cascade of mistreatment with serious consequences. The case presented highlights the challenges encountered in taking care of such patients. It is necessary to understand the pre-testing probability to reach a precise conclusion. Factitious disorder or sample contamination can be yet another challenge in the differential diagnosis of Cushing's work up.

(1)
Raff H Measurement of Late-Night, Salivary Cortisol and Cortisone by LC-MS/MS to Assess Preanalytical Sample Contamination with Topical Hydrocortisone. Clinical Chemistry 58:5 (2012)

Reproductive Endocrinology

CLINICAL STUDIES IN FEMALE REPRODUCTION I

Comparison of Estradiol by Mass Spectrometry Versus Immunoassay in Women Undergoing Menopause: Study of Womens Health Across the Nation (SWAN)

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SAT-027

Serum estradiol (E2) concentrations in midreproductive women are easily measured using a variety of conventional immunoassays (IA). However, when women approach and traverse menopause, E2 eventually drops below levels where IA lacks sufficient sensitivity to accurately measure E2. Liquid chromatography and tandem mass spectrometry (LC/MS/MS) has become the standard method for assessing steroid hormones, especially when circulating concentrations are low. We evaluated the relationship between IA and LC/MS/MS E2 measurements in a cohort of women taken from the Study of Womens Health Across the Nation (SWAN) to assess the degree of agreement between the two methods and to determine the level of E2 at which IA becomes unreliable.

Methods: 315 serum samples that had been previously measured for E2 using IA were re-analyzed using LC/MS/MS performed by one of the authors (RA). In this original set, E2 levels that were below the limit of assay detection (LLD, 6 pg/ml) were interpolated as a random number between 0 and the LLD. Agreement between all 315 samples was assessed using both Pearson and Spearman correlation. The analysis was repeated excluding the subset of specimens that were below the lower limit of detection (LLD) for the IA E2 assay (6 pg/ml; N=176), and a third set of correlations was obtained for specimens that measured <15 pg/ml by IA but were above the 6 pg/ml LLD (N=82).

Results: The overall dataset (N=315) demonstrated excellent agreement between IA and LC/MS/MS with a Pearson's r and Spearmans r of 0.98 AND 0.60, respectively. When the subset of 176 samples above the LLD were assessed, Pearson's r was 0.98 and Spearman's r was 0.81. In contrast, when specimens measuring 6–15 pg/ml by IA were compared to LC/MS/MS, Pearson's r was -0.03 and Spearman's r was 0.09, indicating a complete loss of relationship between the two methods.

Conclusions: The IA used by SWAN (England, Clin Chem 2002; 48: 1584) and LC/MS/MS demonstrate excellent correlation for E2 measurements above 15 pg/ml. However, circulating concentrations of E2 below 15 pg/ml were not accurately measured using IA.

Reproductive Endocrinology

FEMALE REPRODUCTION: BASIC MECHANISMS

NALCN Expression Is Regulated by Progesterone and Estrogen in Human Myometrial Smooth Muscle Cells

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MON-011

During pregnancy, the uterus transitions from a quiescent state to a highly contractile, excitable state. Both ion channels and hormones are essential for this transition. We recently identified that the Na^+ leak channel, non-selective (NALCN) contributes to a leak current in human MSMCs and mice lacking NALCN have prolonged and dysfunctional labor. Additionally, NALCN levels change throughout mouse pregnancy suggesting regulation by hormones of pregnancy, specifically estrogen and progesterone. Here, we tested the hypothesis that P4, a pro-quiescent hormone, and E2, a pro-contractile hormone, regulate NALCN expression and current in the myometrium. In a human immortalized myometrial cells (HM6ERMS2), using qPCR we measured a 2.3 fold decrease and a 5.6 fold increase in NALCN mRNA expression in the presence of E2 and P4, respectively. These findings were also confirmed when NALCN protein expression were measured by immunoblot. Conversely, treatment with the ER antagonist, ICI 182,780, significantly increased NALCN mRNA expression, while treatment with the PR antagonist RU486 significantly decreased NALCN mRNA expression suggesting E2 and P4 work through their respective receptors to regulate NALCN. P4 differentially regulates myometrial activity depending on which progesterone receptor is activated: PRA, promotes contractility, whereas PRB promotes quiescence. Thus to study the effect of each PR, we used a human myometrial cell line stably expressing PRA or PRB, and measured similar increases in NALCN mRNA expression in both cell lines treated with P4. To determine the functional consequences of E2 and P4, we measured NALCN-dependent leak current in MSMCs using whole cell patch clamping. We observed that E2 significantly inhibited while P4 significantly enhanced NALCN current. Finally, we identified estrogen response and progesterone response elements (ERE and PRE) in the NALCN promoter and showed that the PREs contributed to P4 regulation while the ERE did not contribute to the regulation of NALCN expression using luciferase based promoter assays. Overall, our findings show that NALCN is upregulated by P4, the