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Reference interval determination for serum and urine aldosterone for healthy Belgian population

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Background:

Aldosterone measurement is critical for the screening and diagnosis of primary aldosteronism and other disorders of the renin-angiotensin system. Liquid chromatography coupled to triple quadrupole mass spectrometry (LC-MS/MS) has become an essential tool for small molecule quantitation. We have switched from the RIA to LC-MS/MS. Change the method used in the lab means new reference range. So we present a reference interval study for both urine and plasma aldosterone for a healthy Belgian population determined by LC-MS/MS.

Materials and Methods:

➢For the reference interval study, we enrolled **224** healthy Caucasian volunteers (98M: mean age 35 ± 11 y and 126 F: mean age 43 ± 12 y). A subset of **95** healthy volunteers agreed to collect a 24h urine.
➢Exclusion criteria were: prescription of any medications (including oral contraceptives), history of hypertension, abnormal plasma sodium and body mass index (BMI) >30 kg/m².
➢We measured urine sodium concentration on a Cobas c501 (Roche Diagnostic, Manheim, Germany) and calculated daily excretion of NaCl using: the following formula: $Exc_{NaCl} = 58 \times V_{24h} \times [Na]$, where Exc_{NaCl} is the 24h urine excretion of NaCl in mg/d.
➢Aldosterone was measured by LC-MS/MS (TQ5500, ABSciex, Framingham, Massachusetts, USA) (Fig 1)
➢The urine samples were centrifuged; an acid hydrolysis of 18 hours was performed, after deuterium labelled aldosterone was added as internal standard and injected in LC.
➢The plasma samples were centrifuged; deuterium labelled aldosterone was added as internal standard and a liquid-liquid extraction (LLE) was performed. The supernatant was evaporated, dissolved in a mix water/methanol (50/50) and analyzed by LC-MS/MS.

➢Quantitative analysis was performed using multiple reaction monitoring (MRM) transition pairs for sample and internal standard. In negative ion mode, aldosterone can be quantified using the MRM transition at 359.2>189 (quantifier ion) and 359.2>331.1 (qualifier ion).
➢Reference range determination was performed with Medcalc software with the robust method according the CLSI C28-A3.

➢***Results:***

➢The distribution was not normal in our reference population for **urine**, the 95th percentile was **24.6 (90%CI: 21.6-27.6) µg/day** (24 hours) (Fig 2).
➢Mean sodium intake was 8.9 ± 3.2 g/24 hours and was not significantly different (p=0.27) in men and women.
➢ Plasma aldosterone concentrations were not normally distributed for women but well for men.
➢We found a significant difference between levels according to gender (p<0.0001); **the 95th percentile was 175 (90%CI: 160.2-189.5) ng/L for women** (Fig 3) **and 104 (90%CI:92.2-114.5) ng/L for men** (Fig 4).

Figure 1: TQ5500 (Sciex)

Figure 2: Box and Whisker plot for urinary aldosterone reference values

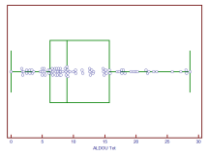


Figure 3: Box and Whisker plot for women plasmatic aldosterone reference values

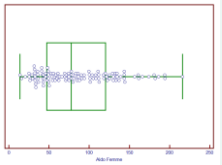
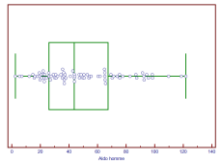


Figure 4: Box and Whisker plot for men plasmatic aldosterone reference values



Conclusions:

We have provided reference intervals on a well-characterized population of normotensive healthy young subjects free of interfering medications. Finally, we urge the Clinical Chemistry community to develop an international standard reference material for aldosterone and a candidate reference method for LC-MS/MS. Once this standard is available, new studies for ARR cut-offs will be required in order to better screen the patients at risk of PA.