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Review

Cortisol assays and diagnostic laboratory procedures in human biological fluids

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Abstract

The overview of cortisol physiology, action and pathology is achieved in relation to the hypothalamic–pituitary–adrenal axis alteration by laboratory investigation. The measurements of cortisol and related compound levels in blood, urine and saliva used to study the physiological and pathological cortisol involvement, are critically reviewed. The immunoassay and chromatographic methods for cortisol measurement in the various biological fluids are examined in relation to their analytical performances, reference ranges and diagnostic specificity and sensitivity. Moreover, blood, urine and saliva cortisol level measurements are described taking into account the diagnostic implications. The deduction is that each method requires the definition of its own reference range and its related diagnostic cut-off levels. Thus, this review, stressing the analysis procedures, could help to understand and compare the results of the different assays.

Keywords: Steroid; Interference; Reference range; Analytical performance; Diagnostic performance

Contents

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Introduction

Cortisol level measurement is used in the assessment of adrenal, pituitary and hypothalamic function and is especially important in the diagnoses of Cushing's syndrome and Addison's disease [1,2]. Total serum cortisol, 24-h urinary free cortisol and salivary free cortisol measurements are utilised in cortisol metabolism study for hyper and hypo-cortisolism identification [3].

In this review, after a brief overview of the physiological actions of cortisol, the various laboratory methodological aspects are illustrated mainly related to blood, urine and saliva cortisol level measurements. Emphasis is made in relation to the diverse results, obtained using various assays, and their comparison is underlined. These differences are more evident when the urine matrix is analyzed, considering that the excreted steroid metabolites are numerous and are comparable to cortisol compounds from the molecular structure point of view [4,5]. The presence of cross-reactions is usually not investigable using immunoassay kits [6,7]. Serum free cortisol is also discussed comparing serum total cortisol levels and focusing the problem related to sample treatment. Recently, salivary free cortisol has offered advantages over serum cortisol [8]. The saliva collection, almost completely non-invasive sampling procedure, avoids the stress-induced rise in adrenal secretory activity associated with blood sampling [9,10]. Salivary, urinary and serum cortisol level measurements in different physiopathological conditions and the corresponding diagnostic implications are critically discussed.

Endocrinology: physiology, signalling pathway and physiopathological changes in cortisol secretion

Physiology

Cortisol (F) is a glucocorticoid hormone secreted by the outer cortex of the adrenal gland. CRH (corticotropin-releasing hormone) and ACTH (adrenocorticotrophic hormone) stimulate cortisol secretion through a feedback control [11] (Fig. 1). This steroid plays a pivotal role in the regulation of most essential physiological processes, including energy metabolism, maintenance of electrolyte balance and blood pressure, immune-modulation and stress responses, cell proliferation and differentiation, as well as regulation of memory and cognitive functions [12–15].

The major fraction of F circulates bound to the plasma protein corticosteroid binding globulin (CBG or transcortin) and to albumin. These prevent the hormone penetrating the membrane of the target cells. CBG is the high affinity specific plasma transport glycoprotein for cortisol and each CBG molecule has a single steroid binding site [16]. The primary role of CBG is to regulate the bioavailability and metabolic clearance of glucocorticoid. The predominant triantennary pattern in pregnancy, with increased sialic acid groups, retarding clearance through the liver sialo-glycoprotein receptor, contributes to the two to threefold rise in circulating CBG of pregnancy [17].

Only 3–5% of the total plasma cortisol (TF) circulates in the unbound free cortisol (free F) form, namely the bioactive form. The free F, lipophilic molecule, passes through capillaries into tissues mainly by passive diffusion [18]. In fact, the concentration in saliva reflects the blood free fraction. Moreover, the F level is approximately 50% of the blood level as a result of 11β-HSD2 activity in the parotid gland [19]. In urine the principal metabolites of cortisol account for more than 95% of cortisol excretion and free F represents only 2–3% of the urinary cortisol metabolites. They are excreted in the urine predominantly as glucosiduronates and sulfates, generated primarily in the liver with a reduction of the A-ring to form tetrahydrocortisol and allo-tetrahydrocortisol through action of β and α-reductases followed by 3α and 3β-hydroxyl-steroid dehydrogenases.

Cortisol levels in the body fluids are characterized by circadian rhythm with a morning maximum, declining levels throughout the daytime, a period of low concentration around midnight and a rise after the first few hours of sleep [20–22]. This circadian rhythm has been demonstrated either in plasma than in urine and saliva [23].

Signalling pathway

There are several levels at which the action of this glucocorticoid can be influenced. At pre-receptor level, 11β-hydrosteroid dehydrogenase 1 (11β-HSD1) modulates glucocorticoid exchange by activating cortisone to cortisol conversion to facilitate glucocorticoid receptor (GR)-mediated action [24]. This enzyme is expressed and plays an important role in many metabolically relevant tissues such as liver, adipose tissue and skeletal muscles [25]. In contrast, 11β-hydrosteroid dehydrogenase 2 (11β-HSD2) plays a pivotal role in aldosterone target tissues (kidney, salivary and sweat glands, distal colon, etc.)