Active renin concentration versus Plasma Renin Activity in the workup of salt-wasting 21-hydroxylase deficiency congenital adrenal hyperplasia

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Abstract:

Congenital adrenal hyperplasia (CAH) is a family of autosomal recessive disorders caused by mutations that encode enzymes involved in adrenal steroid synthesis. More than 90% of cases are caused by 21-hydroxylase deficiency (21OHDCAH). Clinical consequences of 21OHD arise primarily from overproduction and accumulation of precursors proximal to the blocked enzymatic step. These precursors are shunted into the androgen biosynthesis pathway, producing virilization in the female fetus, rapid postnatal growth with accelerated skeletal maturation, precocious puberty, and short adult stature in both males and females. Approximately 75% of patients also have a defect in their ability to synthesize aldosterone. Such salt wasters, especially undiagnosed male infants, may die of shock during the newborn period. Plasma Renin Activity (PRA) is an indirect method of renin estimation and has several methodological problems in many conditions, therefore direct immunoradiometric assay for the measurement of active renin concentration (ARC) has been developed.

Patients & Methods: PRA and ARC were simultaneously measured in 25 patients with salt wasting CAH21OHD and 15 apparently healthy age and sex matched children to evaluate the use of active renin in the management of the salt-loosing form of 21OHD & its potential role for monitoring of mineralocorticoid replacement. During a salt-loosing crisis, we measured blood pressure & drew samples for simultaneous measurement of PRA & ARC. Serum PRA was measured radioimmunoassay in sample volumes of 1000 µl plasma, ARC was measured with immunoradiometric assay using sample volumes of 200 µl plasma. 17-hydroxyprogesterone (17OHP), delta4Androstenedione (Δ4A), Na, K were also measured in the serum.

Results: The age of CAH patients ranged from 0.1-4 years with a mean of 2.7 ±1.4025, while the ages of control group ranged from 0.1–5.3 with a mean of 3.14 ± 2.1057 (p=0.392). Of the CAH patients 11 (44 %) were males, 14 (56 %) females, controls 7 (46.7 %) males and 8 (53.3 %) females. The median value of PRA among patients' was 7.00 ng/ml/h (6.00-7.20) while controls' median was 3.80 ng/ml/h (3.10-5.10) with p value =0.000. The median value of ARC among patients was 93.3 pg/ml (30.9 - 473), while for controls' median was 13.4 pg/ml (6.40 – 26.1) with p value =0.000. We found a highly significant correlation between ARC and PRA among the patients & control (p=0.000, r=0.540). In patients ARC also correlated with the blood pressure during the salt loosing crisis, while PRA did not.

Conclusion: ARC correlates well with PRA. ARC is at least as reliable as PRA to assess the quantity of mineralocorticoid replacement. Unlike PRA, it correlates well with the blood pressure in cases of salt-wasting 21OHDCAH reflecting the circulatory state. In children ARC determination is preferable to PRA determination as it does not rely on endogenous angiotensinogen in addition to the methodological advantages of a smaller sample volume, less laborious technique & the potential of better standardization.

Introduction:

Congenital adrenal hyperplasia (CAH) results from inherited defects in one of the five enzymatic steps required for the biosynthesis of cortisol from cholesterol. Ninty to ninty-five % of CAH cases are caused by 21-hydroxylase deficiency [(1)]with an incidence of approximately 1 in 10000 [(2)]. The fundamental defect among these patients is inadequate cortisol synthesis with accumulation of precursors to the 21-hydroxylase enzyme defect. Those are shunted into the androgen pathway causing prenatal virilization of external genitalia in females. A spectrum of phenotypes is observed. Seventy-five % of patients with classic 21-hydroxylase deficiency have severely impaired 21-hydroxylation of progesterone and thus cannot adequately synthesize aldosterone. This salt-wasting

type (SW) predisposes them to episodically develop potentially life-threatening hyponatremic dehydration [3]. Elevated levels of progesterone and 17OHP had been suggested as mineralocorticoid antagonists, exacerbating the effects of aldosterone deficiency [4]. The potentially fatal SW disease is easily diagnosed by estimation of 17OHP in blood, making it well suited to newborn screening [5]**.** The other form of classic CAH, the simple virilizing type (SV) has an apparently normal aldosterone biosynthesis [5]. Without newborn screening, affected boys are usually identified when signs of androgen excess develop. Later diagnosis is associated with greater difficulty in achieving hormonal control, abnormal puberty, and short stature [7].

Treatment of classic CAH is by cortisone replacement for life with or without replacement of mineralocorticoids, depending mainly on presence or absence of adequate aldosterone synthesis [1,5]. Aldosterone deficiency is diagnosed indirectly by testing renin level. PRA estimation is an indirect estimation for aldosterone synthesis, through its enzymatic, angiotensin I-generating activity on its endogenous substrate. It has been used to monitor mineralocorticoid and sodium replacement. Hypotension, hyperkalemia and elevated renin levels suggest the need for an increase in the dose. Hypertension, oedema, tachycardia, and suppressed PRA are clinical signs of overtreatment. Adjustments in the dose should be made in increments of 0.05 to 0.1 mg, as excessive fludrocortisone may also retard growth [8]. Experimental evidence and theoretical considerations show that a simple relation between Angiotensin II receptor occupancy, in tissue micromilieu, and the circulating levels of Angiotensin II or renin may not exist. Therefore, direct immunoradiometric assay for the measurement of active renin concentration (ARC) has been developed [9].

Aim of the work

We conducted this study to compare the value of using ARC to the use of PRA in the diagnosis of salt-wasting type of 21OH deficiency.

Patient & Methods:

Patients:

The patients were 25 cases of classic salt wasting 21OHD presenting to the Diabetes Endocrine and Metabolic Pediatric Unit (DEMPU) at Abo El Reeche Hospital-Cairo University, suffering from salt-loosing crises. We included 15 apparently healthy children with matched age and sex were included as controls.

We included patients into the study who presented by salt wasting crisis in the form of vomiting with or without diarrhea, dehydration with or without manifestations of circulatory shock, with laboratory evidence of electrolyte imbalance: hyponatremia, hyperkalemia, and/or acidosis with high levels of adrenal androgen precursors, 17OH progesterone and elevated Δ4 androstendione were included in the study. Cases of hyponatremia & hyperkalemia due to causes other than 21-hydroxylase deficiency were excluded.

Clinical work up:

For each patient we performed the following: 1.Careful history taking including pedigree, consanguinity, similar conditions & previous fetal death in the family. 2.General examination was done. We measured & recorded the blood pressure using a standard mercury sphygmomanometer and an appropriately sized cuff based on Korotkoff sounds during the salt-loosing crisis and plotted it against normal values according to Park & Menard [10]. We also assessed the degree of virilization in females and classified it according to Prader classification [11]. 3. Abdominal and pelvic sonography were done at the Specialized Children's Hospital, Cairo University, visualizing the adrenal glands, internal genital organs for presence of uterus & absence of inguinal gonads in virilized females. 4. All patients with ambiguous genitalia were karyotyped. 5.Adrenal precursors including serum 17-OHP, Δ4-androstendione, testosterone, progesterone & DHEA were done basally & after ACTH stimulation as described by Zarkovic [12] to establish the diagnosis of classic CAH in those presenting for the 1st time.

Laboratory methods:

After taking an informed consent & consent forms were reviewed by the ethics committee, 5 ml venous blood sample was collected aseptically and divided into 2 tubes: The first we collected on plain vacutainer from which serum was separated & rapidly frozen to -20 C & kept till analysis time. The second we collected on vacutainer containing EDTA and immediately centrifuged. After we separated the plasma the samples were rapidly frozen. At time of analysis all reagents were allowed to reach room temperature and mixed thoroughly by gentle inversion before use. Samples were rapidly thawed.

Serum was used for Na and K estimation in the serum sample by ion selective electrode NOVA. Fasting blood sugar, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), blood urea and serum creatinine were estimated by Autoanalyser Cynchron Beckman & kits supplied by Beckman. Hormones were estimated by solid phase radioimmunoassay (RIA) using the following commercial kits: 17OHP*(DSL-5000), Δ4*(DSL-3800) & Active renin *(DSL-25100).

ARC was measured with immunoradiometric assay (IRMA) using a serum sample volume of 200 µl, which depends upon non competitive assay. The analyte to be measured is "sandwiched" between two antibodies. The first antibody is immobilized to the insides of plastic tubes. The other antibody is radio-labeled for detection. The analyte present in the standards, controls and unknowns is bound by both the antibodies to form a "sandwich." Unbound reagents are removed by washing the tubes. The amount of renin bound to the tubes is directly proportional to the amount of renin present in the sample. It was done according to the manufacturers recommendations.

Renin estimation was also done by PRA# (Dia Sorin No: CA-1533, CA-1553) for which a plasma sample of 1000 µl was used. This radioimmunoassay (RIA) is based on solid phase competitive binding principles of RIA in which there is competition between radioactive (I^{125}) labeled angiotensin I and nonradioactive antigen for fixed number of antibody binding sites. The amount of I^{125} labelled antigen (tracer) bound to the antibody is inversely proportional to the concentration of the hormone to be assayed. The separation of free and bound antigen is easily and rapidly achieved by decanting or aspiration of antibody coated tubes. The PRA determination involves an initial incubation of plasma to generate angiotensin I, followed by quantitation of angiotensin I by RIA according to the manufacturer's recommendation.

Statistical analysis was done using SPSS version 11 using t-test for comparison and Spearman's rho for correlations. Correlation is considered significant at the 0.05 level (2-tailed).

RESULTS:

Patients presenting with salt-loosing crises: 17 cases presented for the first time, while 8 had been previously diagnosed & had developed a salt-loosing crisis while on treatment. The CAH patients had a mean age of 2.7 ±1.4025 (range 0.1-4 years), while the control group had a mean 3.14 ± 2.1057 (range 0.1–5.3) (p=0.392). Among the CAH patients 11 (44 %) were males and 14 (56 %) females. In the control group 7 were (46.7 %) males and 8 (53.3 %) females. Three females already had corrective genital surgery done, while 11 females presented with ambiguous genitalia. All 11 had no palpable gonads, 6 were staged Prader II (cliteromegaly & fusion of the labia majora) & 5 were staged as Prader III (single opening) according to Prader classification [(11)]. Karyotype was 46XX in all 14 cases. During the salt-loosing crisis blood pressure was below the 5th centile for age in 17/25 (68%) of patients according to Park & Menard [(10)].

Table 1: Biochemical profile in of patients with 21OHD:

Analyte	Mean ±SD (n=25)	Minimum	Maximum
Sodium (mmol/L)	125±5.6	114	130
Potassium (mmol/L)	7.1±0.54	6.1	8.3
BUN (mg/dl)	11.82±4.6481	4.00	20.00
Creatinine (mg/dl)	0.768±0.5014	0.10	1.60
Blood sugar (mg/dl)	91.8±20.6398	68.00	143.00

17OHP (ng/ml)	25.7±6.1	19.3	45.7
Δ4 Androstenedione	5.132±3.47596	0.3	10.00

Table 2: Comparison of PRA & ARC in patients & control:

	Patients (n =25) Median (min-max)	Control (n=15) Median (min-max)	p-value
PRA ng/ml/h	7.00 (6-7.2)	3.80 (3.1-5.1)	.000
ARC pg/ml	93.3 (30.9-473)	13.4 (6.4-26.1)	.000

Figure 1: Comparison of PRA in cases of 21OHD & controls

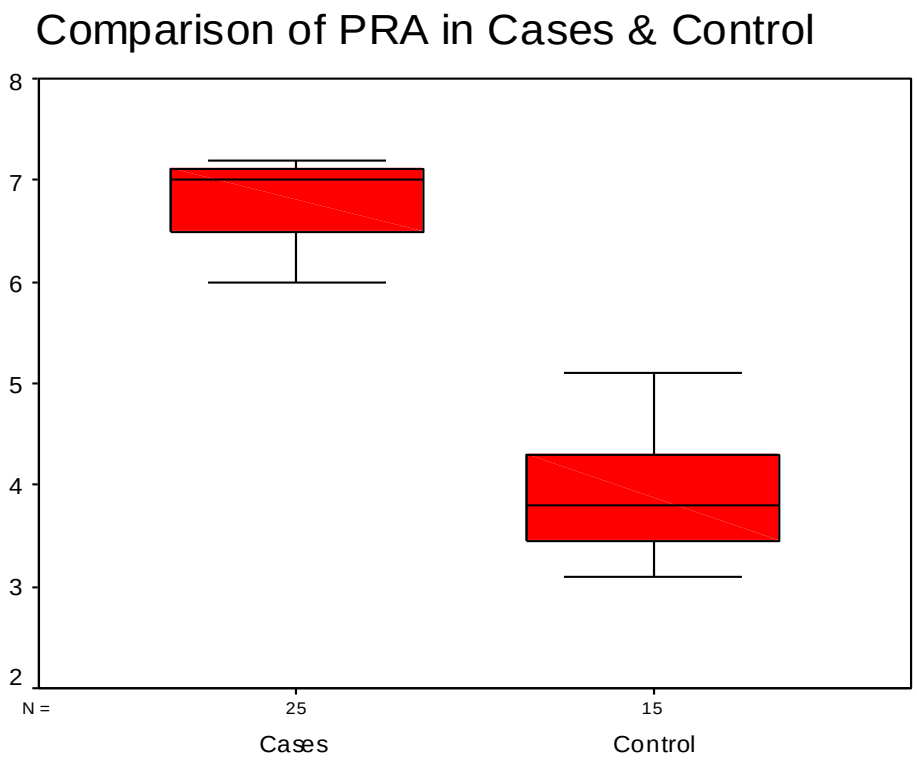


Figure 2: Comparison of ARC in cases of 21OHD & controls

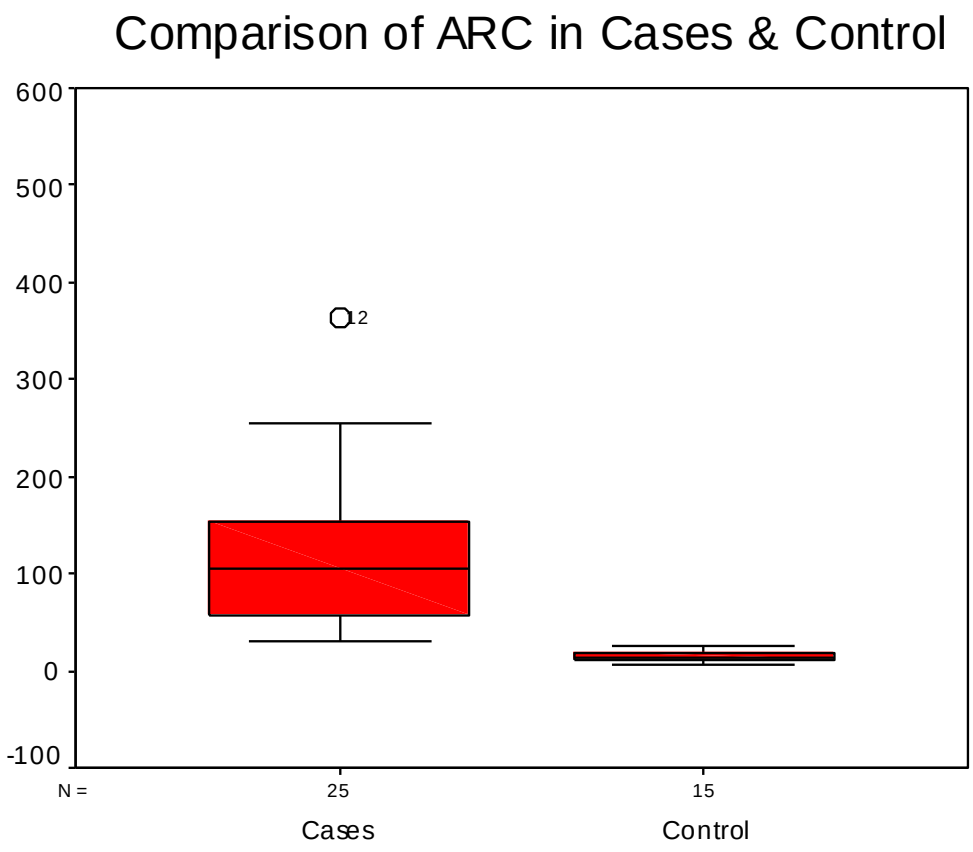


Figure 3 Correlation between PRA and ARC in cases & controls:

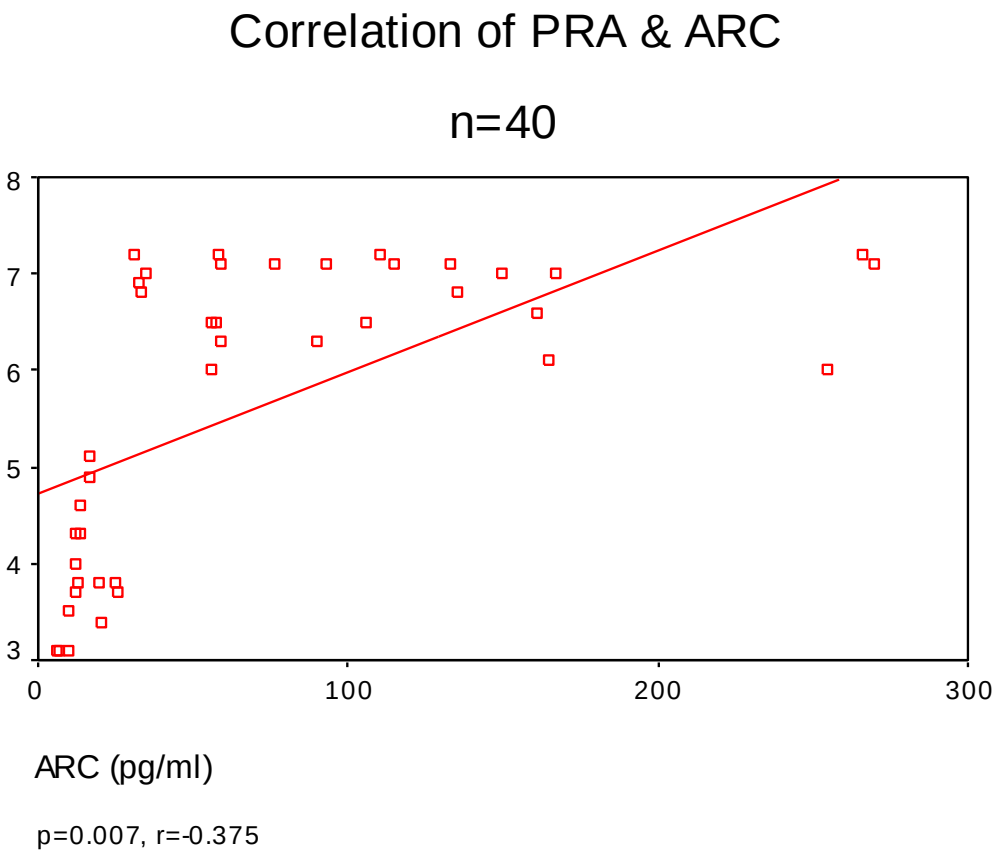


Figure 4: Correlation between ARC and systolic blood pressure in cases:

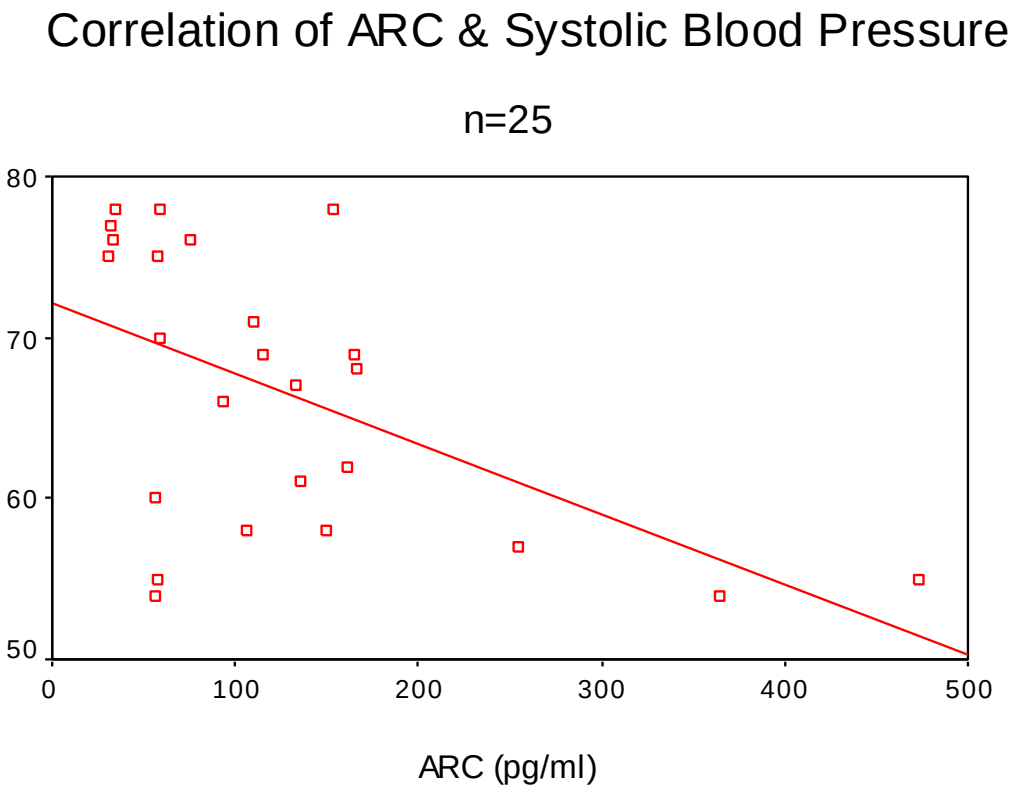
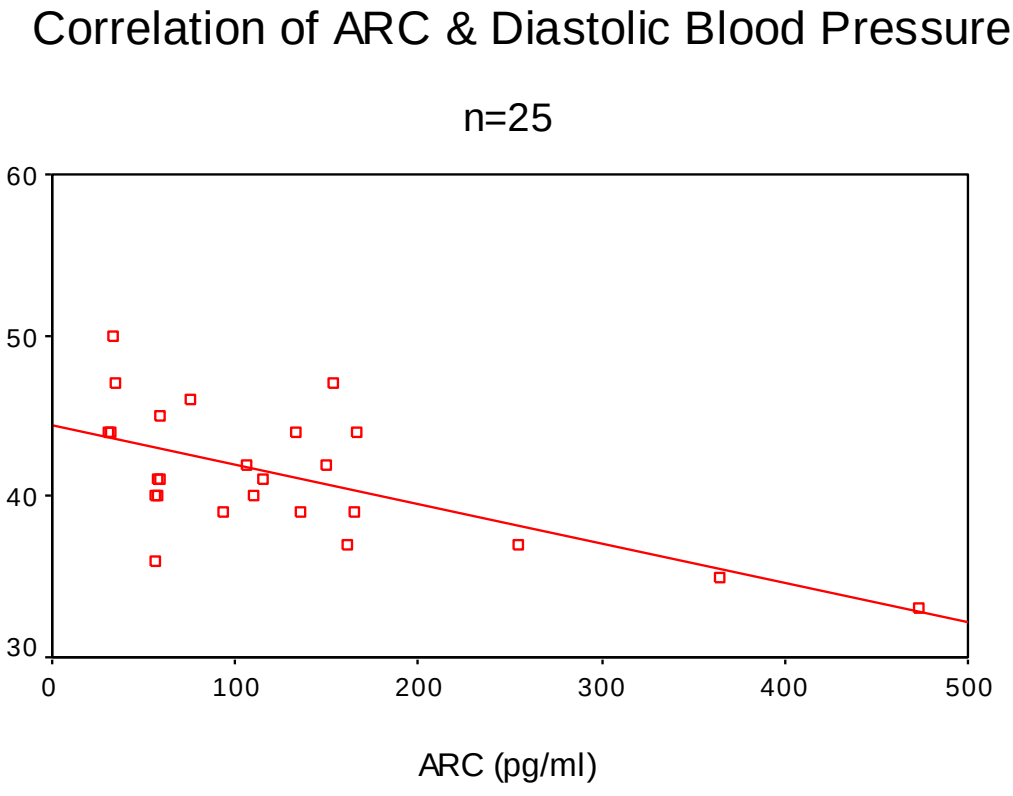


Figure 5: Correlation between ARC and diastolic blood pressure in cases:



We found a significant positive correlation between PRA and ARC (r = 0.540, p=0.000). Using the Receiver Operating Characteristics Curve (ROC) for PRA at a cutoff value of 5.55ng/ml/h there was 100% sensitivity and 100% specificity. For ARC at a cutoff value of 28.6 pg/ml there was 100% sensitivity and 100% specificity.

Systolic & diastolic blood pressure were significantly negatively correlated to ARC (p=0.03, r=-0.428 & p=0.018, r=-0.47 respectively), but not to PRA (systole: p=0.48, r=0.146, diastole p=0.535, r=0.130 respectively).

Levels of 17OHP had a significant positive correlation with PRA (r=0.79, p=0.000), but not with ARC (r=0.188, p-value =0.378), while Δ4Androstendione was not significantly correlated to PRA (r=0.304, p=0.14) nor with ARC (r=0.113, p=0.5).

Discussion

The determination of PRA is widely established. Reference values exist for all age groups, therefore it has been the method of choice for diagnosing salt loss and monitoring mineralocorticoid replacement therapy in 21OHDCAH [(13)]. However, PRA is a function of the ARC concentration and the concentration of angiotensinogen. PRA will over- or underestimate the true ARC concentration, when angiotensinogen concentration is either abnormally high or low [(14)]. It is laborious and has poor intra- and interlaboratory reproducibility and requires several precautions taking into account the age-specific reference ranges for each laboratory. Methodological problems in assay performance and standardization produce variable results in PRA determinations [(15)]. Therefore, immunoradiometric assays for the ARC have been developed. The ARC assay needs a smaller sample volume and shows no relation to the angiotensinogen concentration [(16)].

In this study we have compared use of ARC to the use of PRA in SW cases of 21OHD. The median value of PRA and of ARC were significantly higher in patients than in controls. There was a significant positive correlation between PRA and ARC in both groups. This was found in another study of 39 patients with classical CAH (30 SW, 9 SV) both children and adults on glucocorticoid or dexamethazone therapy respectively, in addition to mineralocorticoid therapy [(17)]. The correlation coefficient was also found very high in normal & in hypertensive patients [(18)] [(19)]. However, there are several conditions in which PRA & ARC dissociate from each other such as in hyperreninemic states as renovascular hypertension, Bartter's syndrome and in essential hypertension having high resting renin levels or in cases of hyporeninemic states as in heart or liver failure (13). PRA is significantly lower in type 1 diabetics than in control subjects, while ARC is similar [(20)]. Extending the incubation time in cases of low renin concentrations, as in salt loading and in primary hyperaldosteronism may lead to cryoactivation of prorenin and therefore overestimation of ARC[(21)]. Cryoactivation of plasma prorenin (which is present in 10-fold greater concentrations than renin) occurs when plasma is liquid at temperatures less than 6 degrees. Therefore, samples should be processed at room temperature and stored completely frozen [(22)]. Limited exposure to 4 °C has minimal or no affect on ARC results [(19, 23)]. However, manufacturers' data show a definite effect on samples stored at 4 °C for 24h or more.

In this study, ARC showed a significant inverse correlation to both systolic and diastolic blood pressure in cases of 21OHDCAH, while no correlation could be found with PRA. This indicates that ARC is superior to PRA in reflecting the circulatory state in cases of salt loss.

Differentiation of the phenotype SW or SV of 21OHDCAH is difficult. Plasma and urinary aldosterone levels should be correlated with PRA and with sodium balance to gain an accurate assessment of phenotype [(16, 17)]. After corticotropin stimulation, SW patients have the highest levels (up to 100,000 ng/dl), followed by patients with simple virilizing disease, who usually have somewhat lower levels (10,000 to 30,000 ng/dl)[(6)]. However, with neonatal screening becoming more widespread, early detection and early onset of therapy makes differentiation between salt losing and simple virilizing forms more difficult. Classical versus nonclassical phenotype can be predicted from genotypes in most cases. However, rare exceptions exist [(24)]. Molecular studies have found the phenotype-genotype correlation not strong [(25)] [(26)].

All classic CAH patients should be treated with fludrocortisone at diagnosis in the newborn period, since such therapy will reduce vasopressin and ACTH levels and lower the dosage of glucocorticoid required. Sodium chloride supplements are often needed in infancy, distributed in several feedings until the child has access to salt [27]. The question how long to continue giving fludrocortisone, remains an issue of debate. The need for continuing mineralocorticoids was based on PRA and blood pressure. Elevated PRA values, which are used to monitor the adequacy of mineralocorticoid and sodium replacement [21], can also be increased in patients with normal aldosterone secretion who have high circulating levels of ACTH, 17-OHP, and progesterone, making poorly controlled simple virilizers having a high 17OHP level biochemically resemble salt wasters. This together with the early onset of therapy after neonatal screening makes the diagnosis of salt loss difficult.

Recently, the ratio of serum aldosterone to plasma renin activity was found to discriminate well between the different groups of disease severity in 21OHD. The lowest ratios, indicative of the least sodium conservation, were seen in the salt-wasting group with increasing ratios in the simple virilizing, nonclassical, and unaffected groups & this ratio remained stable with age [28].

The absence of a correlation between 17OHP and ARC together with the significant negative correlation to the blood pressure may make it useful in diagnosing the salt loss in cases of 21OHCAH. Further studies are needed to compare the level of salt-wasters with uncontrolled simple virilizers to evaluate the ARC in the differentiation of the 2 forms of classic CAH.

The improved immunoradiometric assay is specific for renin and is a simple and precise method. This assay is easier to standardize than the enzyme-kinetic assays that rely on endogenous angiotensinogen, such as the PRA assay. The results obtained with the IRMA are expressed in terms of the internationally recognized human renin standard and, therefore, permit ready comparison between different laboratories. The problem of cryoactivation of prorenin can be overcome by shorter incubation at a higher temperature [23].

The direct monoclonal antibodies method for measuring ARC can be used instead of PRA in studying both clinical and pathophysiological aspects of the renin-angiotensin system in patients with 21OHD CAH. It shows a direct correlation to blood pressure in cases of salt-loosing CAH. It is less laborious and has the potential of better standardization because IRMAs are much less prone to interlaboratory variation.

CONCLUSION:

Active renin concentration (ARC) determines the activation of the renin-angiotensin system as precisely as PRA in assessment of the renin-angiotensin system in 21OHD. It is preferable to PRA in children because of methodological advantages and a smaller sample volume & better standardization. Unlike PRA it is not correlated to 17OHP which is also elevated in uncontrolled simple virilizers making it potentially useful for differentiation of salt wasters from simple virilizers.

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