UNIVERSITY OF PNG
SCHOOL OF MEDICINE AND HEALTH SCIENCES
DIVISION OF BASIC MEDICAL SCIENCES
DISCIPLINE OF BIOCHEMISTRY AND MOLECULAR BIOLOGY
PBL SEMINAR
PITUITARY FUNCTION TESTS: An Overview

PITUITARY FUNCTION GENERAL CONSIDERATION:

- Release of all Pituitary Hormones is Episodic, which reflects the Pulsatile secretion of Hypothalamic Releasing Factors;
- Slower Diurnal Rhythms in secretion are superimposed upon episodic patterns;
- It is important to understand and to consider these patterns when assessing the Pituitary Function;
- To assess the functional state of the Pituitary Gland it is important to:
    - Use simple screening tests to eliminate other courses, before using more complicated Dynamic Tests;
- If Pituitary disorder is suspected, then damage to Pituitary Functions should be assessed;

How is Pituitary Function Assessed?

- If Hypopituitarism is suspected in a patient, the combined Pituitary Function Test (PFT) should be requested;
- Combined PFT is used to assess the Anterior Pituitary reserve for production of the Anterior Pituitary Hormones:
    - ACTH, GH, FSH, LH and TSH;
- Combined Pituitary Function Tests: TRH / GnRH / GHRH / CRH Tests;
- Blood is collected to measurement the following:
    - Blood Glucose, to assess Hypoglycemic response during the test;
    - Basal plasma levels of: FSH, LH, Estradiol or Testosterone, Cortisol, TSH, FT4, HGH and Prolactin;

- Patient is given Intravenous infusion from separate syringes:
    - Insulin (0.10U/kg),
    - TRH (200ug),
    - GnRH (50ug),
- Blood samples are collected at intervals of 0, 20, 30, 60, 90 and 120 minutes for assay of the respective hormones;
- Important to note that the test must be carried out in the presence of a Clinician;
- Throughout the duration of the tests the following must be available for IV administration if needed:
    - Glucose solution,
    - Hydrocortisone,
- Insulin-Induced Hypoglycemic Test MUST be replaced by the GHRH and CRH test to investigate HGH and Cortisol secretion;

How is the Combined PFT Interpreted? (See Figs: 1 – 4)

- Interpretation of the combined PFT follows the same procedure for interpretation of each test when performed separately;
- Request for the PFT by Clinicians is on the decrease, because of the availability of more specific and highly specialized tests;

What are the current biochemical recommendations for assessing Anterior Pituitary Function?

- Current Biochemical recommendations for assessing Anterior Pituitary function:
  - Measurement of the Basal Anterior Pituitary Hormones levels in plasma;
  - Measurement of plasma level of the Hormone produced by the corresponding Primary Target Organ;
  - Stimulation tests of IV administration of GnRH and TRH are outdated;
  - Exceptions include:
    - Investigation for Acromegaly and Cushing's Syndrome;
      - Stimulation or Suppression tests or both must be done;

Outline the biochemical investigation for initial assessment of a patient with suspected Pituitary Dysfunction.

- **Biochemical investigations for initial assessment of Pituitary dysfunction: (First Line methods)**:
  - Basal measurements provide useful diagnostic information:
    - At about 9.00am collect blood sample for basal levels of:
      - Cortisol, FT4, TSH, Testosterone or Estradiol, LH, FSH, Prolactin (ACTH may be included);
      - If Posterior Pituitary dysfunction is also suspected then, measure Osmolality in Serum and Urine;

- **Interpretation of results**:
  - Patient with normal stature,
    - **No clinical evidence of Pituitary disease**,
    - Normal HPT-axis,
    - Normal HPG-axis,
    - Normal Serum and Urine Osmolality,
    - Plasma [Cortisol] > 400nmol/L,

    Such results indicate: Normal Pituitary Function;

- If Plasma [Cortisol] is between 100 – 400nmol/L
  - **Then request for the Synacthen Test to assess the HPA-axis**;
  - **Request for Insulin Stress Test if the result is Equivocal (borderline)**

- **Patient with strong clinical signs for Pituitary dysfunction (Hypopituitarism); or**
- **Abnormal basal results of the patient;**
  - Request for the Insulin Stress Test to assess ACTH and HGH reserve;
    - (Request should not be made if there is contraindication);
  - Note: If Thyroid and ACTH deficiencies are identified on the basal results, patient should be treated before proceeding with other investigations of Pituitary function; **WHY???**

    - Hypothyroidism reduces the ACTH and GH responses to Insulin Stress Test;
- If basal Osmolality of Urine and Plasma are affected;
- Request for the Fluid Deprivation Test;

INSULIN STRESS TEST FOR GROWTH HORMONE AND CORTISOL (IST):

{This test is contraindicated for children and all patients with significant Cardiac problems and for patients with seizures}

What is the Insulin Stress Test (IST)?

- Insulin stress test (IST) is use for assessment of:
    - HGH reserve,
    - Hypothalamic-Pituitary-Adrenal Axis (HPA-axis),
    - Investigation of suspected Hypopituitarism in adults and in stunted children,

Briefly describe the procedure for the IST:

- **Insulin Stress Test is also called the Insulin Hypoglycemia Test (IHT):**
- Patient should be in supine position throughout the duration of the test;
- IV line is inserted in a vein in the back of the patient hand or arm;
- Blood is collected for baseline levels of Glucose, Cortisol and HGH;
- Insulin (0.1U/kg) is administered IV,
- Insulin is expected to reduce the blood glucose level to about 2.2mmol/L or lower;
- Blood samples are collected at intervals of 30, 45, 60 and 90 minutes after the IV injection of Insulin;
- Blood samples are used to assess the HGH and Cortisol response to Insulin Induced Hypoglycemic Stress;
- Blood glucose level must be monitored regularly;
- Essential to achieve significant drop in blood glucose needed to Stress the cerebral tissues, and stimulate the Anterior Pituitary gland;

Special Precautions needed during the IST:

- Clinician must be present throughout the duration of the IST;
- Development of Hypoglycemia may result in discomfort: Shaking, Sweating, Feel Hungry, Tired and Sleepy;
- Glucose injection, should be use to restore the blood glucose to normal if the patient develops severe hypoglycemia;

How are the results of IST (IHT) Interpreted?

- Results of the IST should be rejected if hypoglycemia (2.2mmol/L or lower blood glucose level) was not achieved during the test;
- In apparently healthy individuals, Hypoglycemia causes:
    - Increase in Plasma [HGH] to more than 20m U/L;
    - Plasma [Cortisol] increases to maximum (about 425nmol/L) in 60 to 90 minutes;
- In patients with Severe Pituitary Dysfunction, Hypoglycemia has limited effect:
    - Plasma [HGH] does not increase significantly;
    - Plasma [Cortisol] does not increase significantly;

- In patient with Partial Pituitary Failure, Hypoglycemia causes:
    - Limited increases in Plasma [HGH] and [Cortisol];
- Pre-menopausal women, the test can be performed at any phase of the menstrual cycle, as there are no cycle effects on the Hypothalamic-Pituitary-Adrenal Axis response to Insulin-Induced Hypoglycemia;

TAKE NOTE:

- Both male and female children show subnormal responses to Hypoglycemia and other Dynamic Tests just before Puberty;

How does high plasma Cortisol affect the Pituitary Function Tests?

- High plasma Cortisol suppresses the Hypothalamus and Pituitary Gland;
- High Plasma Cortisol suppresses the LH response to GnRH;
- High Plasma Cortisol suppresses the TSH response to TRH;
- High Plasma Cortisol negatively affects the Increase in Plasma [HGH] in response to induced Hypoglycemia;
- **Note:** Adrenocortical Hyper-function (Example: Cushing's syndrome) causes the release of High Cortisol in Plasma, thus PFT results <u>will not</u> be interpreted correctly;

TRH TEST:

Outline of the procedure:

- Patient should be in supine position throughout the duration of the test;
- IV line inserted into a vein in the back of the patient hand or arm;
- Collect blood to check baseline level of TSH, and FT4;
- Give calculated amount of TRH to stimulate the Anterior Pituitary;
- Collect blood samples at 20 minutes and 60 minutes after the TRH injection;
- Measure TSH and FT4 levels;

Interpretation of the TRH test results (See Fig. 1)

- About 20min after IV injection of 200mcg TRH Serum TSH increases;
- TRH test can be used to exclude Hyperthyroidism in borderline cases or where measures of Serum FT4 and FT3 are equivocal;
- Serum TSH above reference range excludes Hyperthyroidism;
- Absent or Impaired TSH response is consistent with the following:
    - Hyperthyroidism;
    - Grave's ophthalmology,
    - Some Euthyroid Multinodular Goitres,
    - Subclinical Toxic Adenoma,
    - Acromegaly,
    - Hypopituitarism,
    - Cushing's disease
- TSH may also be impaired if too much Thyroid hormone is given to Hypothyroid patients;
- Currently the TRH stimulation test has largely been replaced by highly sensitive TSH assays;

GnRH TEST: (Fig. 2)
Outline of the procedure:

- Patient should be in supine position throughout the duration of the test;
- IV line inserted in vein in the back of the patient hand or arm;
- Collect blood to check base line levels of LH, FSH and the appropriate sex steroid hormone;
- Give calculated amount of GnRH;
- Collect blood samples at 30 minutes and 60 minutes after the GnRH injection;
- Measure LH, FSH and appropriate sex steroid hormone;

Interpretation of the GnRH test results:

- GnRH test involves administration of GnRH and measurements at timed intervals of LH, FSH, and sex steroid (Estradiol, or Testosterone);
- Interpretation should be made in the context of the pubertal stage;
- If there is no response then Gonadotrophin deficiency might be suspected;
    - However this may be unreliable in Pre-Pubertal children, including Uncomplicated Pubertal Delay;
- Exaggerated response may be seen in Precocious Puberty, or in conditions where there is end organ failure to respond - such as Turner's syndrome;

FLUID DEPRIVATION TEST
What is a fluid deprivation test?

- Test to check regulation of fluid balance and concentration power of the kidneys;
- Regulation of fluid balance involves Arginine Vasopressin produced in Posterior Pituitary;
- Test must be done only under medical supervision as it can potentially cause dehydration, fluid and salt imbalance;

Outline of the procedure:

- Test carried out after overnight fasting with no fluid intake;
- Collect blood and urine at 09.00am;
- Allow patient to consume only dry foodstuff without any fluid up until 4.00 pm;
- Obtain Body weight of patient,
- Measure Urine output every hour;
- Collect blood samples every hour;

Interpretation of test results:

- Normal individuals: hourly urine output will drop and will become more concentrated as they had no fluid intake;
- Body weight and blood concentration will remain the same;
- Patient with Diabetes Insipidus due to lack of AVP:
- Production of large volume of urine continues despite no intake of water;
- Urine remains diluted, body weight falls and blood become more concentrated as they become dehydrated;

- At 1600 hr DDAVP (1-Deamino, 8-D-Argenine Vasopressin; structurally similar to natural AVP) will be injected and the patient will be allowed to take fluid;
- Urine and blood collections will continue up until 20.00hr;
- Aim is to assess the body's response to a lack of fluids and then the response after an injection of DDAVP;

Further Interpretation of results

- Results may be interpreted as:
    - Urine Osmolality less than 300mosmol/kg after fluid deprivation, and
    - Greater than 800mosmol/kg after Desmopressin suggests Cranial Diabetes Insipidus;
    - Urine Osmolality less than 300mosmol/kg after fluid deprivation, and
    - Less than 300mosmol/kg after Desmopressin suggests Nephrogenic Diabetes Insipidus;
    - Urine Osmolarity greater than 800mosmol/kg after fluid deprivation, and
    - Greater than 800mosmol/kg after Desmopressin suggests primary Polydipsia;

Fig. 1: TRH Stimulation Test

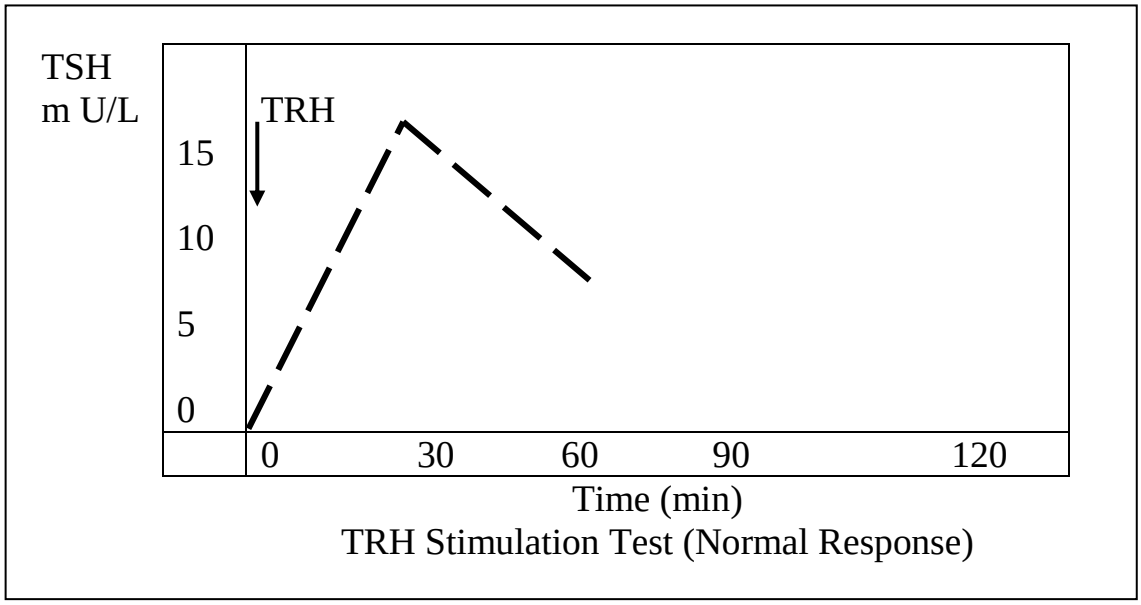

Fig. 2: GnRH Stimulation Test

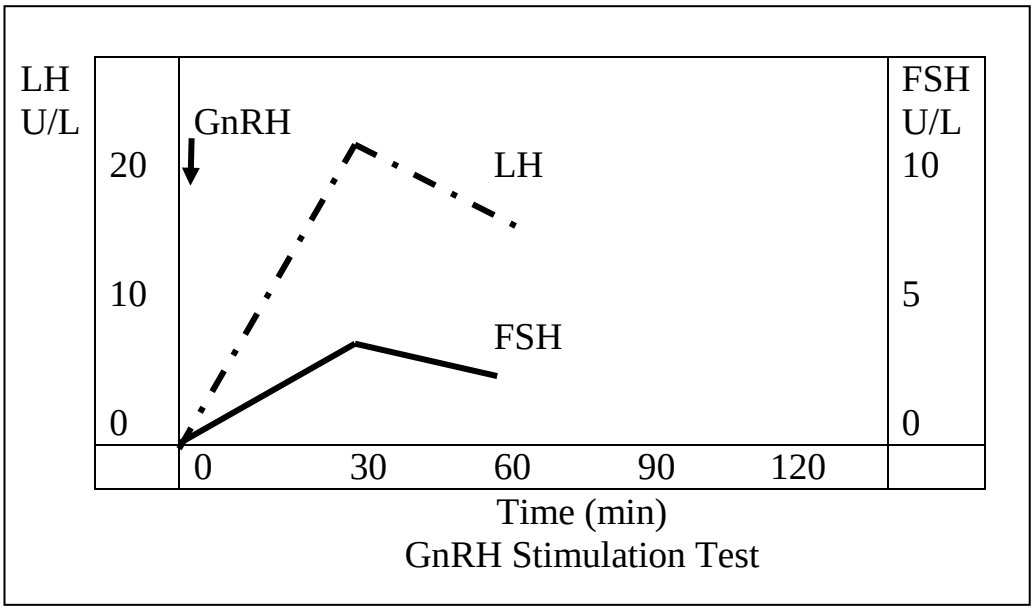

Fig. 3: Section of Insulin Stress Test for HGH reserve;

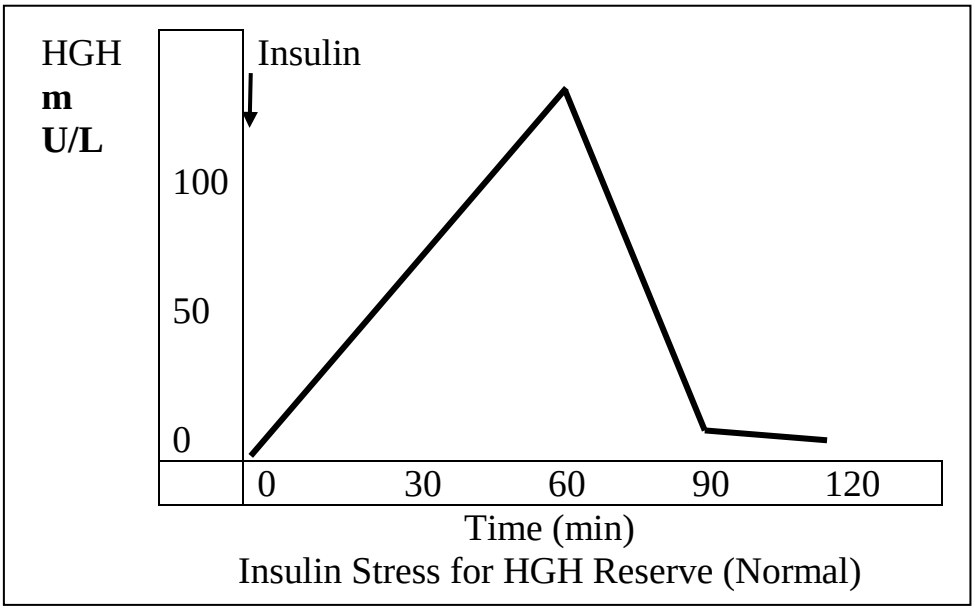

Fig. 4: Section of Insulin Stress Test for Cortisol reserve;