Serum fetuin-A and insulin levels in classic congenital adrenal hyperplasia

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İNTRODUCTİON

Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive diseases that are caused by a defect in adrenal steroidogenesis. Accumulating steroid precursors that shift to androgen synthesis pathway leads to excess androgen production. Consequently, life-threatening salt loss in early infantile period and ambiguous genitalia in females can be observed. Glucocorticoids (GCs) to prevent adrenal insufficiency and to suppress excess androgen production as well as fludrocortisone in case of aldosterone synthesis defect are included in the treatment. However; there are some difficulties in applying these treatment regimens. GC replacement with supra-physiological doses in order to suppress androgen production brings some problems such as iatrogenic cushing syndrome, obesity, developmental retardation, insulin resistance, hypertension, a tendency to develop atherosclerosis and low bone mineral density. Also different problems arise in the presence of unsuppressed excess androgens, including shorter height in the adulthood due to advanced bone age, virilization and menstrual irregularities. In child and adolescent CAH patients followed-up by different clinics it was found that they had an increased rate of obesity and hypertension. In addition increased cardiometabolic risk and early atherosclerosis are observed in these patients. Increased carotid intima media thickness considered as a result of exposure to excess androgens supports early cardiovascular disease (CVD) risk. All of these outcomes demonstrate the need for further optimized treatment and extended follow-up criteria.

Fetuin-A, secreted from the liver, enhances the level of insülin as it is a natural insulin signaling inhibitor glycoprotein. Insulin resistance and high fetuin-A levels are described in patients with obesity and type 2 diabetes mellitus (T2DM). Also insulin levels of obese children were found to be higher than control group. Besides in a different study fetuin-A levels were found to regress after weight loss. Increased fetuin-A levels in obesity and T2DM and results of previous studies support that fetuin-A is associated with insulin resistance. However, how fetuin-A causes insulin resistance has not yet been completely elucidated. However; how fetuin-A causes insulin resistance has not yet been completely elucidated.

Androgens play a pivotal role in non-reproductive tissues, such as the kidneys, heart, and liver, as well as the pancreas. Since the expression of androgen receptors (ARs) has been described in tissues such as the pancreas and liver, this raises the possibility that excess testosterone produces insulin hypersecretion and increased fetuin-A. As with PCOS, excess androgen exposure may also lead to unfavorable outcomes in CAH patients. This in turn raises the question of whether fetuin-A and insulin levels are affected by androgens in CAH patients, because impaired insulin signaling tends to be more common in CAH patients. On the other hand; a high degree of sequence identity in human fetuin-A promoters suggests that the promoter is similarly up-regulated by GC. Therefore, GC supra-physiological doses may enhance the level of fetuin-A protein circulating in serum of CAH patients. Furthermore long term GC therapy can induces hyperinsulinaemia, due to compensation for GC-induced insulin resistance. Due to the potential association between hyperandrogenism and insulin resistance, the aim of this study was to investigate the levels of insulin, fetuin A, and insulin resistance in CAH patients and a matched control group. We also aimed to evaluate relation of levels of insulin, fetuin A, and insulin resistance with GC, androgen levels, BMI within the CAH patients.

PATİENTS AND METHODS

Patients with classic CAH due to 21-hydroxylase deficiency based on biochemical and/or genetic testing at the Dr. Sami Ulus Obstetrics and Gynecology, Children Health and Disease Training and Research Hospital Pediatric Endocrinology, Turkey, were included in the study. Age-, sex-, pubertal stage-, and body mass index (BMI)-matched controls were included as control cases. These subjects were healthy apart from being overweight or obese. Individuals who refused to participate in the study and those with chronic illness were excluded.

RESULTS

Table 1 outlines the clinical features of the groups. Data were organized in terms of gender, and of prepubertal/pubertal status in order to eliminate potential changes in serum lipid profile, glucose, and insulin associated with the production of sex steroids. The prepubertal group consisted of Tanner stage 1 subjects, and the pubertal group of Tanner stages 4 and 5. Twenty-six cases were included in the CAH group and 30 subjects in the control group. Cases included in the pubertal group were subdivided by gender, the male and female subgroups consisting of 14 and 16 patients, respectively. The pubertal control group consisted of 20 boys and 20 girls.
No differences were determined between the groups in terms of age, sex, pubertal stage, or BMI. In the CAH group, 64% of cases were salt-losing type, and 34% were simple virilizing type. All 56 CAH patients were taking glucocorticoid and 33 were also using fludrocortisone in combination. When the CAH patients were classified based on 17-OHP levels, four were suppressed, 23 had acceptable control, 11 intermediate control, and 18 poor control.

No differences were also observed between the groups in terms of SBP, DBP, total cholesterol, LDL, or HDL. However, triglyceride levels were higher in the pubertal CAH patients than in the controls Triglycerides did not correlate with androgens, fetuin-A, daily dosage of fludrocortisones per m^2 and hydrocortisone per m^2, but correlated with insulin level. Prepubertal CAH patients had significantly higher levels of hs-CRP, a marker of inflammation risk, than the controls. However, no difference was observed in terms of triglyceride levels in the prepubertal groups, or hs-CRP levels in the pubertal groups. Our most important finding was that the CAH group had higher fetuin-A levels than the controls . Differences were observed in terms of insulin levels and HOMA-IR values between CAH patients and controls (Table 1). Furthermore, within the CAH group (n = 56), Fetuin-A levels were positively correlated with insulin, HOMA-IR, Androstenedione , DHEAS, total testosterone and free testosterone levels as well as BMI (Tablo 2). We also observed a positive correlation between insulin and fetuin-A, DHEAS, total and free testosterone, triglycerides, fludrocortisones per m^2 and BMI (Table 2). Multiple linear regression analysis performed that free testosterone and insulin levels may predict fetuin-A and free testosterone and fetuin-A levels also may predict insulin levels in CAH patients.

Table 1. Clinical characteristics of adolescents and young adults with CAH due to 21-hydroxylase deficiency and controls

	At prepuberty			At puberty					
	CAH (n: 26, m: 13)	Control (n: 30, m:15)	p value	CAH males (n:14)	Control (n: 20)	p value	CAH Females (n:16)	Control (n:20)	p value
Age, years	7.0±0.53	6.46±2.36	0.36	15.86±2.13	15.09±2.07	0.28	14.6±2.26	14.30±2.08	0.82
Weight, kg	27.85±10.08	26.26±10.02	0.64	61.50±12.91	57.30±9.14	0.37	58.06±15.52	57.46±8.19	0.50
Weight SDS	0.71±1.14	0.39±1.1	0.23	-0.34±1.13	-0.22±0.97	0.74	0.25±1.43	0.63±1.04	0.20
Height, cm	121.16±17.01	118.44±16.70	0.51	161.17±5.34	161.48±7.32	0.78	156.45±5.71	157.82±4.11	0.09
Height SDS	0.09±1.07	0.16±0.90	0.74	-1.34±1.09	-0.69±0.94	0.08	-0.64±1.24	-0.20±0.81	0.10
BMI	18.23±2.62	17.16±2.65	0.16	23.59±4.33	21.86±2.44	0.17	23.54±5.11	23.19±3.14	0.58
BMI SDS	0.85±0.95	0.28±1.16	0.08	0.38±0.98	0.18±0.77	0.50	0.7±1.11	0.84±0.97	0.38
SBP, mm Hg	106.54±10.93	106.16±11.42	0.90	117.85±7.77	115.0±8.58	0.32	117.50±8.36	116.3±8.6	0.62
DBP, mm Hg	64.04±7.35	63.50±7.56	0.82	71.07±4.0	70.25±3.79	0.53	71.25±6.2	70.25±6.17	0.60
Glucose, mg/dL	87.61±10.07	85.26±7.83	0.51	81.64±8.55	82.15±7.81	0.80	82.93±5.47	83.10±5.73	0.95
Insulin, IU/mL	8.47±1.80	3.90±2.66	**<0.001**	11.45±3.21	6.94±2.66	**<0.001**	9.04±2.22	6.86±4.30	**0.008**
HOMA-IR	1.84±0.48	0.83±0.60	**<0.001**	2.31±0.7	1.41±0.55	**0.001**	1.86±0.53	1.40±0.90	**0.01**
AST, U/L	23.42±4.92	23.3±6.24	0.91	22.92±5.15	21.3±5.37	0.32	21.87±5.93	23.00±6.17	0.59
ALT, U/L	21.61±5.27	22.76±5.15	0.44	20.35±5.01	17.45±4.92	0.11	21.43±4.64	23.60±4.99	0.206
Total cholesterol, mg/dl	146.19±46.44	139.22±45.85	0.50	152.71±31.22	145.90±23.36	0.42	161.07±26.24	151.45±15.7	0.25
LDL, mg/dl	85.14±25.66	83.79±24.1	0.82	85.1±20.91	82.45±18.67	0.56	87.87±22.24	84.36±18.37	0.67
Triglycerides, mg/dl	108.46±68.42	78.90±11.04	0.16	95.57±9.88	87.75±7.44	**0.008**	101.0±38.67	71.60±20.3	**0.01**
HDL, mg/dl	48.73±11.03	39±10.68	0.80	48.4±9.31	52.76±13.71	0.482	55.27±15.58	54.69±15.37	0.88
Fetuin A, ng/ml	511.12±112.15	415.70±118.35	**0.002**	877.14±267.61	583.19±246.89	**0.004**	675.38±233.26	512.42±154.54	**0.036**
hs-CRP, mg/L	0.28±0.26	0.15±0.14	**0.03**	0.38±0.32	0.20±0.19	0.08	0.25±0.28	0.19±0.26	0.16
Glucocorticoid dose in hydrocortisone equivalents, mg/m^2/day	12.11±3.66			16.51±5.98			14.06±8.61		
Fludrocortisone dose, mcg/m^2day	56.73±54.66			31.65±26.35			37.47±27.06		
17-OHP, ng/dl	22.43±30.07			64.16±68.30			60.73±88.28		
Androstenedione, ng/dl	0.6±0.7			2.14±2.52			2.55±2.76		
DHEAS (females/males), µg/dl	18.4±24.48			61.30±62.59			64.45±82.16		
Total testosterone (females/males), ng/dl	32.89±23.63			354.82±168.58			108.04±132.15		
Free testosterone (females/males), pg/ml	1.00±0.85			9.11±5.51			4.15±4.39		

Table 2. Correlation of factors influencing fetuin-A, and HOMA-IR

	Fetuin-A ng/mL		Insulin, IU/mL	
	r	p	R	p
Insulin, IU/mL	0.424	**<0.001**	-	**-**
HOMA-IR	0.497	**<0.001**	0.23	0.08
Fetuin-A, ng/ml	-	**-**	0.42	**0.001**
17-OHP, ng/ml	0.318	0.02	0.06	0.7
Androstenedione, ng/dl	0.610	**<0.001**	0.27	0.048
DHEAS, µg/dl	0484	**<0.001**	0.49	**<0.001**
Total testosterone, ng/dl	0.689	**<0.001**	0.48	**<0.001**
Free testosterone, pg/ml	0.625	**<0.001**	0.47	**0.001**
Total cholesterol, mg/dl	-0.081	0.55	-0.02	0.87
LDL, mg/dl	-0.151	0.27	0.02	0.86
Triglycerides, mg/dl	-0.024	0.86	-0.18	**0.18**
HDL, mg/dl	-0.02	0.92	-0.003	0.98
Glucocorticoid dose in hydrocortisone equivalents, mg/m^2/day	0.1	0.5	-0.12	0.38
Fludrokortizon dozu, mcg/m^2day	-0.17	0.34	-0.34	**0.049**
BMI	0.4	**0.002**	0.28	**0.038**

Table 3. Multiple regression analysıs in CAH children, with fetuin-A and insulin as dependent variables

	Dependent Variable: Fetuin-A				
				95.0% CI for B	
	B	t	p	Lower	Upper
Insulin	23.416	2.108	**0.043**	0.789	46.043
17-OH progesterone	-0.338	-0.606	0.549	-1.474	0.798
Androstenedione	19.326	0.907	0.371	-24.061	62.713
DHEAS	-0.497	-0.697	0.491	-1.949	0.955
Free Testosterone	30.561	4.104	**<0.001**	15.394	45.728
BMI	5.112	0.905	0.372	-6.400	16.624

	Dependent Variable: Insulin				
				95.0% CI for B	
	B	t	p	Lower	Upper
DHEAS	0.016	2.045	0.057	-0.001	0.033
Free testosterone	-0.335	-2.297	**0.035**	-0.642	-0.027
BMI	0.129	0.816	0.426	-0.205	0.463
FC dose	-0.005	-0.624	0.541	-0.022	0.012
Fetuin-A	0.011	3.471	**0.003**	0.004	0.017

CONCLUSİON

Our findings demonstrate clear differences in fetuin-A levels between children with CAH and the control subjects. In our study, this level was correlated with insulin, HOMA-IR, androgens and BMI. When we look at the factors affecting fetuin-A, we found that free testosterone level was most effective parameter. It is consistent with the study in the literature. Elevated level of fetuin-A may impede the action of insulin, and leads to insulin resistance. It can be considered that androgens have direct effect on regulation of fetuin-A, and indirect effect on insulin.

On the other hand; we found that fetuin-A and free testosterone levels effect on insulin levels. These differences are likely to have arisen as a result of androgen excess. Similarly, some studies have suggested that insulin and androgen levels are causally related. Hyperinsulinemia may contribute to hyperandrogenism and, conversely, androgens may contribute to insulin resistance. Studies have reported that insulin resistance increases androgen production due to increased ovarian sensitivity in PCOS patients. Insulin hypersecretion can be also stimulated by excess testosterone by ARs expressed in the pancreas in females. However, whilst insulin secretion can be enhanced by androgens, deficiency of those hormones increases insulin resistance, leading to metabolic syndrome in males. Although we did not identify any cases of T2DM in our CAH patients, high insulin levels in these subjects may result from excess androgen exposure, because the receptors, ARs, are expressed in pancreatic cells, thereby resulting in a boost in insulin secretion.

Furthermore, a high degree of sequence identity in human fetuin-A promoters suggests that the promoter is similarly up-regulated by GC. Therefore, GC supra-physiological doses may enhance the level of fetuin-A protein circulating in serum of CAH patients. Furthermore long term GC therapy can induces hyperinsulinaemia, due to compensation for GC-induced insulin resistance. However, although GCs level correlated positively with total testosterone, there was no effect on fetuin a and insulin in multi-regression analysis.

Serum fetuin-A and insulin levels seem to be associated with androgens in CAH patients. Therefore, further studies are needed in larger series

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