Mini Review

Endocrine Labomas

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ABSTRACT

Laboratory endocrinology forms an integral part of 21st century endocrinology. Perhaps, no other specialty of medicine is as closely associated with laboratory as endocrinology. This review intends to highlight the challenges faced by an endocrinologist before interpreting a hormone assay report. This review by no means is holistic but intends to highlight some of the pitfalls of laboratory endocrinology and arouse further interest in this important but neglected section of endocrinology. Lack of standardization, as well as rigorous implementation is some of the major challenges facing endocrine assays in our country. It is essential to be aware not only of the details of the method of analysis of a hormone, the pre-analytical requisites, but also disease-specific analytical issues to prevent unnecessary concern both for the patient, as well as the treating physician, as well as needless investigations. Problems with interpretation of serum prolactin, thyroglobulin, steroid hormone assays, rennin assay and vitamin-D assay have been highlighted.

Key Words: Endocrinology, laboratory, pitfalls, Labomas

INTRODUCTION

Laboratory endocrinology forms an integral part of 21st century endocrinology. Perhaps no other specialty of medicine is as closely associated with laboratory as endocrinology. The advent of radioimmunoassay (RIA) (Dr. Rosalyn Yalow, Noble prize, 1974) revolutionized the field of laboratory endocrinology. RIA has largely been replaced by chemiluminescence assays (CLIA) as of today. Mass spectrometry use, though still not common, has increased rapidly over the last few years. However, it must be remembered that laboratory medicine is not without its fallacies and limitations. This review intends to highlight the modern day laboratory challenges in managing patients with endocrine disorders, the “Endocrine Labomas”, and to be a gentle reminder to all of us that it’s the patient we do treat and not the report.

Pre-analytical errors

More than 50% of all errors in the diagnostic process are due to pre-analytical factors. Up to 20% are believed to be due to sampling errors. Common errors being collection of sample in a wrong tube, incorrect labeling of samples and incorrect transport methods, lack of temperature regulation or delayed delivery of sample to the laboratory. Hemolysis and lipemia commonly interfere with hormonal assessment (1). Certain hormones are intrinsically associated with a great degree of biological (within person) variation, making interpretation difficult [Table 1].

Blood for serum phosphorus should always be collected in the fasting state to avoid the interference from dietary phosphate. Most variety of meals is rich in phosphorus which is rapidly absorbed from gut leading to falsely elevated values if blood sampling is done post-prandial.

Table 1: Within person (Biological variation)

| Hormonal parameter          | Variation expressed as coefficient of variation (%) |
|----------------------------|----------------------------------------------------|
| Aldosterone                | 29                                                 |
| Prolactin                  | 24                                                 |
| TSH                        | 20                                                 |
| Androstenedione            | 16                                                 |
| Testosterone                | 10                                                 |
| Sex hormone binding globulin | 9                                                  |
Pooling of sample is essential for estimation of luteinizing hormone, follicle stimulating hormone and growth hormone. Peptide hormones like ACTH and parathyroid hormone are highly labile and sampling should be done by a pre cooled syringe and a pre cooled EDTA container, the specimen should immediately undergo cold centrifuge to separate plasma from which estimation should be done immediately to prevent erroneous low values.

**Analytical issues**

Immunoassays are the most commonly available and used assay system in modern endocrinology. However, the binding specificity of the diagnostic antibody used depends a lot on the care and attention given by the manufacturer in selecting the reagents. Often the standards used are limited cause of their lack of reproducibility, e.g. standards for pituitary hormones are generally derived from purified pituitary extracts, which contain a mixture of peptides leading to different binding affinities of the antibody to the different isoforms. Antibody interference is also a major problem in up to 1% of all immunoassays. Most commonly it is due to heterophile antibodies (e.g. human anti-mouse antibody, anti rabbit antibody), or due to endogenous circulating antibodies.

Understanding hormonal assays

With the mushrooming of innumerable laboratories using different assay systems, it is important for a smart clinician to discern the quality and validity of the assay used to measure a hormone. Sensitivity of an assay refers to how well an assay works at very low levels of analyte concentration approaching zero. Because an assay may occasionally give a signal even when the analyte is absent, the minimum level of concentration of analyte that can be statistically differentiated from zero by the assay is called Minimal Detectable Concentration (MDC). However, it must also be considered that lower the analyte concentration more is the variability of result on repeated testing. The minimum concentration of analyte where the intra-assay coefficient of variation is <20 % is called Limit of Quantitation (LOQ). Assay results falling between MDC and LOQ should be reported as qualitatively positive or negative. Sensitivity of an assay is modulated by the method used like radiation, fluorescence, light, amplification and detection mechanisms.

**Pituitary labomas**

A unique problem with old 2 site immunometric assay is that when the analyte levels are very high, separate analyte molecule may attach to the 2 different sites, leading to failure to formation of sandwich, hence, failure of detection of analyte, leading to falsely low values, known as Hook’s effect. Clinically, this problem is rarely faced in a patient with prolactinoma who have falsely low values on testing due to Hook’s effect. Repeated estimation in serial dilutions unmasks the otherwise very high prolactin levels. Fortunately, with modern immunometric assays, Hook’s effect is unusual, and as for prolactin, it usually does not occur for prolactin concentrations <20,000 ng/ml.

In some patients with hyperprolactinemia high molecular weight forms of PRL are primarily detected. These forms of PRL are the big PRL (molecular weight 50-60 K DA) and the big-big PRL (macroprolactin) with a molecular weight greater than 100 K DA.

Another problem with prolactin, which is usually detected incidentally in an asymptomatic patient or patient with minor symptoms, is macroprolactinemia. Prolactin normally circulates in three forms, monomeric PRL (molecular weight 23 kDalton) which is the usual form and has the highest biologic activity, big prolactin (molecular weight 50-60 kDalton) and big-big prolactin (called macroprolactin, molecular weight 150-170 kDalton). The last two forms seem to result from complexes of monomeric prolactin with prolactin-binding antibodies and have reduced biologic activity due to their difficulty in crossing the capillary wall. The PRL-binding antibodies act as a buffer system for the monomeric PRL molecules and are responsible for pseudo-hyperprolactinemia with a normal prolactin assay. The prevalence of macroprolactinemia is believed to range from 10-30 % of hyperprolactinemic patients, depending on the method employed and the definition used. The most validated method for the detection of macroprolactinemia is gel filtration chromatography, which however, is expensive and difficult to perform. A simpler and more commonly available test is incubation of the suspected serum with polyethylene glycol (PEG), which leads to precipitation of macroprolactin. In general, recovery rates less than 40% are attributed to macroprolactinemia.

**Thyroid labomas**

Serum thyroglobulin (Tg) is commonly done in the follow up and prognostication of patient of thyroid cancer. However, a clinician should be aware of the technical limitations when asking for a Tg assay, as a result of the interference due to anti-Tg antibodies which may be observed in as many as 40% of patients with thyroid malignancy. Tg estimation in the background of positive anti-Tg antibodies can lead to falsely high values in RIA (competitive assays) and falsely low values in two-site sandwich immunometric assays.
**Adrenal and gonadal labomas**

**Steroid hormone assay pitfalls**

Assay of steroid hormone is important in a variety clinical setting from diagnosing congenital adrenal hyperplasia, precocious puberty and polycystic ovarian syndrome to androgen deficiency in males. The major problem with steroid hormone assay is the cross reactivity with other similar structurally homologous circulating steroids due to lack of 100% specificity of the steroid antibodies used in the immunoassays. Hormones like cortisol and DHEAS are produced in large quantities with levels in microgram per deciliter, whereas as 17-OH-progesterone (17OHP), an important steroid for diagnosing 21-hydroxylase deficiency is present in much lower levels of nanogram per deciliter. Hence, direct immunoassay of the serum from patient without necessary processing can lead to falsely elevated reporting of 17OHP even with as little as 1% cross reactivity with cortisol or DHEAS. This problem is magnified when assessing 17OHP as a part of newborn screening program for CAH or in a patient suspected to have CAH due to either genital ambiguity in females or suggestive clinical features in male babies. In the newborns and the neonatal period, delta-5 steroids like DHEAS and 17-OH-pregnenelone are high, a result of hyperactive and hyperplastic adrenal fetal zone, causing apparent but not genetic 3 beta hydroxysteroid dehydrogenase deficiency.[8]

Another common problem is the lack of reliability of serum testosterone immunoassays to detect low levels of testosterone necessary for diagnosing androgen excess in women with PCOS or hyperandrogenism in children. Liquid chromatography tandem mass spectrometry (LC-MS/MS) is the investigation of choice for estimation of steroids, but is not available in most of the research centers in our country; leave alone in the clinical practice. An important pitfall is asking for assay for free testosterone, which is highly unreliable. A better and more practical alternative is calculation of free testosterone index, which is 100X total testosterone/sex hormone binding globulin.[9,10]

Similarly, estradiol assay should be avoided unless done by liquid chromatography tandem mass spectrometry, as commercial immunoassays are highly unreliable.

**Assessing rennin activity for diagnosing autonomous hyperaldosteronism**

Diagnosing primary (autonomous) hyperaldosteronism is dependent on calculation of aldosterone rennin ratio (ARR). Assays currently available for measuring plasma rennin activity (PRA) and direct rennin assay (DRA) lose their precision when rennin levels are low.[11] As a result of this loss of sensitivity, and to avoid overinflating the ARR when levels of renin are very low, the lowest renin value that can be included in the ratio is often fixed at a minimum (0.2 ng/ml/hr for PRA and 0.36 ng/ml for DRA).[12]

The DRA is becoming popular because the samples are handled at room temperature. However, freezing or exposing samples to low temperatures during this assay can artificially raise the value owing to cryoactivation.[13,14] By contrast, when using the PRA assay, handling plasma at room temperature can lead to angiotensinogen consumption, angiotensin-I generation and high blank values, which can result in underestimation of the levels of renin.

If the samples are properly collected for each assay, the DRA and PRA values show a good correlation.[15]

**Bone and mineral labomas**

Vitamin D estimation in the clinical practice has increased exponentially in the last few years following increased awareness of the classical (bone) and non-classical effects of vitamin-D deficiency. The most accepted measure of vitamin D status is circulating 25 (OH) D concentrations. However, there is little consensus on which assay method should be used. Commonly used assays include competitive protein-binding assay, RIA, enzyme immunoassay, chemiluminescence immunoassays, HPLC, and LC-MS/MS, each with its own advantages and disadvantages. However, there is significant inter-assay and inter-laboratory variability in measurements. It has been demonstrated that using a deficiency cut-point of 20 ng/ml, 57% of samples assessed using a chemiluminescence immunoassay were classified as deficient compared with 41% of samples assessed using LC-MS/MS; a 20% misclassification rate. Similar rates of misclassification were seen at 30 ng/ml. This has implications for clinical practice and decision limits for vitamin D supplementation, suggesting that cut-points should be assay specific rather than universal and that greater harmonization between laboratories is required.[16]

**Summary and Conclusions**

Pre-analytical errors thus constitute a significant proportion of endocrine labomas, an aspect we often tend to ignore. Special care should be taken during interpretation of a serum prolactin report keeping in mind the patient’s relevant drug history, symptoms and assay used. Estimation of serum thyroglobulin should always be accompanied with anti-thyroglobulin antibody measurement in the same assay method for correct interpretation. Steroid assays by non LC-MS/MS methods are associated with significant proportion of cross reactivity and hence, should be done only when needed and interpreted with caution. Rampant use of serum vitamin D should be avoided as most of the assays are associated with poor reproducibility, and are
often not standardized. Lack of standardization, as well as rigorous implementation is some of the major challenges facing endocrine assays in our country. It is essential to be aware not only of the details of the method of analysis of a hormone, the pre-analytical requisites but also disease specific analytical issues to prevent unnecessary concern both for the patient, as well as the treating physician, as well as needless investigations.

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