UMEÅ UNIVERSITET

Umeå University Medical Dissertations, New Series No 2237

# DIAGNOSING HYPER- AND HYPOCORTISOLISM USING SALIVA SAMPLES

## Pitfalls and How To Avoid Them

Nils Bäcklund

# Akademisk avhandling

som med vederbörligt tillstånd av Rektor vid Umeå universitet för avläggande av filosofie doktorsexamen framläggs till offentligt försvar i lärosal A, byggnad T9, fredagen den 9 juni, kl. 09:00.

Avhandlingen kommer att försvaras på svenska.

Fakultetsopponent: Professor Kerstin Landin-Wilhelmsen, Institutionen för medicin, Göteborgs Universitet

Department of Public Health and Clinical Medicine

**Organization**
Umeå University
Department of Public Health
and Clinical Medicine

**Document type**
Doctoral thesis

**Date of publication**
17 May 2023

**Author**
Nils Bäcklund

**Title**
Diagnosing hyper- and hypocortisolism using saliva samples – Pitfalls and how to avoid them

**Abstract**
**Background:** Diseases caused by excess cortisol secretion (Cushing's syndrome [CS]) and insufficient cortisol secretion (adrenal insufficiency [AI]) are rare. Both entities have substantial diagnostic delay, high morbidity, and high mortality whilst good treatment options exist.
**Aims:** This thesis aims to improve diagnostic tests for CS and AI by analyzing cortisol and its inactive metabolite cortisone in saliva samples.
**Methods and results:** Papers 1, 2, and 4 investigate putative confounding factors and calculate reference limits in healthy individuals for salivary cortisol and cortisone. These reference limits are used to categorize test results as pathological or healthy. Using the reference limits from Papers 1 and 2, diagnostic accuracy for CS was calculated and found to be highest for saliva cortisone. There was no difference in late-night salivary cortisol/cortisone collected at 22:00 hours instead of 23:00 hours, but factors such as age >70 years, hypertension, and diabetes may increase salivary cortisol and cortisone levels. When several analytical methods for salivary cortisol and cortisone were tested, salivary cortisone analyzed by liquid chromatography-tandem mass spectrometry showed highest diagnostic accuracy together with salivary cortisol analyzed with the second-generation Roche immunoassay. In Paper 4, we found that mean salivary cortisol and cortisone concentrations were slightly lower in women using oral contraceptives (OCs) during a short Synacthen test. However, OCs did not significantly affect the lower reference limits, making salivary samples useful in AI diagnosis for women using OCs. Paper 3 is composed of investigations of some potential sources of preanalytical error. This showed that salivary cortisol was increased substantially by 1 week of daily liquorice intake corresponding to 100 g of confectionary liquorice, an effect that was sustained for up to 1 week. Salivary cortisol was also increased by 0.5 % blood contamination, which could be detected by visual inspection, and was strongly increased by topical hydrocortisone on the hands when sampling (>1000-fold increase), which was avoided by use of gloves and detected by an increased cortisol:cortisone ratio. Importantly, salivary cortisone was not affected by liquorice or blood and very modestly increased by topical hydrocortisone (+32 %).
**Conclusion:** Salivary hormone measurement is patient friendly and has high diagnostic accuracy for several analytical methods using robust reference intervals. Salivary cortisone is considerably less affected by preanalytical confounding factors and has a high diagnostic accuracy for CS when used for late-night sampling and after a dexamethasone suppression test. It should be considered the primary measurand in diseases affecting the HPA axis.

**Keywords**
Salivary cortisone, salivary cortisol, LC-MS/MS, immunoassay, Cushing's syndrome, adrenal insufficiency, diagnostic accuracy

| Language | ISBN | ISSN | Number of pages |
|---|---|---|---|
| English | print: 978-91-8070-045-0<br>PDF: 978-91-8070-046-7 | 0346–6612 | 72 + 4 papers |