Serum Prolactin as a Diagnostic Marker of Fibroid

Thesis

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Candidate

✍ **Joseph Mikhael Rizk**

List of Contents

List of Abbreviations

Abbr.	Full-term
DMEM	: Dulbecco's modified Eagle's medium
ECM	: Extracellular matrix
EGF	: Epidermal growth factor
ELFA	: Enzyme linked fluorescent assay
ERα	: Estrogen receptor alpha
FSH	: Follicle stimulating hormone
GnRH-a	: GnRH analogue
HBEGF	: Heparin-binding epidermal growth factor
HMGA2	: High-mobility group AT-hook 2
IGF-1	: Insulin-like growth factor-1
LH	: Luteinising hormone
MAO-I	: Monoamine oxidase inhibitor
MED12	: Mutation of mediator complex subunit 12
mRNA	: Messenger Ribonucleic acid
NPY	: Neuropeptide Y
PDGF	: Platelet-derived growth factor

List of Abbreviations *(Cont.)*

Abbr.	Full-term
PHDA	: Periventricular hypophyseal
POMC	: Proopiomelanocortin
PRL	: Prolactin
SMC	: Smooth muscle cells
SSRI	: Selective serotonin reuptake inhibitors
TGF	: Transforming growth factor
TH	: Tyrosine Hydroxylase
THDA	: Tuberohypophyseal
TIDA	: Tuberoinfundibular
TRH	: Thyroid releasing hormone
VGCC	: Voltage-gated Ca^{2+} channels
VIP	: Vasoactive intestinal peptide

List of Tables

List of Figures

Introduction

Uterine fibroids are benign tumors originating from the smooth muscle of the myometrium of the uterus. They are the most common tumor affecting the reproductive organs and are found in up to 25% of women. Fibroids may be symptomatic or asymptomatic. The severity of symptoms typically depends on size, number of fibroids, and tumor location. Fibroids that grow towards the cavity (submucous fibroid) generally lead to an abnormal bleeding pattern. Intramural and subserous tumors are more likely to cause problems (pressure, difficulty urinating, dyspareunia, dysmenorrhea) as a result of their size. Location (of submucous fibroids) and size (of intramural and subserous fibroids) may also contribute to decreased fecundity ***(TAYLOR et al. 2015).***

Prolactin was initially identified as a pituitary gland hormone, but several studies have demonstrated that prolactin isn't only produced by the pituitary gland, but it is also produced by uterine tissues, including endometrium, myometrium and uterine fibroids ***(Baban 2009).***

It had been found that is a relation between fibroid and ectopic production of prolactin ***(Abdullaeva et al. 2012).***

Prolactin has multiple functions, including lactation and maternal-infant bonding, parental and sexual behavior. Various factors, including gender, sexual activity, childbirth, stress, smoking, and drugs, can affect the release of prolactin. Most available antipsychotic drugs can therefore cause elevations in prolactin secretion. This increase is associated with a variety of adverse effects: amenorrhea; galactorrhea, acceleration of osteoporosis and weight gain in women ***(Rajkumar 2014).***

Aim of the Work

Aim of the study is to assess the changing in serum prolactin in women with uterine myomas.

Chapter (1):
Prolactin

Prolactin is an anterior pituitary hormone composed of 199 amino acids with glycosylated and non-glycosylated forms. The neural control of prolactin secretion, and indeed the whole hypothalamo-prolactin axis, continues to prove itself a bit different from other hypothalamo-pituitary systems. Not only is the hypothalamic regulation predominantly inhibitory, as opposed to stimulatory, it also involves a catecholamine neurotransmitter, dopamine, rather than the more typical peptide hypothalamic hormones involved in regulating all other pituitary systems. Prolactin is also the only anterior pituitary hormone that does not have an endocrine target tissue, and therefore lacks a classical hormonal feedback system. It is regulated, instead, by a short loop feedback whereby prolactin itself stimulates the secretion of the inhibitory factor, dopamine. Finally, despite its rather simple and one-dimensional name, prolactin does much more than simply promotes lactation. It is now recognized as a pleiotrophic hormone with arguably the widest range of physiological actions of any extracellular signalling molecule in the body ***(Grattan 2015).***

Dopamine as a prolactin-inhibitory hormone

Even after the clear demonstration of inhibitory regulation of prolactin secretion in the 1950s, the search for the inhibitory hormone mediating this action was controversial. All hypothalamic hormones identified to date had been peptides, and the expectation was that 'prolactin-inhibitory factor' would also be a peptide. Initial clues that this might not be the case came from observations that drugs such as reserpine, which depletes endogenous catecholamines, induced pseudopregnancy in rats, indicative of elevated prolactin. It was assumed, however, that the functional role of catecholamines was as neurotransmitters acting in the hypothalamus to regulate the release of a hypothalamic hormone. Based on the evidence of dopaminergic nerve terminals in the median eminence, McLeod proposed that dopamine may be released into the pituitary portal system, and thereby acting as a hypothalamic hormone (as distinct from its neurotransmitter role in other systems). He demonstrated that dopaminergic agonists were effective at suppressing prolactin secretion in vivo, and perhaps more importantly, that dopamine could inhibit prolactin secretion from isolated pituitary glands. Dopamine was subsequently detected in the pituitary portal blood, and Porter's group (and others) found that variations of levels of dopamine in the portal blood accounted for changes in prolactin secretion in

various physiological conditions ***(BEN-JONATHAN 1980; BEN-JONATHAN et al. 1980; MACLEOD et al. 1970).***

Dopamine receptors were identified on lactotroph cells in the anterior pituitary. The observation that mice lacking the dopamine D2 receptor are hyperprolactinemic, clearly demonstrated the critical role of dopamine in suppressing endogenous prolactin secretion ***(Kelly et al. 1997).***

The dopamine neurons that control prolactin secretion are located within the arcuate nucleus of the hypothalamus. While it seems likely that they serve functionally as a single population, these neurons have been subdivided into three sub-populations based on the anatomy of their projections: the tuberoinfundibular (TIDA), tuberohypophyseal (THDA), and periventricular hypophyseal (PHDA) dopaminergic neurons. TIDA neurons arise from the dorsomedial arcuate nucleus and project to the external zone of the median eminence. The other two populations have their cell bodies located slightly more rostrally, but their projections pass in the hypothalamo-hypophyseal tract through the median eminence to the hypophysis ***(Demaria et al. 2000; Freeman et al. 2000).***

The THDA neurons originate in the rostral arcuate nucleus and project to the intermediate and neural lobes of the pituitary gland, while the PHDA neurons originate in the

periventricular nucleus, with axons terminating in the intermediate lobe. The TIDA neurons produce the classical hypothalamic hormone secretion into the pituitary portal blood vessels, while THDA and PHDA neurons contribute to basal regulation of prolactin secretion, after transport of dopamine to the anterior pituitary gland through short portal vessels from the neurohypophysis. While anatomically distinct, there is considerable overlap in their dendritic fields, and all three populations appear to be regulated similarly. For example, all are stimulated by prolactin ***(Demaria et al. 2000; Freeman et al. 2000).*** Hence, it is reasonable to consider them as a functional unit of prolactin-inhibiting neurons ***(Demaria et al., 2000).***

Electrophysiological studies of hypothalamic dopamine neurons in the rat have described TIDA neurons as exhibiting a robust oscillation between hyperpolarized and depolarized states, with periodicity of about 20s, and a spontaneous firing rate of ~4Hz during the depolarized 'up-state'. Remarkably, the TIDA neurons were found to show a synchronous pattern of firing suggestive of an interconnected network, dependent on functional expression of gap junctions ***(Lyons et al. 2010).***

Taking advantage of transgenic technologies to label dopaminergic neurons with fluorescent tags, studies of TIDA electrical activity have also been completed in brain slices

from mice **(*Brown et al. 2012; Dow and Brown 2012; Romano et al. 2013),*** showing a similar pattern of firing to that seen in the rat, although only a small proportion of TIDA neurons showed the phasic oscillations in this model. Importantly, one of these latter studies demonstrated that patterns of firing of an individual TIDA neuron were reflected in the pattern of dopamine release from the population, as measured using *in vivo* amperometry in the median eminence ***(Romano et al. 2013).*** These data support the concept postulated by others, that the neurons act as a synchronous network to release dopamine in a pulsatile or phasic fashion ***(Lyons et al. 2010).***

Of the five dopamine receptors, the two members of the D2-like receptor family, D2 and D4 are found in the pituitary gland and it is through these D2-like receptors that dopamine acts to inhibit lactotroph cell function ***(Ben-Jonathan and Hnasko 2001).*** Uniquely among anterior pituitary cells, lactotrophs display spontaneous electrical activity in the absence of hypothalamic stimulation and Ca^{2+} influx through voltage-gated Ca^{2+} channels (VGCC) stimulates to prolactin secretion. This accounts for the high levels of basal prolactin secretion, and is consistent with a regulatory mechanism primarily based on inhibition. Dopamine inhibits calcium influx resulting in membrane hyperpolarization and reduced prolactin secretion ***(GREGERSON 2003a; GREGERSON 2003b).***