which is termed as big big PRL (Tietz, 2006). The PRL polypeptide is arranged in a single chain of amino acids with three highly conserved intramolecular disulfide bonds between six cysteine residues. According to nuclear magnetic resonance spectroscopy, PRL folds into four antiparallel alpha helices, similar to the tertiary structure of growth hormone (GH) and other close relatives (Keeler *et al.*, 2003). Figure 1.1 below shows illustration of PRL proteins as they exist in the serum as three forms: monomeric 23 kDa PRL (>95%), "big PRL," consisting of PRL aggregates and "big big PRL," or macroprolactin, consisting of PRL bound to immunoglobulin G (IgG). Red hatching indicates the relative locations of the three disulfide bonds. A single N-glycosylation site has been identified on human

PRL at codon 31. Two putative phosphorylation sites (not depicted) have been proposed at serines 163 and 194 (Keeler *et al.*, 2003).

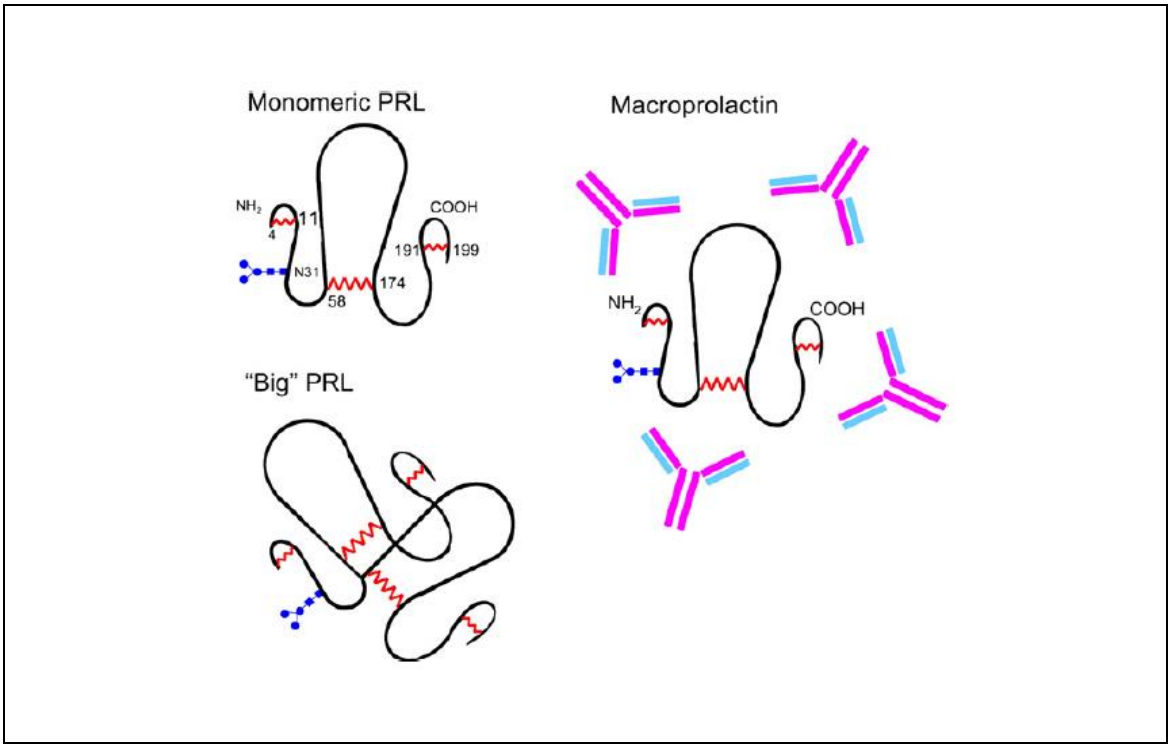

Figure 1.1: Schematic illustration of prolactin proteins. (Keeler et al., 2003)

The monomeric form is considered as the most bioactive form that found in the circulation and demonstrate the greatest response to thyrotrophin releasing hormone (TRH), the hypothalamic releasing factor that stimulates the pituitary to release PRL (Tietz, 2006).

Hyperprolactinemia (HyperPRL) is the most common hypothalamic-pituitary disorder encountered in clinical endocrinology. The clinical syndromes of hyperPRL are galactorrhea, oligomenorrhea or amenorrhea and infertility in women and reduced libido, oligospermia or impotence or both and also galactorrhea in men. HyperPRL has

an estimated prevalence of 15% in women with secondary amenorrhea, a condition that affects at least three per cent of women of reproductive age (Gibney *et al.*, 2005c).

In general, big big PRL also known as macroprolactin is a non-bioactive PRL isoform usually composed of a PRL monomer and an IgG molecule (Kavanagh-Wright *et al.*, 2009; Richa *et al.*, 2010). It is have a prolonged clearance rate similar to that of immunoglobulins. This isoform is clinically non-reactive but it interferes with immunological assays used for the detection of PRL (Richa *et al.*, 2010). When the serum of a patient with hyperPRL contains mostly macroprolactin the condition is termed macroprolactinemia. Because some of the laboratories fail to screen hyperprolactinemic sera for macroprolactinemia this may lead to misdiagnosis and unnecessary medical and surgical intervention (Beltran *et al.*, 2008) or delayed diagnosis and inappropriate treatment.(Olukoga *et al.*, 1999; Suliman *et al.*, 2003; Gibney *et al.*, 2005a).

Screening of hyperprolactinemic sera for the presence of misleading concentrations of macroprolactinemia is readily performed in biochemistry laboratories although the procedures have not been automated. The most widely employed method is to treat the hyperprolactinemic sera with polyethylene glycol (PEG) which precipitates out high-molecular weight constituents including immunoglobulins. Re-assay of the sera for PRL will then identify those sera which yield values within the relevant normal range indicative of macroprolactinemia and not true hyperPRL (Joseph McKenna, 2009).

There are few methods available to remove macroprolactin namely, gel filtration chromatography (GFC), PEG, protein A (PA), protein G (PG), anti-human IgG (anti-hIgG), and ultrafiltration (UF). These methods deplete macroprolactin from sera before

immunoassay. Among all the methods available, PEG is the suitable and practical method (Kavanagh *et al.,* 2006). Generally PEG precipitation is used to differentiate macroprolactinemia from true hyperPRL. This method is simple, cheap and rapid for the detection of macroprolactinemia (Leslie *et al.*, 2001). This method has been extensively validated against GFC and has obtained good correlation (Vieira *et al.*, 1998; Fahie-Wilson, 1999; Olukoga *et al.*, 1999).

GFC technique is robust and reproducible. It is often considered as the gold standard. There are four main disadvantages. First, with a low affinity antibody complex there exists the potential for dissociation of PRL from the autoantibody during the lengthy gel filtration run, thereby this leads to an underestimation of the macroprolactin content in serum. In practice, dissociation to any appreciable extent does not take place, indicating that macroprolactin is probably a high affinity complex. Secondly, there is considerable inherent cumulative imprecision associated with measuring the levels of PRL and macroprolactin in 30 to 40 discrete fractions to obtain an estimate of the percentage of macroprolactin present. Third, procedural loss of PRL immunoreactive material through adsorption or denaturation during the gel filtration runs if selective. It could be a disproportionate loss of either PRL or macroprolactin, which would lead to either under- or overestimation of the individual isoforms present. Fourth, the labour-intensive nature and expense of GFC usually preclude its widespread use in all except for research laboratories (Gibney *et al.*, 2005c).

CHAPTER 2: LITERATURE REVIEW

2.1. Prolactin

2.1.1. Gene, primary structure and species specificity

PRL belongs to the somatotropin/PRL family, a large family of proteins that includes GH, placental lactogens (PL), PRL-like and PRL-related proteins, proliferins and proliferin-related proteins. The human PRL gene is located on chromosome 6p22.2-p21.3 and consists of five coding exons, one noncoding exon and four introns (Truong *et al.*, 1984). It is believed that PRL, GH and PL arose from duplication of a common ancestral gene ~ 400 million years ago (Niall *et al.*, 1971; Cooke *et al.*, 1981).The divergence of the PRL and GH lineages occurred; 400 million years ago as quoted by Cooke *et al* (1981).

In the human genome, a single gene was found on chromosome six that encoded PRL (Owerbach *et al.*, 1981). The PRL gene is 10 kilobase in size and is composed of five exons and four introns (Cooke *et al.*, 1981; Truong *et al.*, 1984). Transcription of the PRL gene is regulated by two independent promoter regions. The proximal 5,000 base pairs (bp) region directs pituitary-specific expression (Berwaer *et al.*, 1991), while a more upstream promoter region is responsible for extrapituitary expression (Berwaer *et al.*, 1994). The human PRL complementary DNA is 914 nucleotides long and contains a 681-nucleotide open reading frame encoding the PRL prehormone (pre-PRL) of 227 amino acids. During PRL processing, the 28-amino acid signal peptide is proteolytically cleaved (Shome and Parlow, 1977), resulting in a mature human PRL. (Sinha, 1995). Figure 2.1 below shows schematic diagram of the

human PRL 5' gene regulatory region, gene and messenger RNA transcript. The PRL gene consists of five exons, designated by the numbers [1e5]. The region from e5800 bp to zero indicates the region known to regulate pituitary PRL synthesis whereas the region from e8789 to e5800 is referred to as the "superdistal" or "extrapituitary" promoter region. Thirteen Pit1-binding sites are present in the regulatory region. A single degenerate estrogen response element has been identified at e1189 bp. The extrapituitary PRL mRNA is ~150 bp longer than the pituitary transcript, and has a different 5'untranslated region, but transcription from either promoter produces identical protein coding sequences (Melmed, 2010).

The PRL molecule is arranged in a single chain of amino acids with three intramolecular disulfide bonds between six cysteine residues (Cys^{4}-Cys^{11}, Cys^{58}-Cys^{174}, and Cys^{191}-Cys^{199} in humans) (Cooke *et al.*, 1981) as shown in Figure 2.2. The sequence homology can vary from the striking 97% among primates to as low as 56% between primates and rodents (Sinha, 1995). In humans (Shome and Parlow, 1977). PRL consists of 199 amino acids with a molecular mass of 23 kDa whereas in rats (Cooke *et al.*, 1980) pituitary PRL consists of only 197 amino acids.

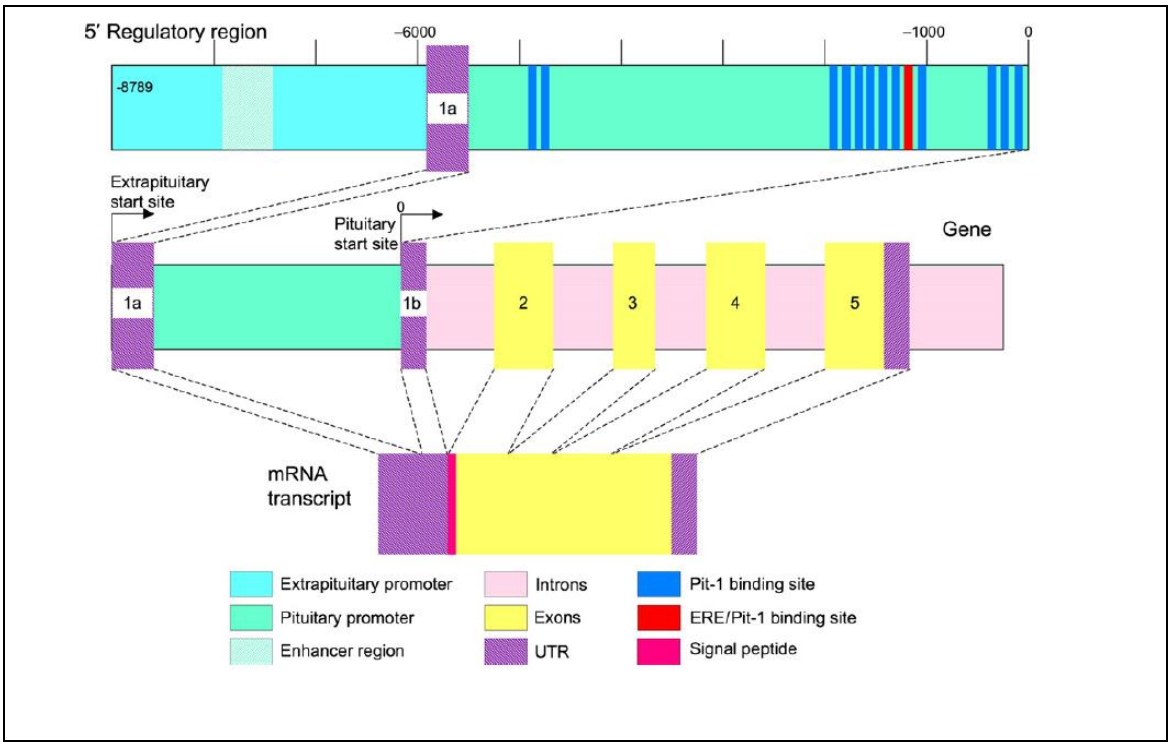

Figure 2.1: Schematic diagram of the human prolactin 5' gene regulatory region, gene and messenger RNA transcript (Melmed, 2010)

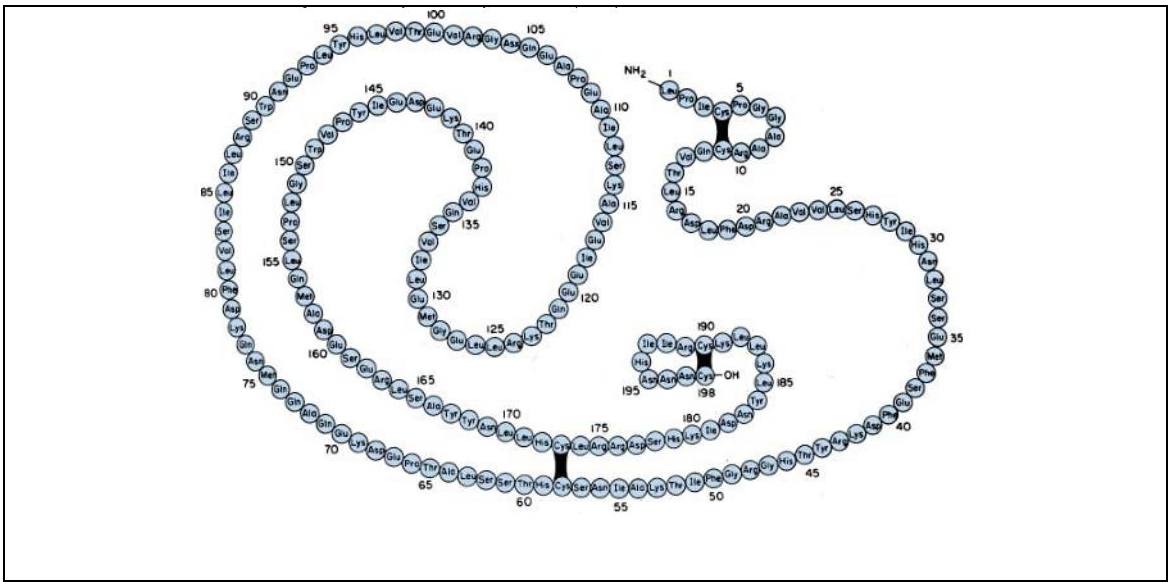

Figure 2.2: Amino acid sequence of prolactin. Three cysteine disulfide bands are located within the molecule (C. Matthew Peterson, 2004)

2.1.2. Secondary and tertiary structure of prolactin

Studies on the secondary structure of PRL have shown that 50% of the amino acids chain is arranged in α-helices, while the rest of it forms loops (Bewley and Li, 1972). The tertiary structure of PRL was predicted by homology modelling approach (Goffin *et al.*, 1995), based on the structural similarities between PRL and other helix bundle proteins, especially GH (Abdel-Meguid *et al.*, 1987; De Vos *et al.*, 1992). According to the current three-dimensional model, PRL contains four long α-helices arranged in antiparallel fashion (Abdel-Meguid *et al.*, 1987; De Vos *et al.*, 1992).

2.1.3. Prolactin variants

Although the major form of PRL found in the pituitary gland is 23 kDa, variants of PRL have been characterized in many mammals, including humans. PRL variants can be as a result of alternative splicing of the primary transcript, proteolytic cleavage and other posttranslational modifications of the amino acid chain (Freeman *et al.*, 2000).

The majority of PRL variants are of posttranslational processing of the mature molecule in the anterior pituitary gland or the plasma. These include dimerization and polymerization, phosphorylation into 14,16 and 22 kDa forms, glycosylation, sulfation, and deamidation (Freeman *et al.*, 2000).

2.2. Prolactin synthesis and secretion patterns

PRL consists of 199 amino acids with three intramolecular disulfide bonds. It is synthesized as a prehormone with a molecular weight of 26 kDa (Sinha, 1995). When

the prehormone is proteolytically cleaved, the resulting mature polypeptide has a molecular weight of 23 kDa, and this monomeric form accounts for the majority of total PRL in the serum of normal subjects and most patients with hyperPRL (Freeman *et al.*, 2000).

PRL is secreted episodically by the anterior pituitary and is primarily under tonic inhibitory control by hypothalamic dopamine traversing the portal venous system to impinge on lactotroph D2 receptors (Freeman *et al.*, 2000; Melmed, 2003). There is an innate pulsatility to pituitary PRL secretion with an interpulse interval of about eight minutes, as determined by studies of media obtained from primate pituitaries cultured in vitro (Stewart *et al.*, 1985).

Figure 2.3 shows the nature of PRL secretion throughout the day in a single individual superimposed upon the range from five normal individuals. The pattern shows the episodic nature of secretion and the nocturnal rise (Melmed, 2010).

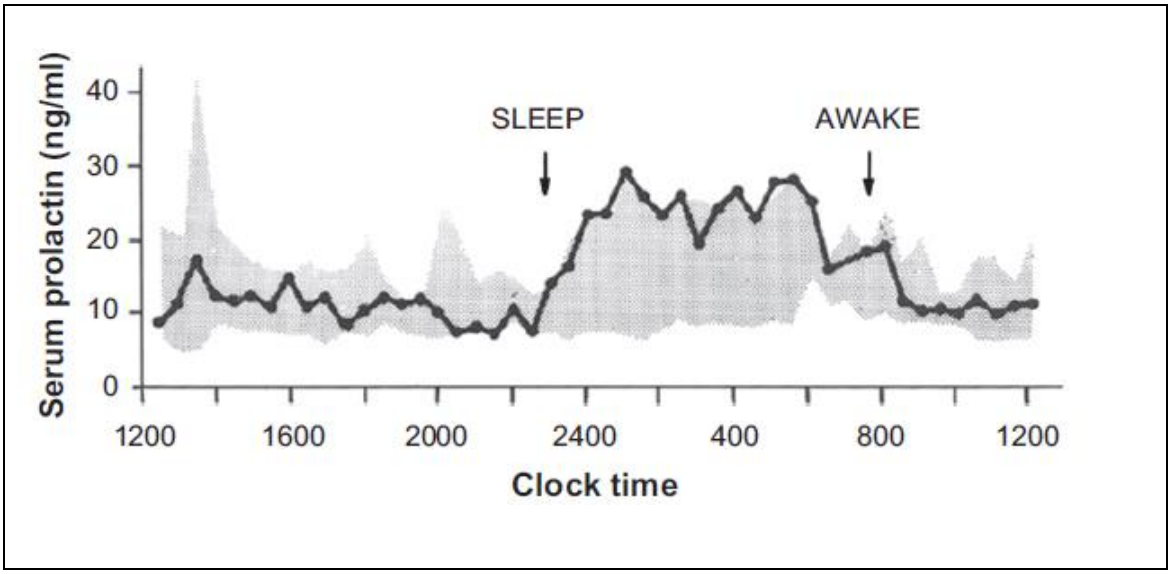

Figure 2.3: Nature of prolactin secretion (Melmed, 2010).

When plasma is sampled from normal individuals in whom hypothalamic function is superimposed upon this innate pulsatility, it becomes apparent that there are four to14 secretory episodes per day. Using cluster analysis, 13 to 14 peaks per day in young subjects were found with a peak duration of 67 to 76 minutes, a mean peak amplitude of three to four ng/ml and an interpulse interval of 93 to 95 min (Veldhuis and Johnson, 1988). An elevation of basal PRL levels in hypothalamic tumour is due to an increase in pulse amplitude and not pulse frequency (Samuels *et al.*, 1991).

2.2.1. Sleep with prolactin

An increase in the amplitude of the PRL secretory pulses begins about 60 to 90 minutes after the onset of sleep; the secretory pulses increase with non-rapid eye movement sleep resulting in highest concentration and fall prior to the next period of rapid eye movement sleep (Parker *et al.*, 1974). When subjects are kept awake to reverse the sleep-waking cycle, PRL levels do not rise until sleep begins (Sassin *et al.*, 1973). Thus, the diurnal variation of PRL secretion is not an inherent rhythm but depends on the occurrence of sleep. Interestingly, the diurnal variation of PRL with the sleep-induced rise persists despite other powerful physiologic influences such as breastfeeding (Stern and Reichlin, 1990).

2.2.2. Meals with prolactin

There is an increase in circulating PRL levels of 50 to 100% within 30 minutes of meals that is due to the amino acids generated from the protein component of the meals, phenylalanine, tyrosine and glutamic acid being the most potent in this regard

(Carlson *et al.*, 1989). Carlson *et al*. (1989) have provided evidence that this stimulatory action of these amino acids is centrally mediated by showing that large neutral amino acids such as valine inhibit the transport of phenylalanine across the blood-brain barrier and blunt the stimulatory action of this amino acid.

2.2.3. Pregnancy with prolactin

Physiological levels of PRL are higher during pregnancy and lactation than otherwise and the mean serum levels are higher in women than in men (Delitala, 1998).

2.2.4. Other factors

Factors inducing PRL synthesis and secretion include estrogen, thyrotropin-releasing hormone, epidermal growth factor, and dopamine receptor antagonists (Melmed *et al.*, 2011).

2.3. Metabolic clearance and production rates of prolactin

Using a labelled PRL method, the metabolic clearance rate has been found to be 46 ± 4 and 40 ± 6 ml/min/m^2 and the calculated production rates using the labelled PRL method were 200 ± 63 and 536 ± 218 mg/day/m^2 in two studies (Cooper *et al.*, 1979; Sievertsen *et al.*, 1980) . Studies in patients with chronic renal failure have shown the metabolic clearance rate to be reduced by 33% (Sievertsen *et al.*, 1980); increased

uptake by the liver has been found in nephrectomised rabbits (Falconer and Vacek, 1983).

2.4. Regulation of prolactin

Similar to other anterior pituitary hormones, PRL is under dual regulation by hypothalamic hormones delivered through the hypothalamic–pituitary portal circulation. Under most conditions the predominant signal is inhibitory, preventing PRL release, and is mediated by the neurotransmitter dopamine. The stimulatory signal is mediated by the hypothalamic hormone thyrotropin-releasing hormone (TRH). Increased anterior pituitary hormone production can occur from a PRL-producing adenoma or from inflammation (hypophysitis). However, conditions that result in impaired dopamine delivery or enhanced TRH signalling, or both, will also result in increased PRL release. In general, medications result in increased PRL production through their anti-dopaminergic properties. Chest-wall injury and breast stimulation serve as peripheral triggers of autonomic control, which impinge on central neurogenic pathways that attenuate dopamine release into the hypophyseal portal circulation. Furthermore, the amount cleared by the kidneys influences the concentration of PRL in the blood. (Vallette-Kasic *et al.*, 2002a; Serri *et al.*, 2003) (Figure 2.4)

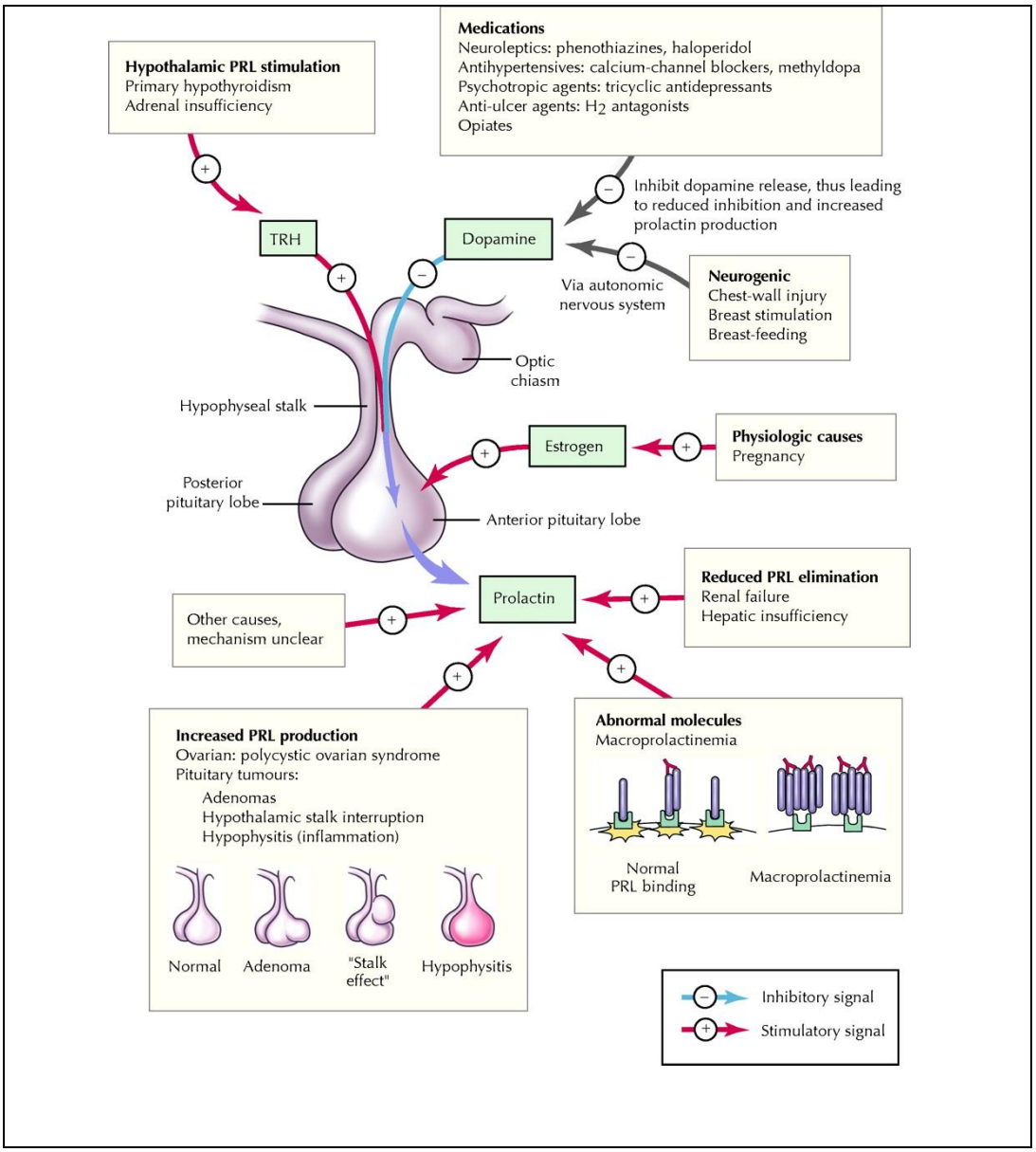

Figure 2.4: Prolactin regulation.(Serri *et al.*, 2003)

2.5. Physiological action of prolactin

PRL helps in controls of the initiation and maintenance of lactation. However, it needs estrogens, progestins, corticosteroids, thyroid hormone and insulin in order for an appropriate expression of PRL action. PRL induces ductal growth, development of the breast lobular alveolar system and the synthesis of specific milk proteins, including casein and γ-lactalbumin. PRL also has effects on the immune system and important in

the control of osmolality and various metabolic events, including the metabolism of subcutaneous fat, carbohydrate metabolism, calcium and vitamin D metabolism, fetal lung development and steroidogenesis (Tietz, 2006).

2.6. Hyperprolactinemia

HyperPRL is a condition of elevated PRL levels in blood which could be physiological, pathological, or idiopathic in origin. Similarly elevated PRL levels could be associated with severe clinical manifestations on one side of the spectrum or be completely asymptomatic on the other side (Majumdar and Mangal, 2013).

HyperPRL has an estimated prevalence of 15% in women with secondary amenorrhoea (Bergh *et al.*, 1977) a condition that affects at least three per cent of women of reproductive age (Münster *et al.*, 1992).

Physiological conditions associated with an increased PRL release include pregnancy, puerperium, nursing, stress, pain, physical exercises, coitus, nocturnal sleep, high-protein meals and late follicular and luteal phase of menstrual cycle (Kasum *et al.*, 2012).

Whereas pathological hyperPRL or true hyperPRL can be results from a lactotroph adenoma, conditions that increase TRH, empty sella syndrome, intracranial tumours compressing the pituitary stalk or hypothalamus, dopamine receptor D2 antagonist and PRL stimulative drugs, repetitive mechanical stimulation of breast, chest wall trauma, hepatorenal disease and primary hypothyroidism, or conditions in which reduced clearance of PRL occurs, such as renal failure (Gibney *et al.*, 2005c; Kasum *et al.*, 2012). Usually pathological hyperPRL is caused by biologically active PRL. It is

associated with suppression of gonadotrophin secretion and gonadal activity (Thorner, 1977).

Idiopathic hyperprolactinemia (IH) can be defined as the presence of elevated serum PRL levels in a patient in the absence of demonstrable pituitary or central nervous system disease and of any other recognized cause of increased PRL secretion (Martin *et al.*, 1985). The major cause of IH is due to macroprolactinemia, in which most circulating PRL forms large protein complexes (more than 150 kDa). The patients with macroprolactinemia have non-pathologic gonadotropin and gonadal activity and clinically characterized by the lack of hyperPRL-related symptoms such as amenorrhea and galactorrhea (Fraser *et al.*, 1989; Hattori *et al.*, 1992a; Hattori, 2003).

The clinical manifestations of hyperPRL are relatively few and easy to recognize. Typical symptoms of hyperPRL can be seen in premenopausal women and in men, but not in postmenopausal women. In premenopausal women causes hypogonadism, manifested by infertility, oligomenorrhoea, or amenorrhoea and less often by galactorrhoea. Excluding pregnancy, hyperPRL accounts for approximately 10 to 20% of cases of amenorrhoea. The mechanism appears to involve inhibition of luteinizing hormone (LH) and perhaps follicle-stimulating hormone (FSH) secretion, via inhibition of the release of gonadotropin-releasing hormone (GnRH). As a result, serum gonadotropin concentrations are not supranormal, typical of other causes of secondary hypogonadism (Snyder *et al.*, 2006).

The symptoms of hypogonadism due to hyperPRL in premenopausal women correlate with the magnitude of the hyperPRL. Postmenopausal women, by definition, are already hypogonadal, and hyperPRL does not change that situation. Galactorrhoea is rarely seen in postmenopausal women because postmenopausal women are markedly

hypoestrogenemic. HyperPRL in these women is recognized only in the relatively unusual situation when a lactotroph adenoma becomes so large as to cause headaches or visual impairment. HyperPRL also causes hypogonadotropic hypogonadism in men, which is manifest by reduced libido, impotence, infertility, gynecomastia, or rarely galactorrhoea (Snyder *et al.*, 2006).

2.6.1. Macroprolactinemia

Macroprolactinemia is characterized by presence of macroprolactin as the main molecular form of PRL in sera and the lack of symptoms. Macroprolactin has a molecular mass of more than 150 kDa, usually contributes a small, though variable amount to circulating levels (Suh and Frantz, 1974; Smith and Norman, 1990). Majority of cases, macroprolactin consists of an antigen–antibody complex of monomeric PRL and IgG (Hattori *et al.*, 1992b; Hattori and Inagaki, 1997b; De Schepper *et al.*, 2003; Kavanagh-Wright *et al.*, 2009; Hattori *et al.*, 2010).

2.6.1.1. Prevalence of macroprolactinemia in the general population

Study in healthy Japanese adult done in 1990 found that 0.4% (40 subjects) had hyperPRL. A quarter of these hyperprolactinaemic subjects were macroprolactinemic, eight subjects were female (Ichihara and Miyai, 1990). None of these 10 subjects had symptoms or signs of endocrine dysfunction. However other study done by Bjoro *et al* in 1995 identified only one female patient with macroprolactinemia. These findings suggest that macroprolactinemia probably exists in the general population at a prevalence of 0.2% in women, but only 0.02% in men.

Another study by Hatorri *et al* (2009) suggests shows that macroprolactinemia is more common, with a prevalence of 3.68% and no difference in prevalence between males and females.

2.6.1.2. Prevalence in the hyperprolactinemia patients

The prevalence of macroprolactinemia among hyperPRL patients varies depending upon the assay used to measure PRL and the subgroup of patients selected for the studies. Few studies showed variation in prevalence of macroprolactinemia in hyperprolactinemic populations between 15 and 46% (Bjoro *et al.*, 1995; Vieira *et al.*, 1998; Olukoga and Kane, 1999; Leslie *et al.*, 2001; Smith *et al.*, 2002b). The lowest incidence which was 15% (Olukoga and Kane, 1999) and highest incidence which were 46% (Hauache *et al.*, 2002) as shown in Table 2.1.

A study regarding screening for macroprolactinemia among hyperPRL done in UK found that prevalence of macroprolactinemia reported varied from 15 to 46% (Gibney *et al.*, 2005c). Prevalence for macroprolactinemia in UK and US were estimated about five to 15% of all cases of hyperPRL (Joseph McKenna, 2009). Macroprolactinemia has been most frequently described and investigated in women because serum PRL is more frequently measured in females as one of the investigation for infertility (Radavelli-Bagatini *et al.*, 2013).

Table 2.1: Prevalence of macroprolactinemia in studies in which all hyperprolactinaemic samples were screened for macroprolactin

Reference	Country	N	Threshold(mIU/L)	Prevalence (%)
Bjoro *et al*. 1995	Norway	605	1000	25
Fahie-Wilson and Soule 1997	England	69	700	25
Viera *et a*l. 1998	Brazil	1220	540	36
Olukoga and Kane 1999	UK	188	430	15
Leslie *et al*. 2001	UK	1225	700	26
Smith *et al*. 2002	Ireland	300	700	24
Hauache *et al*. 2002	Brazil	113	620	46
Strachan *et al*. 2003	UK	273	700	21

Source : Clinical relevance of macroprolactin from Clinical endocrinology N, number of subjects with hyperprolactinemia; Threshold, level of total PRL above which screening for macroprolactin was undertaken (Gibney *et al.*, 2005c).

2.6.1.3. Macroprolactinemia in men

HyperPRL in men may be associated with reduced testosterone, decreased libido, galactorrhoea and impotence. These symptoms are at least partially due to suppression of the hypothalamic–pituitary–gonadal axis, although a direct effect of PRL may also occur. Few reports exist concerning macroprolactinemia in male subjects, but those that exist suggest reduced in vivo bioactivity (Gibney *et al.*, 2005c). A study in 1996 reported six male subjects who were evaluated for sexual dysfunction and found to

have elevated PRL levels but normal testosterone levels and no evidence of any abnormality of the pituitary on MRI scanning (Guay *et al.*, 1996). When submitted to GFC, serum PRL from these subjects proved to be predominantly macroprolactin. A surprising feature of this study was that six subjects with macroprolactinemia were identified from a total of 326 consecutive patients with impotence. This represents a prevalence of two per cent or approximately 100 times that expected for men in the general population.

The same group subsequently reported on two men with pituitary macroadenomas in whom the majority of circulating PRL was macroprolactin (Tritos *et al.*, 1998). These men had normal sexual function and normal tests of nocturnal penile tumescence and rigidity. However, both had extremely high levels of circulating PRL (> 10 000 mU/l) and there was evidence that both were tumours secreted PRL. These findings can be explained by the concept that antibodies directed against PRL may have reduced the bioavailability of PRL and thus producing the clinical manifestations of hyperPRL.

2.6.1.4. Macroprolactinemia in children and adolescent

In children hyperPRL has been described infrequently. This may reflect the less common measurement of PRL in children. However, there has been a series of five children with macroprolactinemia reported. Two of the children had three CT or MRI scans each in search of pituitary lesions with abnormalities detected. Fideleff *et al* (2000) reported five patients (one male and four females) aged 11 to 18 years with asymptomatic hyperPRL who underwent repeated evaluations over a period of three

months to eight years. In all of these cases increased levels of either macroprolactin or big PRL were identified (Fideleff *et al.*, 2000).

A study in Turkey involving six children and adolescent with macroprolactinemia revealed that one patient was asymptomatic and the other five had non-specific symptoms (headache, menstrual disturbance, short stature, increased hair growth or early puberty). The researcher concluded that macroprolactinemia should be taken into consideration in the differential diagnosis of hyperPRL in childhood although the patient presented with or without relevant clinical symptoms (Tutunculer *et al.*, 2006).

2.6.1.5. Macroprolactinemia in elderly

There has been limited number of published study available on macroprolactinemia among elderly. Only one study had described the prevalence of macroprolactinemia among elderly. The study which involved a large number of hospital worker (1330 worker) reported the prevalence of macroprolactinemia among elderly age 60 to 73 years old was 9.1%. The prevalence of macroprolactinemia in the older age group was higher compared to those in lower age group (2.8% in those aged 18 to 39 years old and 4.8% in those aged 40 to 59 years old). The study also observed a statistically significant difference in proportion of patients with macroprolactinemia between those aged 60 to 73 years old and those aged 18 to 39 years old.

2.6.1.6. Macroprolactinemia in pregnancy

During pregnancy, there is a great increment of serum PRL level occurs, and the presence of big big PRL has been reported in a range of eight to 38% of total PRL (Suh and Frantz, 1974; Farkouh *et al.*, 1979; Pansini *et al.*, 1985). Jackson *et al*. reported two women with persistent macroprolactinemia who became pregnant in spite of hyperPRL, and macroprolactin was the predominant isoform during pregnancy (Jackson *et al.*, 1989).

2.6.1.7. Clinical features of macroprolactinemia

There are two mechanisms that may be responsible for macroprolactin to have minimal bioactivity in vivo. Firstly the high molecular mass complex is likely to be restricted to the intravascular compartment by virtue of its size. Secondly steric hindrance by the bound anti-PRL auto-antibody may prevent binding of PRL to its receptor (Hattori *et al.*, 2007).

The evidence of minimal bioactivity suggests that macroprolactin is unlikely to be a cause of symptoms mirroring the hyperprolactinaemic syndrome. Few studies have reported that patients with macroprolactinemia have lower frequency of symptoms and a better prognosis than other hyperprolactinemic patients. It is because of its high molecular mass, which makes macroprolactin confined to the vasculature and hence exhibits limited bioactivity in vivo. One study found a low prevalence of symptoms of menstrual irregularity (23.6%), galactorrhoea (1.8%), and headache (10.9%) (Hattori *et al.*, 2009).These findings have been replicated in other studies (Vieira *et al.*, 1998; Olukoga and Kane, 1999; Smith *et al.*, 2002a; Vallette-Kasic *et al.*, 2002b).

Gibney *et al.* (2005) stated that macroprolactinemic patients could not be differentiated from true hyperprolactinemic patients on the basis of clinical features alone. Although oligomenorrhoea or amenorrhoea and galactorrhoea were more common in patients with true hyperPRL ($P < 0.05$), they were also frequently present in macroprolactinemic patient.

Oligomenorrhoea or amenorrhoea in true hyperPRL is characterized by low levels of oestradiol and low or inappropriately normal concentrations of FSH and LH, reflecting the hypothalamic origin of the disturbance. However study in 2003 had found that plasma levels of oestradiol and LH proved significantly higher in macroprolactinemic compared with true hyperprolactinemic subjects (Suliman *et al.*, 2003) (Table 2.2).

It is likely that the association of the relatively common symptoms of galactorrhoea and oligomenorrhoea and the biochemical finding of macroprolactinemia were observed in this and other studies (Olukoga *et al.*, 1999; Leslie *et al.*, 2001; Vallette-Kasic *et al.*, 2002a; Fahie-Wilson *et al.*, 2005; Gibney *et al.*, 2005c) was coincidental. A study done by Olukoga (2002) had suggested that macroprolactin can be responsible for some patients with macroprolactinemia to have symptoms consistent with hyperPRL, for which no other cause can be found.

Table 2.2: Clinical and laboratory data in true hyperprolactinemic and macroprolactinemic groups[a]

Characteristics	Hyperprolactinemia (n= 100)	Macroprolactinemia (n=32)
Age (yr)	35 (19–69)	29 (18–59)
Total PRL (mIU/liter)	1315 (514–6775)	1145 (517–3390)
PRL after PEG precipitation (mIU/liter)	992 (393–5776)	240 (99–384)
FSH, IU/L	5.7 (0.5)	7.1 (2.1)
LH, IU/L	5.3 (0.5)	10.1(2.4)
Estradiol pmol/L	162 (33)	284 (48)
Clinical features		
Oligomenorrhoea or amenorrhoea	73%	59%
Galactorrhoea	54%	22%
Infertility	7%	22%
Headache	7%	5%

[a] Data are mean (SE). Reference intervals: prolactin, 78–564 mIU/L; prolactin after PEG precipitation, 70–403 mIU/L; FSH, 2–25 IU/L; LH, 2–50 IU/L; estradiol, 110–1470 pmol/L.

Source : Frequent Misdiagnosis and Mismanagement of Hyperprolactinemic Patients before the Introduction of Macroprolactin Screening: Application of a New Strict Laboratory Definition of Macroprolactinemia (Suliman *et al.*, 2003).

Suliman *et al*. (2003) pointed out that the presence of a substantial proportion of macroprolactin does not exclude a coincident elevated concentration of bioactive monomeric PRL, which could explain the appearance of symptoms in cases of macroprolactinemia, as in those cases reported by Olukoga and Kane (1999). Nevertheless, there is no doubt that symptoms of the hyperprolactinaemic syndrome are commonly found in clinical practice in subjects with macroprolactinemia. Gibney *et al*. (2005) reviewed the clinical features in 310 patients with macroprolactinemia from eight series and found that zero to 45% presented with galactorrhoea, six to 40% with subfertility and 15 to 80% with oligomenorrhoea or amenorrhoea. However, there was no convincing evidence that these symptoms were caused by macroprolactin. In the retrospective study of Suliman *et al*. (2003), the prevalence of oligomenorrhoea or amenorrhoea and galactorrhoea was significantly lower (57% and 29% respectively) in patients with macroprolactinemia relative to those with true hyperPRL (84% and 63%).

Moreover, there have been a substantial number of reports of patients who presented with symptoms similar to those of the hyperprolactinemic syndrome, were found initially to have hyperPRL, but on follow-up and further investigation were found to be ovulating or had conceived spontaneously and the hyperPRL was attributable to macroprolactin (Carlson *et al.*, 1992; Hattori *et al.*, 1993; Hattori *et al.*, 1994; Hattori, 1996; Hattori and Inagaki, 1997a; Olukoga *et al.*, 1999). Given that measurement of PRL is prompted by symptoms of the hyperprolactinemic syndrome and these symptoms are not specific (Gibney *et al.*, 2005c).

HyperPRL was found in a minority of patients who present with symptoms suggestive of the hyperprolactinemic syndrome and macroprolactinemia in only 14 of 955 (1.5%). Hence, if the symptoms in these cases of macroprolactinemia are coincidental and do not exhibit a cause and effect relationship, a similar prevalence of