EVALUATION OF CHEMILUMINESCENCE TECHNIQUE FOR DETERMINATION OF FOLLICLE STIMULATING HORMONE AND LUTEINIZING HORMONE

Thesis
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By

MANAL ABDEL BAKY MAHMOUD
(M.B., B.Ch)

Supervisors

PROF.DR. SAWSAN HOSNY HAMZA
Prof. of Clinical Pathology
Faculty of Medicine, Ain Shams University

DR. HANZADA IBRAHIM ABDEL FATTAH
Assist. Prof. of Clinical Pathology
Faculty of Medicine, Ain Shams University

DR. NASHWA AHMED EL-BADAWI
Lecturer of Clinical Pathology
Faculty of Medicine, Ain Shams University

CLINICAL PATHOLOGY DEPARTMENT
FACULTY OF MEDICINE
AIN SHAMS UNIVERSITY
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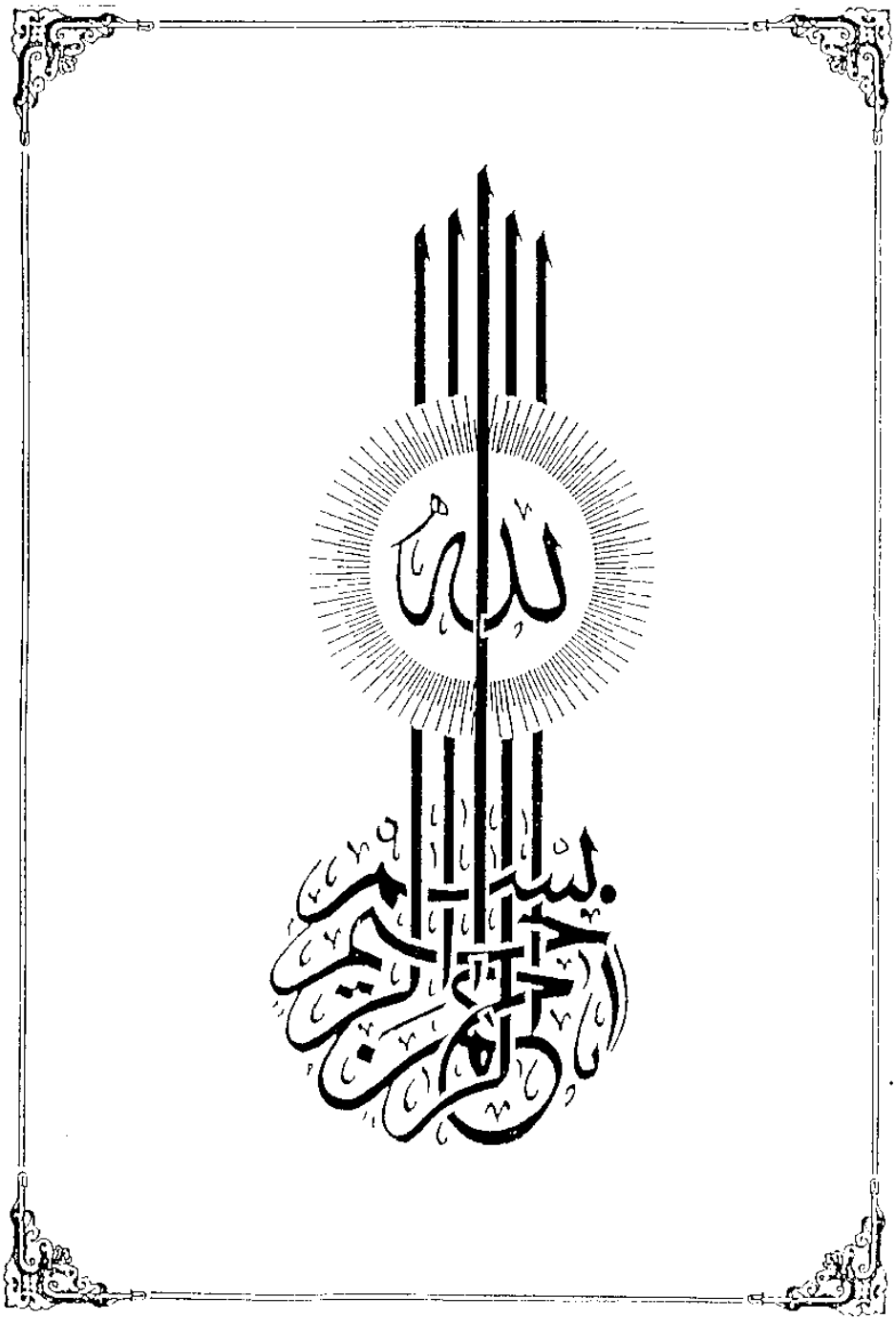

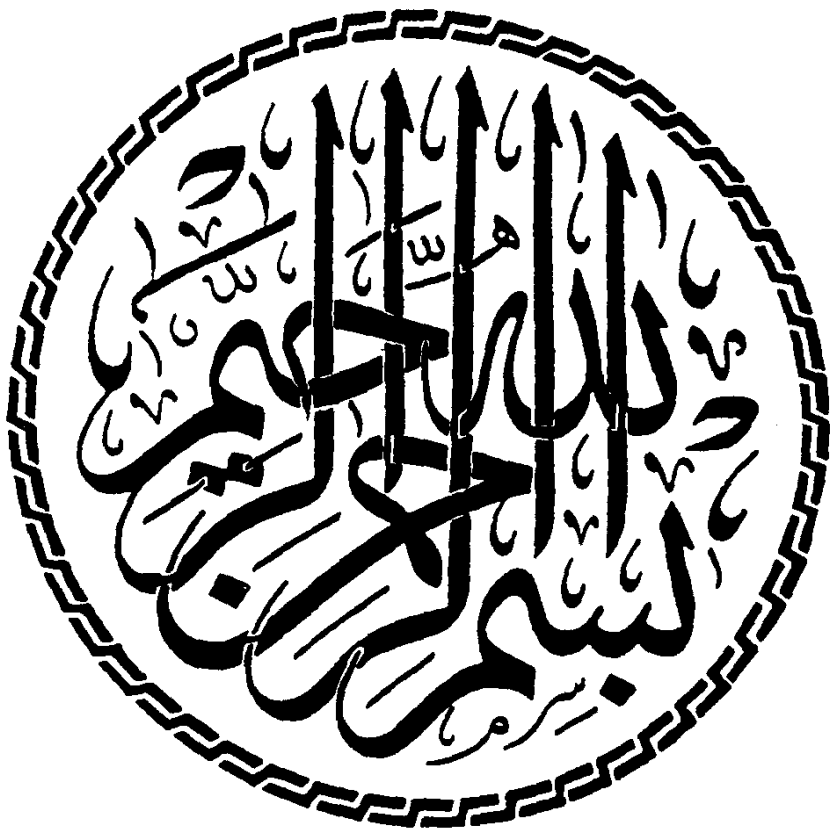

CONTENTS

INTRODUCTION AND AIM OF THE WORK

INTRODUCTION

The analysis of FSH and LH is an important tool in the differential diagnosis of hypothalamic, pituitary or gonadal dysfunction (Wide et al., 1973). The development of immunoassay techniques during the last three decades has permitted a sensitive quantitation of FSH and LH (Monroe, 1984).

Problems of performance and stability are common to all radioimmunoassays. Several disadvantages are encountered in the technique including counting time (several minutes), inconsistency and radiation hazards (Duddly, 1990).

Other non isotopic immunoassay techniques have been introduced. The most popular was enzyme immunoassay (EIA) which solved some of the previously mentioned problems. However, new problems were encountered with the use of EIA technique. The most critical factors for reaction are the time and temperature accuracy (Beastall et al., 1987).

Recently, chemilumincent compounds were introduced as labels for immunoassays. These compounds have several advantages over radioimmunoassay and even (EIA) techniques. These include safety, no centrifugation, very short counting time, long reagent stability, temperature independant

reactions and incubation at room temperature (Simpson and Cambell, 1981).

AIM OF THE WORK

Evaluation of chemiluminescence technique as regards sensitivity, specificity, precision and accuracy compared to the enzyme linked immunosorbant assay (ELISA). The study parameters will include FSH and LH. These measurements will be done by both chemiluminescence and ELISA.

REVIEW OF LITERATURE

GONADOTROPIC HORMONES

The control of gonadal function and reproduction in primates and other mammalian species is exerted predominantly through three gonadotropic hormones secreted by the adenohypophpysis. These include the two glycoproteins, Lutropin (Luteinizing hormone (LH) or interstitial cell-stimulating hormone) and follitropin (follicle-stimulating hormone (FSH), and the polypeptide hormone prolactin (Catt et al., 1986).

The general biological roles of LH and FSH are the stimulation of testicular and Ovarian function via the regulation of gametogenesis and steroid hormones synthesis in the gonade (Catt et al., 1978).

Structure of Gonadotropic hormones:-

In terms of molecular structure, LH and FSH have many chemical similarities. Both hormones are glycoproteins composed of two polypeptide subunits designated alpha and Beta that are bound together in a noncovalent association of high affinity. These two gonadotropins, together with thyroid-stimulating hormone (TSH), constitute one of the three groups of well characterized anterior pituitary hormones.

It is of particular interest that the alpha subumit of LH, FSH and TSH, together with that of human chorionic gonadotropin (hcG), is common in all these hormones (Pierce et al., 1971).

Within a species, the alpha subunits of the several glycoprotein hormones possess essentially the same amino acid sequence. The B subunit differs in its amino acid sequence in each hormone and carries the information that dictates the specific hormonal activity to be expressed upon association with the subunit (Kessler et al., 1979).

General chemical features of the LH and FSH molecules include the locations of the oligosaccharide (carbohydrate) moieties in both subunits, with two oligosaccharide groups on the alpha subunit, one in human LH-B subunit and two in human FSH-B subunit. The carbohydrate groups are found at specific locations in the structure of each subunit and have been defined most clearly for the HCG molecule (Kessler et al., 1979). The carbohydrate content of FSH is less well defined.

The constituent monosaccharides in both LH and FSH are manose, galactose, fucose, glucosamine, galactosamine and neuraminic acid (Sialic acid). Each oligo saccharide is branched and is apparantly heterogenous at its periphery,

with branches terminating mainly in neuraminic acid and fucose and, probably, galactose. In LH, some of the oligosaccharide branches also contain a terminal sulfated amino sugar. The sialic acid content varies widely among the glycoprotein hormones, from 20 residues per molecule in hcG and 5 in FSH to only 1 or 2 in LH (Parson's et al., 1980).

The function of the carbohydrate content is not fully known, except that removal of the terminal neuraminic acid residues drastically shortens the half-life of the circulating hormone in blood (Vanhall et al., 1971).

The carbohydrate groups also influence the ability of the glycoprotein hormones to combine with and activate their receptor sites in the testis and the ovary (Tsuruhara et al., 1972 and Sairam, 1980).

Bother alpha and B subunits are glycosylated at specific residues and are highly cross-linked internally by disulfide bonds, five in the alpha subunit and six in each B subunit.

There are no interchain disulfide bonds or other covalent bonds between the alpha and B subunits. The reason for the requirement of the alpha- B combination for expression of hormonal activity is not known. The individual

subunits have low or negligible binding activity in radioligand receptor assays and in vitro bioassay for LH and hcG (Catt et al., 1973 and Williams et al., 1980).

Two major roles can be envisioned for the alpha subunit after combination with the B subunit. One is that the combined alpha subunit contains some or all of the recognition sites necessary for combination with the receptor. The other is that an active conformation of the B subunit is achieved only after its combination with the alpha subunit (Ward, 1978 and Parsons et al., 1981).

The molecular weight of LH is approximately 28,000, that of FSH is 33,000, and the molecular weight of the common alpha subunit is 14,00. The uncertainty in exact molecular weights results from heterogenity of the attached carbohydrate groups and minor uncertainties concerning amino acid sequences (Catt et al., 1986).

Biosynthesis:

The biosynthesis of FSH and LH occurs by the usual process of ribosomal synthesis of the peptide chains with post transitional modifications prior to the final step of hormone secretion. These alterations include the cleavage of presequences from the amino terminus of the alpha and B

subunit polypeptides and the subsequent addition of carbohydrate residues (Catt et al., 1986).

Secretion:

FSH and LH are secreted from the anterior pituitary, where they are produced in the basophilic cells. Their secretion is regulated by three factors:

1- Central nervous system control.
2- Steroid hormones (Ovarian steroids and testicular & steroids).
3- Inhibin (Luhibin).

I- Central nervous system Control:

The secretion of anterior pituitary hormones is controlled by the central nervous system through hypothalamic regulator. The hypothalamic releasing hormones stimulate both synthesis and release of anterior pituitary hormones.

Release of both FSH and LH is stimulated by the hypothalamic peptide known as gonadotropin-releasing hormone (GnRH), also termed LH-releasing factor (LRF), LH-releasing hormone (LHRH), and gonadoliberin (Catt et al., 1986).

The gonadotropins are released intermittently in a pulsatile manner from the pituitary gland in both sexes.

This pulsatile nature is the direct result of episodic secretory discharges of GnRH into the hypophyseal portal blood, a process that is governed by a pulse generator located in the arcuate region of the medial basal hypothalamus (Wilson et al., 1984). The amplitude of pulses is less pronounced fro FSH than for LH due to its lower plasma concentration and longer circulating half-life which make its pulsatile changes less obvious (Pierce et al., 1981).

An optimal frequency of GnRH pulsatile stimulation of the pituitary is essential to maintain appropriate plasma levels of of LH and FSH. Changes in the frequency of GnRH stimulation markedly influence both the concentrations and the ratios of LH and FSH in plasma (Catt et al., 1986).

In females, the pulses of LH and FSH increase in frequency and amplitude toward the time of the midcycle surge (Channing et al., 1980).

In males, episodic LH secretion first becomes detectable during sleep just before the onset of puberty (Wu et al., 1980). The increased LH pulse frequency and amplitude is important in the development of pituitary and testicular functions during puberty (Tauber et al., 1980). In the adult male, LH pulsatile secretion is no longer