Evaluation of insulin resistance in Turkish girls with premature pubarche using the homeostasis assessment (HOMA) model

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SUMMARY: Evliyaoğlu O, Berberoğlu M, Adıyaman P, Aycan Z, Öcal G. Evaluation of insulin resistance in Turkish girls with premature pubarche using the homeostasis assessment (HOMA) model. Turk J Pediatr 2007; 49: 165-170.

Premature pubarche can be benign, or it can be an early marker of insulin resistance in some girls. The aim of this study was to evaluate insulin resistance in prepubertal girls presenting with premature pubarche (n=19; mean age=6.93±1.78) as compared to age- and Tanner stage-matched controls (n=10; mean age=7.55±1.32) using indirect insulin resistance parameters. Two groups were compared by means of oral glucose tolerance test (OGTT), indirect insulin resistance (IR) parameters [homeostasis assessment (HOMA) models: HOMA-IR, glucose/insulin (G/I)] and serum lipid profiles. In the girls with premature pubarche, mean baseline insulin level and HOMA-IR value were significantly higher and G/I ratio was lower than in the control group. IR was observed in 42.1% and in 31.6% according to HOMA-IR value and G/I ratio, respectively. Even at diagnosis, IR was increased in a significant portion of the girls with premature pubarche.

Key words: premature pubarche, insulin resistance.

Premature pubarche in girls is defined as the early appearance of pubic hair before eight years of age. The usual etiology of premature pubarche is premature adrenarche, which is the early occurrence of adrenal androgen levels appropriate to normal pubarche. The development of pubic hair is non-to-slowly progressive[1]. Premature pubarche does not alter normal pubertal development and final height. However, recent studies have shown that it may not be a benign phenomenon; these girls may develop insulin resistance (IR) and related diseases[2,3]. Even at diagnosis, incidence of IR is increased in girls with premature pubarche[4,5].

In a number of studies, indirect IR parameters were used to evaluate IR in suspected girls. Baseline glucose/insulin (G/I) ratio was found to be highly sensitive and specific for measurement of insulin sensitivity[5-8]. Insulin secretion and resistance were assessed by the homeostasis assessment (HOMA) models[7,9,10]. By using the indirect parameters of IR, we aimed to evaluate IR in prepubertal girls with premature pubarche, and tried to find a marker indicative of girls at risk for development of IR syndrome.

Material and Methods

Nineteen girls with premature pubarche were enrolled in this study. The criteria for entry included the appearance of pubic hair in prepubertal girls before the age of eight years. Girls with breast development, an enzymatic defect of adrenal steroidogenesis and obesity were excluded. Birth weight and gestational age data were obtained from hospital records and transformed in SD scores. Birth weight SDS <-1.75 was accepted as small for gestational age (SGA)[11]. Family history of type 2 diabetes mellitus (DM) was obtained. The control group consisted of 10 prepubertal age-matched otherwise healthy girls admitted to hospital with unrelated and nonspecific symptoms. All children underwent anthropometric measurements using the growth standards of Tanner and Whitehouse[12]. Bone age was

assessed with the method of Greulich and Pyle[13]. Body mass index (BMI) was calculated according to the following formula: weight (kg)/height (meters)^2. Height was expressed as z-score for chronological age[14]. Informed parental consent and assent from minors were obtained.

Late onset congenital adrenal hyperplasia due to 21-hydroxylase (21-OH) and 3β-hydroxysteroid dehydrogenase (3β-HSD) deficiencies were ruled out in all subjects before their inclusion in the study by means of adrenocorticotropic hormone (ACTH) testing (Synacten, Ciba-Geigy, Basel, Switzerland; 0.25 mg/m^2 intramuscular), with 17-hydroxyprogesterone (17-α-OHP), dehydroepiandrosterone sulfate (DHEA-S), and cortisol and androstenedione determinations at 0, 30, and 60 minutes[15,16]. The plasma 17-α-OHP response to ACTH was assessed using published normogram standards[17]. Stimulated dehydroepiandrosterone (DHEA)/Δ^4-A ratios for 3β-HSD were evaluated following the data reported[18].

All subjects underwent an oral glucose tolerance test (OGTT) (1.75 g/kg body weight, maximum 75 g glucose), after three days on a high carbohydrate diet (300 g/day) and an overnight fast. Blood sampling was performed at 0, 30, 60, 120 and 180 minutes after oral glucose administration for glucose and insulin measurements[19]. Glucose intolerance was defined as plasma glucose level between 140 mg/dl and 200 mg/dl at min 120-OGTT[20]. IR was assessed from fasting serum insulin and plasma glucose concentrations by the HOMA models using the following formulas:

1. HOMA-IR: (fasting insulin in mU/L) x (fasting glucose in mmol/L /22.5)[10]

2. G/I ratio: fasting glucose in mg/dl / fasting insulin in mU/L[5,21]

The cut-off for HOMA-IR was considered to be the 95% confidence interval of the control group; we designated a child positive for IR if her value was above 2.96. This value had a specificity of 100% and sensitivity of 37%. Positive predictive value was 100% and negative predictive value was 45%. In our study group, we presumed that there were also children with normal insulin sensitivity and our aim was to select the girls who undoubtedly had IR. Thus, despite the low sensitivity and high negative predictive value, because of the high specificity and positive predictive value, we chose 2.96 as a cutoff value. As previously proposed, a G/I ratio lower than 6 was also accepted as IR[7,8].

Glucose metabolism (OGTT, IR parameters) and lipid profiles of the girls with premature pubarche were compared with the control group. Among the children with premature pubarche, IR was identified by using indirect resistance parameters, and insulin resistants were compared with the nonresistants by their anthropometric measurements, birth weight SDS, OGTT-plasma glucose levels, OGTT-serum insulin and baseline insulin derived parameters, baseline and ACTH stimulated cortisol, 17-α-OHP, DHEA-S, androstenedione levels, and serum lipid profiles.

Hormonal Assays

Serum 17-α-OHP levels were measured by radioimmunoassay (RIA) method (ImmuChem™ Coated Tube 17-α-Hydroxyprogesterone 125/RIA Kit, ICN Biomedicals, Inc, Costa Mesa, CA[22]), and serum DHEA-S and cortisol levels were measured by electro-chemiluminescence immunoassay "ECLIA" (Roche Diagnostics GmbH, D-68298 Mannheim[23]). Serum androstenedione levels were measured by RIA method (DSL-3800 ACTIVE™ Androstenedione Coated-Tube RIA Kit, Diagnostic Systems Laboratories, Inc, Texas[24,25]). Serum insulin levels were measured by ^{125}I RIA (Coat-A-Count Insulin) (Diagnostic Products Corporation Los Angeles, CA). All hormone determinations in each individual were measured in duplicate within a single assay.

Biochemical Measurements

Serum cholesterol levels were measured by CHOD-PAP-method (Enzymatic Colour Test for Clinical Chemistry Analyzers, Olympus System Reagent, Ireland[26]). Serum high-density lipoprotein (HDL)-cholesterol was measured by Immunoinhibition Method (Enzymatic Colour Test for Clinical Chemistry Analyzers, Olympus System Reagent, Ireland[27,28]). Serum triglyceride levels were measured by GPO-PAP method (Enzymatic Colour Test for Clinical Chemistry Analyzers, Olympus System Reagent, Ireland[29]). Serum very low density lipoprotein (VLDL)–cholesterol and low density lipoprotein (LDL)-cholesterol levels were estimated by the following formulas[30]: VLDL-cholesterol=triglyceride/5; LDL-cholesterol=total cholesterol-HDL-triglyceride/5.

Statistical Analysis

Results are expressed as the mean±SD. Serum levels among independent groups were compared by the Student's t test and the Mann-Whitney U test for nonparametric variables. Correlations were made using Pearson's or Spearman's where appropriate, and a value of $p<0.05$ was considered statistically significant.

Results

All of the girls had normal growth and weight with appropriate bone age for chronological age. All bone ages were within 2.5 SD of the mean chronological age. Four (21.1%) of them were SGA. Nine had a family history of type 2 DM. Baseline and stimulated levels of cortisol and adrenal androgens of the girls with premature pubarche are shown in Table I. In the girls with the family history of type 2 DM, plasma glucose and serum insulin levels (baseline and min-120-OGTT), baseline serum insulin derived parameters and serum lipid patterns were comparable to those without family history. Consequently, those with the history of type 2 DM were not omitted from the study.

Of the girls with premature pubarche, three (15.8%) had glucose intolerance at OGTT. Incidences of IR were 42.1% (n=8) and 31.6% (n=6) according to HOMA-IR value and G/I ratio, respectively. None of the girls in the control group had glucose intolerance or IR.

When the girls with premature pubarche were compared with the control group, mean baseline serum insulin level and HOMA-IR value were significantly higher, and G/I ratio was lower. Serum lipid patterns were indifferent between the two groups (Table II).

Table I. Baseline and ACTH-Stimulated Cortisol and Adrenal Androgens in Girls with Premature Pubarche

Baseline cortisol (μg/dl)	12.24±4.83	(337.70±133.25 nmol/L)
Δ cortisol** (μg/dl)	19.21±12.77	(530.00±352.32 nmol/L)
Baseline 17OHP (ng/ml)	0.70±0.36	(0.02±0.01 nmol/L)
Stimulated 17OHP* (ng/ml)	3.02±1.21	(0.09±0.03 nmol/L)
Baseline DHEAS (μg/dl)	45.12±39.17	(1.17±1.01 nmol/L)
Stimulated DHEAS* (μg/dl)	56.37±44.31	(1.46±1.15 nmol/L)
Baseline androstenedione (ng/ml)	0.50±0.26	(0.02±0.01 nmol/L)
Stimulated androstenedione* (ng/ml)	0.97±0.60	(0.03±0.02 nmol/L)

Values are expressed as mean±SD. *ACTH stimulated peak levels. **Increment in cortisol after ACTH stimulation. ACTH: Adrenocorticotropic hormone. 17OHP: 17-hydroxyprogesterone. DHEAS: Dehydroepiandrosterone sulfate.

Table II. Clinical Characteristics, OGTT-Plasma Glucose, Insulin Levels, Baseline Serum Insulin-Derived Parameters and Serum Lipid Profiles of the Girls with Premature Pubarche and Controls

	Premature pubarche (n=20)	Control (n=10)	p value
Age at admission (years)	6.93±1.78	7.55±1.32	
Bone age (years)	6.87±2.05		
Age at onset of symptoms (years)	6.42±1.51		
Height SDS	0.40±0.85	0.56±0.88	
BMI SDS	0.33±1.19	0.97±0.23	
Baseline insulin (mU/L)	13.34±9.94	6.0±3.74	0.02
OGTT-120-min insulin (mU/L)	45.93±47.83	25.48±13.49	
Baseline glucose (mg/dl)	92.78±12.58 (5.15±0.69 mmol/L)	88.6±10.96 (4.92±0.61 mmol/L)	
OGTT-120-min glucose (mg/dl)	101.26±27.73 (5.62±1.54 mmol/L)	99.70±21.29 (5.54±1.18 mmol/L)	
HOMA-IR	2.98±2.03	1.33±0.92	0.02
G/I	13.68±16.42	24.50±23.27	0.03
Total cholesterol (mg/dl)	163.77±23.30 (4.24±0.6 mmol/L)	153.85±23.3 (3.98±0.60 mmol/L)	
Triglyceride (mg/dl)	92.61±40.14 (0.92±0.40 g/L)	107.71±46.83 (1.07±0.46 g/L)	
LDL (mg/dl)	92.72±21.03 (2.40±0.54 mmol/L)	81.42±27.45 (2.11±0.71 mmol/L)	
VLDL (mg/dl)	18.44±8.02 (0.18±0.08 g/L)	19.14±8.51 (0.19±0.08 g/L)	
HDL (mg/dl)	52.77±11.69 (1.36±0.30 mmol/L)	53.57±12.98 (1.39±0.34 mmol/L)	
T-chol/HDL	3.17±0.77	3.01±0.88	

Values are expressed as mean±SD.
OGTT: Oral glucose tolerance test. BMI: Body mass index. HOMA-IR: Homeostasis assessment of insulin resistance. G/I: Glucose-insulin ratio. LDL: Low density lipoprotein. VLDL: Very low density lipoprotein. HDL: High density lipoprotein.

Comparison of the insulin resistant and non-resistant groups (HOMA-IR >2.96 vs <2.96, G/I <6 vs >6) in the girls with premature pubarche:

There was no significant difference between the BMIs of the patients with and without IR. In the insulin resistant groups (HOMA-IR >2.96 and G/I <6), mean baseline and min-120-OGTT serum insulin levels were significantly higher (Fig. 1). Additionally, in the insulin resistant group based on G/I ratio, mean plasma glucose level at min–120-OGTT was higher. There was no difference in baseline and stimulated adrenal steroids between insulin resistant and non-resistant groups.

In the girls with premature pubarche, min-120-OGTT serum insulin level was positively correlated with baseline serum insulin, cholesterol levels, and HOMA-IR value, and negatively correlated with G/I ratio (Fig. 2).

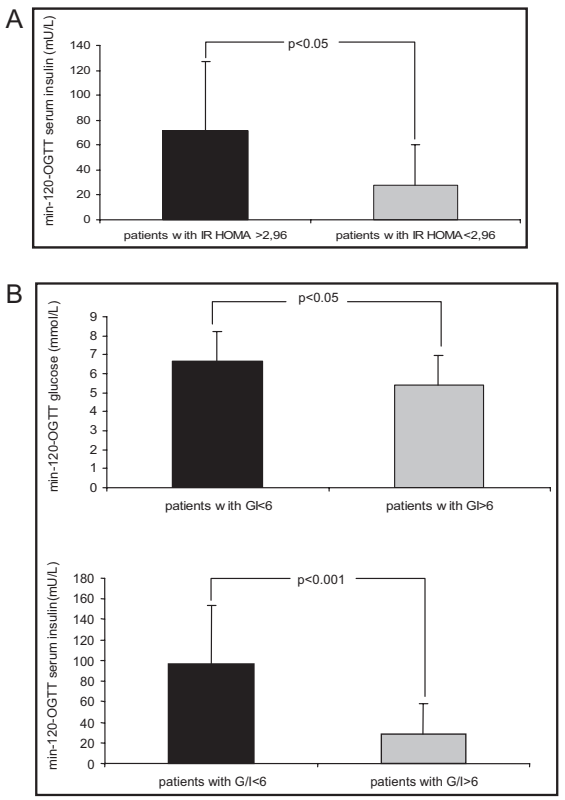

Fig. 1. A. Min-120-OGTT serum insulin levels of the girls with premature pubarche with HOMA-IR >2.96 compared with the girls with HOMA-IR <2.96. B. Min-120-OGTT plasma glucose and serum insulin levels of the girls with premature pubarche with G/I ratio <6 compared with the girls with G/I ratio >6.

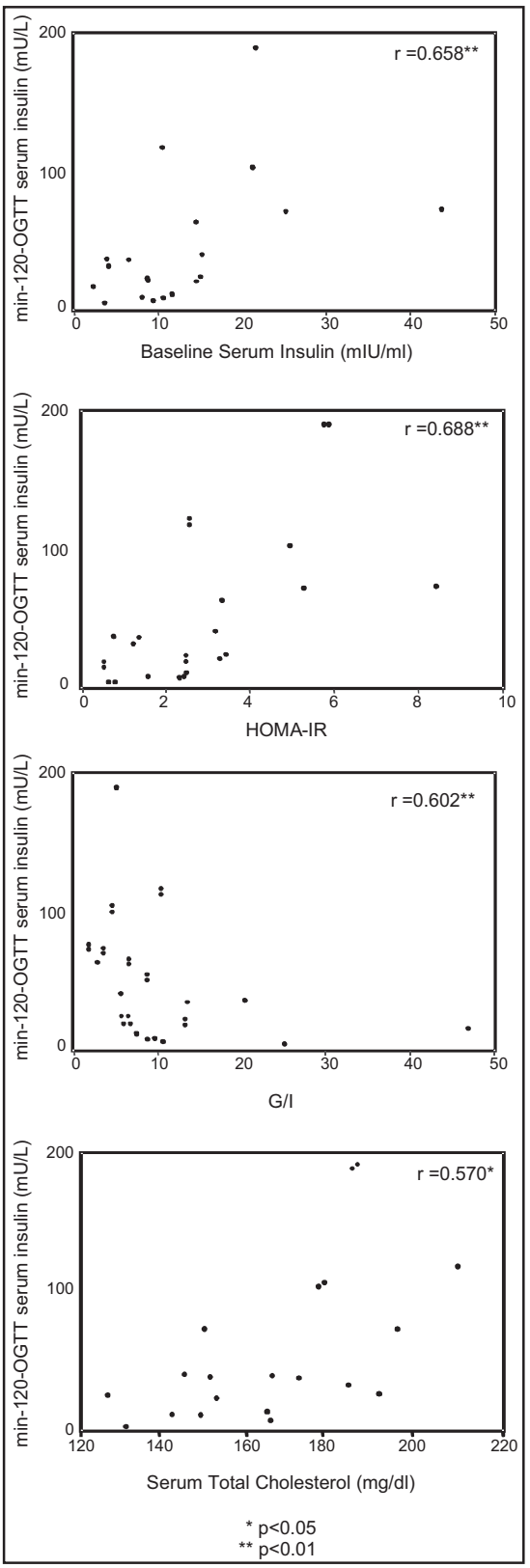

Fig. 2. Correlations between min-120-OGTT serum insulin level and HOMA-IR value, G/I ratio and serum cholesterol level in the girls with premature pubarche.

Discussion

Our results indicate that even at diagnosis, incidence of IR is increased in the girls with premature pubarche. They have increased mean serum baseline insulin level as well as higher HOMA-IR value and lower G/I ratio compared to the control group. Although it has been established that the incidence of IR is increased in the prepubertal girls with premature pubarche, in the previous studies, mean baseline serum insulin levels were comparable with those of the prepubertal control groups[4,5].

In the girls with premature pubarche, serum insulin level at min-120-OGTT was positively correlated with baseline serum insulin level and HOMA-IR value, and negatively correlated with G/I ratio. This finding is interesting in that by determining fasting serum insulin and derived parameters, one can obtain an idea about min-120-OGGT serum insulin levels without performing an OGTT. Indirect IR parameters can be used as a screening test for the evaluation of IR.

In a study of Ibanez et al.[31], lipid patterns of premature pubarche girls showed that hyperinsulinemia was accompanied by increased triglyceride levels compared with a Tanner stage- and age-matched population. Contrary to this finding, in a recent study of Meas et al.[32], there was no statistical difference in serum lipid levels between post-pubertal Caucasian girls with premature pubarche and the control group. This study showed no difference between the lipid patterns of the prepubertal girls with premature pubarche and the control group. However, there was a positive correlation between min-120-OGTT serum insulin and total cholesterol levels. Because of the multifactorial interaction, data about IR and serum lipid profile seem to be conflicting.

When the girls with premature pubarche were evaluated according to their IR parameters, mean baseline serum insulin levels were significantly higher in the insulin resistant groups compared to the non-resistant groups, in agreement with the previous studies[4,5]. Additionally, in insulin resistant groups, mean min-120-OGTT serum insulin levels were also higher. Postprandial hyperlipidemia is a major risk for the development of atherosclerosis, which has been shown to be associated with IR[33]. High min-120-OGTT insulin levels in the study group might further increase the incidence of related complications in the follow-up. In the studies above, OGTT was not performed, thus min-120-OGGT insulin levels were not determined. Mean plasma glucose levels were similar in insulin resistant and non-resistant groups according to HOMA-IR value, in agreement with the previous studies[4,5,34]. However, in the insulin resistant group, according to G/I ratio, min-120-OGTT plasma glucose level was significantly higher. To the best of our knowledge, there is no study showing any effect on plasma glucose in insulin resistant girls with premature pubarche.

We did not find a significant difference between the ACTH-stimulated androgen levels of the girls with high and low IR. But we did not study 17-OH-pregnenolone and free testosterone levels, which were higher in the reduced insulin sensitivity groups in the previous studies[4,5]. Also, contrary to the above studies, there was no difference in the BMIs between the high and low IR groups, which suggested that the IR was not related to the body mass. Although in the previous studies, adrenal androgens and high BMI were associated with IR, such an association was not found in this study. The underlying mechanism in IR is very complex, including the polygenetic nature of the syndrome[35]. In this study, adrenal androgens, which we could not study, and genetic or other undefined factors might have played a role in the occurrence of IR.

Even at diagnosis, IR is increased in a significant number of girls with premature pubarche. IR should be investigated in girls with premature pubarche. Indirect insulin sensitivity parameters seem to be useful tools for evaluating glucose metabolism, and choosing the candidates for OGTT. Assessment of patients at risk for IR may permit initiation of preventive measures as well as therapy.

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