THE EFFECT OF ORAL SODIUM LOADING AND SALINE INFUSION ON DIRECT ACTIVE RENIN IN HEALTHY VOLUNTEERS

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Abstract

Context. In patients with suspected primary aldosteronism (PA), the aldosterone-to-renin ratio (ARR) is the most frequently recommended screening test. Further evaluation is based on hormonal changes during volume expansion. Both analyses are critically dependent on an accurate estimation of renin concentration. Direct active renin (DAR) is a novel laboratory technique used for plasma renin assessment.

Objective. The objective of this study was to evaluate DAR for use in PA diagnostic work-ups.

Subjects and Methods. The study enrolled 69 healthy volunteers. Blood sampling was conducted before and after an increase in oral salt intake. Furthermore, a subset of 32 individuals underwent a saline infusion suppression test. DAR and serum aldosterone were measured in all blood samples. To calculate the ARR, serum aldosterone and DAR were expressed in ng/L.

Results. ARR values [median (range); 97.5 percentile] associated with normal and elevated oral salt intake were 8.4 (0.6-37.7); 26.3, and 6.8 (1.1-37.7); 19.6, respectively. DAR and serum aldosterone concentrations [median (range); 97.5 percentile] after saline infusion suppression were 2.9 (2.7-10.7); 7.2 ng/L and 30 (30-72); 54 pmol/L, respectively.

Conclusions. The observed values may be useful in excluding a diagnosis of PA.

Key words: primary aldosteronism, direct active renin

INTRODUCTION

Primary aldosteronism (PA), with an estimated prevalence of 5-10%, is a relatively common form of secondary arterial hypertension. The cardiovascular consequences of PA go beyond blood pressure elevation (1,2). The identification of PA cases is important in daily clinical practice because PA patients can profit from specific medical or surgical treatments.

The currently recommended diagnostic algorithm for PA consists of detection, confirmation and subtype differentiation of the cases (3).

The assessment of plasma renin
plays a key role in this process. The aldosterone-to-renin ratio (ARR) is used to select patients that are subsequently referred for further evaluation. Low renin levels serve also as a marker for adequately performed suppression tests that are used to confirm the presence of autonomous aldosterone secretion, i.e., the diagnosis of PA.

Plasma renin can be measured either as plasma renin activity (PRA) or direct active renin concentration (DAR). Although both techniques have their limitations (4), DAR might be the preferred method because of fewer limitations in pre-analytical processing and higher reproducibility of the results when renin concentrations are low (5, 6).

However, the data describing the use of DAR in diagnosing PA are limited. In order to better interpret the laboratory data from patients investigated for suspected PA, we performed a study in healthy volunteers to evaluate the DAR-based values of both screening and confirmation tests.

**MATERIAL AND METHODS**

**Subjects**

The study enrolled 69 healthy volunteers who were recruited from medical students and the physicians of our hospital. The study was approved by the local ethics committee, and all participants gave consent to the study procedures.

The main inclusion criterion was the absence of any chronic disease that could potentially influence the renin-angiotensin-aldosterone system. Excluded were those with a history of elevated blood pressure (≥140/90). Chronic medications, with the exception of antihistamines and contraceptives, were not allowed. All individuals underwent blood pressure measurements on two occasions during the course of the study.

**Blood sampling before and after oral sodium loading**

Blood sampling for plasma renin and serum aldosterone was performed both with normal and increased salt intake, which was achieved by administration of 6 g of sodium chloride daily (in capsules) three days before the sampling in addition to normal diet.

The adequacy of oral sodium intake was assessed either by elevated urine sodium content (>220 mmol/day) or reported consumption of the entire prescribed amount of sodium chloride. All blood sampling was performed in the morning (7-10 am). Before the blood was taken, the participants had to be in the upright position for at least 30 min.

Blood samples were taken in the sitting position just after the individuals were in the upright position for at least 30 min.

**Saline suppression testing**

Saline suppression testing was performed after oral sodium loading (as described before) because the short-lasting saline infusion alone may not be sufficient to suppress renin and aldosterone secretion in all individuals. The subjects were in the supine position during the test. The procedure started in the morning (between 7-8 AM). After two hours of bed rest, two litres of normal saline infusion were given over a period of four hours. At the end of the infusion, blood samples were taken (in supine position).
Laboratory measurements
Plasma renin and serum aldosterone were measured in all the samples. The material was always processed and subsequently frozen within one hour after the blood was taken. The analysis of hormonal concentrations was performed within two weeks after sampling.

The measurement of plasma DAR was performed by the Renin III Generation radioimmunometric assay (CIS biointernational, Gif sur Yvette Cedex, France). The range of detection of the method was 2.7-340 ng/L. The observed intra-assay coefficients of variation (CVs) were 9.5% and 3.2% for renin concentrations of 7.6 and 347.3 ng/L, respectively. The observed inter-assay CVs were 13.3% and 5.2 % for renin concentrations of 5.9 and 281 ng/L, respectively. When the estimated renin concentration was below the lower limit of detection of the assay, the renin concentration was considered the lowest detectable value (2.7 ng/L).

Serum aldosterone concentrations were measured using a commercially available radioimmunoassay (Coat-A-Count aldosterone kit, Siemens/DPC, Los Angeles, California). The detection range of the assay was 30 - 3880 pmol/L. The observed intra-assay CVs were 10.2% and 4.31% for aldosterone concentrations of 160 and 550 pmol/L, respectively. The observed inter-assay CVs were 10.3% and 7.6 % for aldosterone concentrations of 155 and 1560 pmol/L, respectively.

When the estimated aldosterone values were below the lower detection limit, the individuals were considered to have the lowest detectable serum aldosterone concentration (30 pmol/L).

To calculate the ARR, the concentrations of aldosterone and renin were expressed in ng/L. A coefficient of 0.361 ng/pmol was used to convert the concentration of aldosterone to mass units.

Statistical analysis
MedCalc software (version 9) was used for the statistical analysis of the acquired data.

Values of hormonal concentrations and ARR values were expressed as the mean standard deviation. The 97.5th percentile reflected the upper level of normal values.

RESULTS
The principal characteristics of the study group are given in Table 1. The blood sampling was performed according to the study protocol in all 69 participants. However, adequate sodium intake was achieved in only 52 individuals. Sodium excretion exceeded 220 mmol/day in 26 of the 37 individuals who collected 24-hour urine samples. Twenty-six of the 32 participants who did not provide any urine sample reported the ingestion of the entire given dose of sodium chloride (3x6 g/day).

Some form of intolerance to the oral sodium load was reported by 26 (37%) of the 69 individuals participating in the study. Twenty-four (35%) experienced dyspepsia, and vomiting was reported by 11 of these individuals (16% overall). Two participants (both females) developed oedema.

The results of the DAR, serum aldosterone and ARR assays are presented in Tables 2 and 3. Sodium loading led to a statistically significant decrease in both renin and aldosterone concentrations that
resulted in no significant change in the ARR. No difference in renin concentrations was observed when males and females were compared.

Saline infusion suppression testing was performed according to the study protocol in 32 individuals. No complications related to the procedure were noted. The results of the suppression testing are presented in Table 4. After the saline infusion, DAR and aldosterone concentrations were below or at the lower detection limits of the assays used in 15 (47%) and 24 (66%) of the tested individuals, respectively. DAR < 7.2 ng/L was found to be the value reflecting an adequate suppression of renin secretion.

### DISCUSSION

Our study describes the effect of oral sodium loading and subsequent saline infusion on plasma DAR and ARR in healthy individuals. The obtained data are relevant for the interpretation of laboratory results of the patients examined for suspected PA. Although assessing PRA may be preferred from the pathophysiological point of view, difficulties in pre-analytical processing may reduce the accuracy of PRA measurements in routine clinical practice (5). Moreover, the imprecision of PRA estimations can be higher when the plasma renin level is low (e.g., in PA) (6, 7). Modern DAR radioimmunoassays are

| Table 1. Principal characteristics of the study group |
|-------------------------------------------|-------------------------------------------|
|                                            | Entire group                              | Subgroup that underwent saline infusion suppression testing |
| Number of subjects (women)                | 69 (23)                                   | 32 (6)                                       |
| age (years)                               | 27 (±7)                                   | 33 (±6)                                      |
| Body mass index (kg/m²)                   | 22.5 (±2.1)                               | 24.5 (±1.9)                                 |
| Systolic blood pressure (mmHg)            | 117 (±10)                                 | 115 (±10)                                   |
| Diastolic blood pressure (mmHg)           | 77 (±7)                                   | 74 (±7)                                      |

The data are expressed as means ( ± standard deviation).

### Table 2. Aldosterone, renin and aldosterone-to-renin ratio in 69 subjects with normal (i.e., not supplemented) salt intake

<table>
<thead>
<tr>
<th></th>
<th>median (range)</th>
<th>97.5 percentile</th>
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<tbody>
<tr>
<td>aldosterone (pmol/L)</td>
<td>290 (110-1850)</td>
<td>1 143</td>
</tr>
<tr>
<td>direct active renin (ng/L)</td>
<td>13.3 (4.7-130.3)</td>
<td>118.9</td>
</tr>
<tr>
<td>aldosterone-to-renin ratio a</td>
<td>8.4 (0.6-37.7)</td>
<td>26.3</td>
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</table>

a To calculate the aldosterone-to-renin ratio, aldosterone and renin concentrations were expressed in ng/L. A factor of 0.361 was used to convert aldosterone concentrations from pmol/l to ng/l.
characterised by good reproducibility even with low renin levels. For these reasons, we prefer to use DAR to estimate plasma renin in our centre.

Until now, DAR has not been a well-established method in the PA diagnostic process, and the data from healthy volunteers are limited (8, 9). The current study describes not only the results of the PA screening tests, but also the impact of saline suppression on hormonal levels.

It is important to note that our results are uniquely related to the above-mentioned radioimmunoassays. The observed values for DAR and serum aldosterone are not automatically applicable to assays from different manufacturers. Differences between the assays used to estimate hormonal concentrations might result in variations in the results (10). A recent update on PA by Stowasser (11) points out this diagnostic aspect.

The participants in our study were generally younger than patients who are usually tested for the presence of PA. However, the range of ARR values obtained from younger individuals should not decrease the sensitivity of this screening test in older patients, as ARR tends to increase with age. Moreover, sensitivity is definitely preferred over specificity in all screening procedures.

In our study, the values obtained from healthy volunteers for ARR and serum aldosterone after saline infusion are much lower than the cut-offs that are currently used in the PA diagnostic work-up (3). The main reason for this discrepancy is probably the fact that these cut-offs are usually determined empirically based on observations of patients with confirmed (rather than excluded) PA.

According to our experience, there is a “grey zone” between the upper limits of the normal values and the currently recommended cut-offs where

Table 3. Aldosterone, renin and aldosterone-to-renin ratio in 52 subjects after an increase in oral salt intake

<table>
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<tr>
<th></th>
<th>median (range)</th>
<th>97.5 percentile</th>
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<tbody>
<tr>
<td>aldosterone (pmol/l)</td>
<td>220 (90-1850)</td>
<td>1000</td>
</tr>
<tr>
<td>direct active renin (ng/l)</td>
<td>11.7 (4.7-75.6)</td>
<td>57.6</td>
</tr>
<tr>
<td>aldosterone-to-renin ratio a</td>
<td>6.8 (1.1-37.7)</td>
<td>19.6</td>
</tr>
</tbody>
</table>

a To calculate the aldosterone-to-renin ratio, aldosterone and renin concentrations were expressed in ng/L. A factor of 0.361 was used to convert aldosterone concentrations from pmol/l to ng/l.

Table 4. Aldosterone and renin concentrations after saline infusion suppression testing in 32 volunteers

<table>
<thead>
<tr>
<th></th>
<th>median (range)</th>
<th>97.5 percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td>aldosterone (pmol/L)</td>
<td>30 (30-72)</td>
<td>55</td>
</tr>
<tr>
<td>direct active renin (ng/L)</td>
<td>2.9 (2.7-10.7)</td>
<td>7.2</td>
</tr>
</tbody>
</table>
PA patients are mixed in among those with essential hypertension. Clinical diagnostic protocols must always strike a balance between futile over-testing of individuals with essential hypertension and missing those with surgically correctable forms of PA.

CONCLUSION

Our study evaluated the DAR-based hormonal values useful for PA diagnostic work-ups. Although values taken from healthy individuals cannot be used to reliably confirm the diagnosis, they might be of great value in excluding autonomous aldosterone secretion in patients with suspected primary aldosteronism.

Acknowledgements

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References