PITUITARY FUNCTION TESTS: An Overview

University of Papua New Guinea
School of Medicine & Health Sciences,
Division of Basic Medical Sciences,
Discipline of Biochemistry & Molecular Biology,
PBL MBBS III

VJ Temple
Pituitary Function General Consideration

- Release of all Pituitary Hormones is Episodic; it reflects the Pulsatile secretion of Hypothalamic Releasing Factors;
- Slower Diurnal Rhythms in secretion are superimposed upon episodic patterns;
  - Important to understand and consider these patterns when assessing Pituitary Function;
- To assess functional state of 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;
Fig. 1: Hypothalamic-Anterior Pituitary Axis: Showing Releasing Hormones and corresponding Anterior Pituitary hormones

<table>
<thead>
<tr>
<th>Hypothalamus</th>
<th>TRH</th>
<th>CRH</th>
<th>GnRH</th>
<th>GHRH</th>
<th>Dopamine</th>
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<tr>
<td>Anterior Pituitary</td>
<td>TSH</td>
<td>ACTH</td>
<td>LH</td>
<td>FSH</td>
<td>GH</td>
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<td>Prolactin</td>
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<td>Targets</td>
<td>THYROID</td>
<td>ADRENAL CORTEX</td>
<td>GONADS</td>
<td>Liver &amp; Other tissues</td>
<td>Mammary Glands &amp; Other tissues</td>
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How is Pituitary Function Assessed?

• If Hypopituitarism is suspected in a patient, combined Pituitary Function Test (PFT) should be requested;

• Combined PFT is used to assess the Anterior Pituitary reserve for production of Anterior Pituitary Hormones
  • ACTH,
  • GH,
  • FSH,
  • LH,
  • TSH;

• Combined Pituitary Function Tests include:
  • TRH / GnRH / GHRH / CRH Tests;
• Blood is collected to measure the following:
  ▪ Blood Glucose,
    • To assess Hypoglycemic response during the test;
  ▪ Basal plasma levels of:
    • FSH,
    • LH,
    • Estradiol (in female patients)
    • Testosterone (in male patients),
    • Cortisol,
    • TSH,
    • FT4,
    • HGH,
    • Prolactin;
• Patient is given **IV infusions** 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;
• **NB:** PFT 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? (Figs: 2 – 5)

• Interpretation of the combined PFT follows the same procedure for interpretation of each test when performed separately;

• IMPORTANT TO NOTE:
  • Request for 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:
  • Measure plasma levels of Basal Anterior Pituitary Hormones;
  • Measure plasma level of Hormone produced by the corresponding Primary Target Organ;
  • Stimulation tests of IV administration of GnRH and TRH are outdated;
  • Exceptions include:
    • Investigations 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 for diagnostic information:
    - At 9.00am collect blood sample for basal levels of:
      - Cortisol,
      - TSH & FT4,
      - Testosterone or Estradiol,
      - LH & FSH,
      - Prolactin (ACTH may be included);
    - If Posterior Pituitary dysfunction is suspected then, measure Osmolality in Serum and Urine;
Interpretation of the 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 Synacthen Test to assess 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:
  • Request for Insulin Stress Test to assess ACTH and HGH reserve;
    • Do not make request if contraindication in patient is suspected;
• **NB**: If Thyroid hormones and ACTH deficiencies are identified on the basal results, patient should be treated before proceeding with other investigations of Pituitary function; **WHY???
  • Hypothyroidism reduces ACTH and HGH responses to Insulin Stress Test;

• If basal Osmolality of Urine and Plasma are affected;
  • Request for the Fluid Deprivation Test;
What is the Insulin Stress Test (IST)?

- **IST** is also called Insulin Hypoglycemia Test (**IHT**):
- It is used for assessment of:
  - HGH reserve,
  - Hypothalamic-Pituitary-Adrenal Axis (**HPA-axis**),
  - Investigation of suspected Hypopituitarism in adults and in Stunted children,
What is the procedure for the IST (Figs 2 & 3)?

• Patient should be in supine position;
• IV line inserted into vein in back of hand or arm;
• Blood is collected for baseline levels of:
  • Glucose,
  • Cortisol,
  • HGH;
• Insulin (0.1U/kg) is administered IV,
• Blood samples are collected at intervals of 30, 45, 60 and 90 minutes after IV injection of Insulin;
• Blood samples are used to assess **HGH** and **Cortisol** response to Insulin Induced Hypoglycemic Stress;
• Blood glucose level must be monitored regularly;
  • Insulin is expected to reduce blood glucose level to about **2.2mmol/L or lower**;
• Essential to achieve significant drop in blood glucose needed to Stress the Cerebral tissues, and stimulate the Anterior Pituitary gland;
What are the special precautions needed during the IST?

- Clinician must be present throughout the IST;
- Development of Hypoglycemia by patient may result in discomfort:
  - Shaking,
  - Sweating,
  - Feel Hungry,
  - Tired,
  - 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 Interpreted?

• Results of 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 patient with Partial Pituitary Failure, Hypoglycemia causes:
  • Limited increases in Plasma [HGH],
  • Limited increase in plasma [Cortisol];
In patients with Severe Pituitary Dysfunction, Hypoglycemia has limited effect:

• Plasma [HGH] does not increase significantly;
• Plasma [Cortisol] does not increase significantly;
• Pre-menopausal women, the test can be performed at any phase of the menstrual cycle, because there are no cycle effects on the HPA-Axis response to Insulin-Induced Hypoglycemia;
• **NB:** Both male and female children show subnormal responses to Hypoglycemia and other Dynamic Tests just before Puberty;
Fig. 2:

Insulin Stress for HGH Reserve (Normal)
Fig. 3: Insulin Stress Test for Cortisol reserve
How does high plasma Cortisol affect Pituitary Function Tests?

• High plasma Cortisol suppresses:
  • Hypothalamus,
  • Pituitary Gland;
  • LH response to GnRH;
  • TSH response to TRH;
• High Plasma Cortisol negatively affects increase in Plasma \([\text{HGH}]\) in response to induced Hypoglycemia;
• **NB**: Adrenocortical Hyper-function (Cushing’s syndrome) causes release of High Cortisol in Plasma, thus PFT results will not be interpreted correctly;
What is the procedure for the TRH test (Fig. 4)?

• Patient should be in supine position;
• IV line inserted into vein in back of hand or arm;
• Collect blood for baseline level of **TSH** and **FT4**;
• Give calculated amount of TRH to stimulate the Anterior Pituitary;
• Collect blood at 20 and 60 minutes after TRH injection;
• Measure **TSH** and **FT4** levels in blood samples;
How are the results of the TRH test interpreted?

• Plasma [TSH] increases after injection of 200mcg TRH
• TRH test can exclude Hyperthyroidism in borderline cases or where Plasma [FT4] and [FT3] are equivocal;
• Plasma [TSH] above reference excludes Hyperthyroidism,
• Absent or Impaired TSH response is consistent with:
  • Hyperthyroidism; Grave's ophthalmology,
  • Some Euthyroid Multi-nodular Goiters,
  • Subclinical Toxic Adenoma,
  • Acromegaly, Hypopituitarism, Cushing's disease,
• TSH may be impaired if too much Thyroid hormones are given to Hypothyroid patients;
• **NB:** TRH stimulation test has largely been replaced by the highly sensitive TSH assays;
TRH Stimulation Test (Normal Response)
What is the procedure for the GnRH TEST? (Fig. 5)

- GnRH test involves infusion of GnRH and assay at timed intervals of LH, FSH and sex steroid (Estradiol, or Testosterone);
- Patient should be in supine position;
- IV line inserted in vein in back of hand or arm;
- Collect blood for base line levels of LH, FSH, and the appropriate Sex steroid hormone,
- Give calculated amount of GnRH;
- Collect blood samples at 30 and 60 minutes after the GnRH injection;
- Measure LH, FSH and appropriate Sex hormone;
How are the results of the GnRH test interpreted?

• 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;
Fig. 5:

GnRH Stimulation Test

LH (U/L)

FSH (U/L)

Time (min)

0 30 60 90 120

0 5 10

GnRH

LH

FSH
What is fluid deprivation test?

• Fluid Deprivation Test:
  • Test to check regulation of fluid balance and power of the kidney tubules to concentration the urine;
    • Regulation of fluid balance involves Arginine Vasopressin (AVP) produced in Posterior Pituitary;
  • Test must be done only under medical supervision as it can potentially cause dehydration, fluid and salt imbalance;
What is the procedure for the Fluid Deprivation Test?

- 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;
How is the fluid deprivation test results interpreted?

- **Healthy individuals:**
  - Hourly urine output will drop and will become more concentrated due to lack of 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;
• **Additional procedure:**
  • At 4.00pm DDAVP (1-Deamino, 8-D-Argenine Vasopressin; structurally similar to natural AVP) will be injected and patient allowed to take fluid;
  • Urine and blood collections will continue up until 20.00hr;
• Aim is to assess body’s response to lack of fluids and the response after 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**;
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