The relationships between cortisol levels, insulin levels, and thyroid hormones with 24-h urinary sodium excretion in never treated essential hypertensive patients

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Original Article

Abstract

BACKGROUND: To study the relationship between cortisol, insulin, and thyroid hormone levels with 24-h urinary sodium (Na) excretion levels in essential hypertensive patients.

METHODS: All patients underwent history taking, physical examination, blood pressure (BP) measurement, 12 lead electocardiographic evaluation, routine urine analysis, biochemical analysis including measurement of cortisol, insulin, and thyroid hormone levels, 24-h urine collection to measure urinary Na and protein excretion and creatinine clearance.

RESULTS: In total, 68 newly diagnosed hypertensive patients were included. Spearman correlation analysis revealed that 24-h urinary Na excretion was correlated with insulin levels ($\rho = -0.473$, $P < 0.0001$), serum cortisol levels ($\rho = -0.404$, P= 0.0010) and creatinine clearance ($\rho = 0.407$, P: 0.0010). Linear regression of independent factors has revealed that systolic BP ($B = 0.004$, CI = 0.001-0.008, P = 0.0170), body mass index ($B = 0.014$, CI = 0.005-0.023, P = 0.0030), being male ($B = 0.077$, CI = 0.001-0.153, P = 0.0480), creatinine clearance ($B = 0.003$, CI = 0.001-0.006, P = 0.0120) and insulin levels ($B = -0.008$, CI = −0.014 to −0.002, P = 0.0070) were independently related with logarithmically converted 24-h Na excretion.

CONCLUSION: In conclusion, we found that insulin but not cortisol and thyroid hormone levels were independently related with 24-h urinary Na excretion in newly diagnosed essential hypertensive patients.

Keywords: Cortisol, Hypertension Insulin, Sodium, Thyroid

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Introduction

High blood pressure (BP) is a major public health challenge and one of the most important preventable risk factor for stroke, cardiovascular, and renal disease.[1] Experimental, observational, and clinical data have indicated that dietary salt intake is closely related with BP[2] and various guidelines recommend to lower Na daily intake.[3,4] It is well-known that 24-h urine sodium (Na) excretion is an appropriate and most reliable method to estimate daily Na consumption.[5,6] Various hormones influence Na handling in renal tubules. Apart from renin angiotensin and sympathetic system other hormones been shown to play a role in tubule handling of Na. For example, insulin,[7,8] glucocorticoids (GCs),[9] and thyroid hormones[10-12] have all shown to be antinatriuretic and increase Na reabsorption along nephron segments. However, to the best of our knowledge no study has evaluated the relationship between insulin, cortisol, and thyroid hormone levels with 24-h urinary Na excretion in hypertensive patients comprehensively although these hormones can be measured easily and routinely in everyday clinical practice. Hence, the current study is conducted to analyze the relationships between insulin, cortisol, and thyroid hormone levels with 24-h urinary Na excretion in never treated newly diagnosed essential hypertensive patients.

Materials and Methods

The current study was conducted in the outpatient nephrology unit of a State Hospital. The study was in accordance with the declaration of Helsinki and Local Ethical Approval and informed consent was obtained before enrolment. Study population consisted of patients with newly diagnosed hypertensive that was hitherto treated. All patients firstly underwent following procedures: history

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taking, physical examination, BP measurement, 12 lead electocardiographic evaluation routine urine analysis, fasting blood samples for biochemical analysis (including measurement of insulin, cortisol, and thyroid function tests), 24-h urine collection to measure urinary Na and protein excretion and creatinine clearance. An information leaflet along with a urine container was given to all subjects, and they also received a verbal explanation about how to collect a proper 24-h urine sample. After excluding the first morning urine sample of the collection day, urine was collected over 24 h, which included the first urine sample of the next morning. During the sampling period, subjects were instructed to keep urine samples in a dark and cool place. At the end of the collection period, the urine containers were taken to the laboratory within 2-4 h. Since erroneous estimations of salt intake may occur according to problems in collecting 24-h urine samples participants with urinary creatinine out of reference levels were excluded.[13]

Patients with diabetes mellitus, coronary artery disease, heart failure, rhythm problems, liver disease, nephrotic syndrome, urinary tract infection were excluded. None of the patients reported any alcohol intak.

BP measurement

Seated clinic BP was measured manually with a mercury column sphygmomanometer and an appropriate size cuff after 5 min of rest according to American Heart Association guidelines.[14] Hypertension was defined as systolic BP between ≥ 140 mmHg and diastolic BP ≥ 90 mmHg.[3]

Laboratory analysis

The routine laboratory parameters were measured after 10-12 h of fasting. The laboratory parameters including fasting blood glucose, urea, creatinine, uric acid, Na, potassium, hemoglobin, albumin, calcium, phosphorus, total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, thyroid stimulating hormone (TSH), free triiodothyronine (FT3), free thyroxine (FT4), insulin, and cortisol levels. Twenty four hours urinary Na and protein levels were also measured.

The levels of fasting glucose, urea, creatinine, and uric acid, total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides were determined by using commercially available assay kits with an autoanalyzer (Architect® c16000, Abbott Diagnostics, Abbott Park, IL, USA). Hemoglobin was measured by automated blood analyzer (CELL-DYN 3700 cell counter Abbott Diagnostics Division, Abbott Laboratories, IL, USA). Serum Na and potassium and urine Na were measured by direct potentiometric method by ion specific electrodes. Twenty four hours protein excretion was measured by benzethonium chloride method by (Architect® c16000, Abbott Diagnostics, Abbott Park, IL, USA). Albumin was measured by bromcresol purple method. TSH, FT3, FT4 insulin, and cortisol levels were assayed by direct chemiluminescence method (Advia Centaur XP, Siemens, Dublin, Ireland).

Statistics

Statistical analysis was performed using SPSS for Windows (version 15.0; SPSS Inc., Evanston, IL, USA). Results were considered statistically significant if two-tailed P value was < 0.05. Data were checked for normality. Pearson's correlation coefficient r and Spearman's correlation coefficient ϱ was used for correlations. Linear regression analysis was performed to analyze the independent factors related with logarithmically converted 24-h urinary Na excretion. Variables tested for significance included age, sex, smoking status, body mass index, systolic BP, diastolic BP, 24 h creatinine clearance and protein excretion, TSH, FT3, FT4 insulin, and cortisol.

Results

Initially, 94 patients were enrolled. One patient with coronary artery disease, one patient with heart failure, three patients with diabetes, two patients with chronic liver disease, two patients with nephrotic syndrome, two patients with atrial fibrillation, two patients with urinary tract infection, five patients who did not want to participate, and eight patients with incomplete 24-h urine calculation were excluded from the study. The final patient population consisted of never treated 68 newly diagnosed hypertensive patients. The demographic and laboratory parameters of the patients were shown in table 1.

Spearman correlation analysis revealed that 24-h urinary Na excretion was correlated with insulin levels ($\varrho = -0.473$, $P < 0.0001$), serum cortisol levels ($\varrho = -0.404$, $P = 0.0010$), and creatinine clearance ($\varrho = 0.407$, $P = 0.0010$). There was also a negative correlation between logarithmically converted 24-h urinary Na excretion and logarithmically converted serum insulin ($r = -0.487$, $P < 0.0001$) (Figure 1).

Linear regression of independent factors (as mentioned above) has revealed that systolic BP, BMI, gender, creatinine clearance, and insulin levels were related with logarithmically converted 24-h Na excretion (as a dependent parameter) (Table 2).

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Table 1. The demographic and laboratory parameters of 68 essential hypertensive patients

Parameter*	Mean ± SD	n (%)
Smoker/non-smoker (n)		19/49
Male/female (n, %)		34/34 (50, 50)
Body mass index (kg/m^2)*	27.90 ± 5.30	
Age (year)*	49.70 ± 14.20	
Systolic blood pressure (mmHg)*	147.90 ± 11.70	
Diastolic blood pressure (mmHg)*	92.90 ± 8.70	
Serum glucose (mmol/l) (mean ± SD)*	5.82 ± 0.86	
Serum urea (mg/dl)*	30.00 ± 13.50	
Creatinine (µmol/l)*	74.30 ± 17.70	
Hemoglobin (g/l)*	128.90 ± 13.70	
Sodium (mmol/l)*	139.40 ± 4.40	
Potassium (mmol/l)*	4.65 ± 0.65	
Albumin (g/l)*	42.70 ± 4.80	
Total cholesterol (mmol/l)*	5.03 ± 1.18	
LDL-C (mmol/l)*	3.08 ± 0.87	
HDL-C (mmol/l)*	1.15 ± 0.35	
Triglyceride (mmol/l)*	1.80 ± 1.07	
Uric acid (µmol/l)*	390.20 ± 179.60	
Thyroid stimulating hormone (mU/l)*	1.92 ± 1.24	
FT3 (pg/ml)*	2.94 ± 0.83	
FT4 (ng/dl)	1.23 ± 0.18	
Insulin (µU/ml)*	11.70 ± 7.90	
Cortisol (nmol/l)*	454.70 ± 160.10	
24-h urinary Na excretion (mEq/day)*	143.80 ± 57.40	
Creatinine clearance (ml/min)/1.73 m^{2*}	82.00 ± 20.90	
24-h urinary protein excretion (mg/day)*	222.30 ± 334.40	

* Mean ± SD; LDL-C: Low-density lipoprotein cholesterol; HDL-C: High-density lipoprotein cholesterol; FT3: Free triiodothyronine; FT4: Free thyroxine; SD: Standard deviation

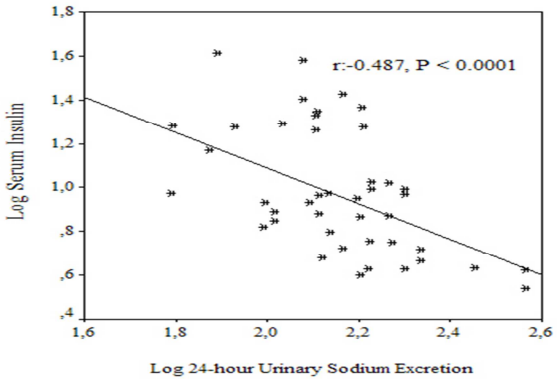

Figure 1. The scatter plot graphic between logarithmically converted 24 h urinary sodium excretion and logarithmically converted serum insulin levels

Table 2. Independent factors related with logarithmically converted 24-h Na excretion

Parameter	Beta	P
Age	0.309	0.189
Gender	0.267	0.048
Body mass index	0.428	0.003
Smoking status	−0.226	0.338
Systolic blood pressure	0.357	0.017
Diastolic blood pressure	0.023	0.889
Creatinine clearance	0.406	0.012
24-h urinary protein excretion	0.053	0.774
Insulin	−0.430	0.007
Cortisol	−0.531	0.098
Thyroid stimulating hormone	0.173	0.397
FT3	0.369	0.099
FT4	−0.048	0.799

FT3: Free triiodothyronine; FT4: Free thyroxine

Discussion

In the current study, to the best of our knowledge, we firstly evaluated the relationship between 24-h urinary Na excretion with insulin, cortisol and thyroid function tests in newly diagnosed essential hypertensive patients who were hitherto treated. Although all these hormones have been shown to be antinatriuretic, we demonstrated that only insulin levels had an independent relationship with 24-h urinary Na excretion.

It is well-known that GCs, whether endogenous, as in Cushing syndrome, or exogenous, through pharmacologic provision, induce hypertension.[9] Traditionally, GC have commonly been believed to increase BP by the activation of the mineralocorticoid receptor in the kidney.[15,16] Surprisingly in the current study, we found no relationship between urinary Na excretion and cortisol levels. We do not know exactly the cause of our findings; however, it was recently speculated that hypertension caused by GCs was not due to the excess reabsorption of Na and water but due to actions on smooth muscle.[9] Clinical experience demonstrates that the hypertension induced by steroids occurs much too rapidly to be accounted for solely, if at all, by increased renal Na reabsorption and excess renal salt reabsorption does not seem to be required for GC to induce hypertension.[17,18] In addition, there were also conflict results in the literature regarding the relationship between urinary free cortisol and hypertension. While some studies have demonstrated that urinary free cortisol were related with hypertension,[19,20] others did not demonstrate these relationships.[21] These points argue convincingly that GC have a wide range of hemodynamic effects distinct from their presumed

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activation of renal mineralocorticoid receptor, thus increasing the understanding of the role of GC in the regulation of BP is of interest.[9] Thus, due to above-mentioned issues we might not find any relationship between cortisol levels and 24-h urinary Na excertion.

Insulin has been shown to increase Na reabsorption by the kidney as well as reduced Na excretion independently of blood glucose levels, filtered load of glucose, glomerular filtration rate, renal blood flow, and plasma aldosterone levels.[22] Insulin, when provided in the perfusion bath for isolated, perfused tubule studies, has been shown to increase Na reabsorption in the proximal tubule[23] and the thick ascending limb.[24,25] Another study had also demonstrated that insulin infusion increased activity of distal renal tubular Na transport pathways including Na-Cl cotransporter and epithelial Na channel possibly through trafficking into the apical membrane.[7] Thus, all these previous findings were in accord with our findings, which demonstrated that insulin levels were independently associated with 24-h urinar Na excretion.

It was shown that hypothyroid rats show defect in tubular Na reabsorbtion.[11,26] The mechanism underlying this defect remains undefined, being variously attributed to relative deficiency of adrenocortical hormones and defective distal Na reabsorption.[27] Thyroid hormones have also a significant role in controlling kidney growth and function. The hormones are important regulators of renal plasma flow, glomerular filtration rate, concentration and dilution of urine, oxygen consumption, and the reabsorption of phosphate, calcium and Na. Thyroid hormones stimulate Na^+, K^+-ATPase activity and changes in renal Na^+, K^+-ATPase activity closely parallels alterations in net transport of Na. It has also been proposed that thyroid hormones augment renal Na^+, K^+-ATPase activity by an adaptive mechanism responding to changing resorptive Na loads. Both mechanisms, the induction of Na pump elements and the adaptive response to increased filtered Na, could operate together in mediating the action of thyroid hormones on Na reabsorption. Despite all these considerations we found no relationship between thyroid hormones and 24-h Na excretion in the current study. We do not have full explanation for our findings, but speculations can be made. Firstly, we measured the levels of hormones for only once and temporal relationships cannot be speculated. Secondly, we do not specifically include hypothyroid patients, but we treat FT3, FT4, and TSH as continuous variables in our analysis.

This study has limitations that deserve mention. Firstly, since our study is cross-sectional, cause and effect relationship cannot be suggested. Secondly, since daily variability can be observed in urinary Na excretion of individuals and the collection of urine samples and hormones were performed for only once temporal relationships cannot be suggested. Thirdly, our study sample is relatively small. Still, we believe that because our study group was composed of special patients that included newly diagnosed essential hypertensive patients who were not receiving any antihypertensive medication such as diuretics the effects of medication were potentially ruled out.

Conclusion

We found that insulin but not cortical and thyroid hormone levels were independently related with 24-h urinary Na excretion in newly diagnosed essential hypertensive patients.

Acknowledgment

No financial and conflict of interest were acknowledged.

Conflict of Interests

Authors have no conflict of interests.

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