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The Effect of Metabolic and Hormonal Parameters on Microalbuminuria in Adolescents with Type 1 Diabetes Mellitus

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SUMMARY

Introduction The prevalence of microalbuminuria (MA), the most important early marker of incipient nephropathy in patients with type 1 diabetes mellitus (T1DM), increases during puberty, the period of exaggerated physiological insulin resistance.

Objective To assess the prevalence of MA and the relationship between MA and metabolic risk factors and pubertal hormones in adolescents with T1DM.

Methods In a cross-section study involving a group of 100 adolescents of both sexes of mean age 14.90±2.18 years and with mean duration of T1DM 5.99±3.64 years, we assessed the presence of MA. In all patients, we determined albumin-to-creatinine ratio (ACR) in two or three morning first-void urine samples in the period up to 6 months. Persistent MA was confirmed in the patients with the finding of ACR rating 2.5-25 mg/mmol in males and 3.5-25 mg/mmol in females in two out of three first morning urine samples.

Results MA developed in 16 (16.0%) patients. Predictors of MA determined by using multiple logistic regression were high HbA1c (OR 4.6; 95% CI 2.1-10.0), higher night-time SBP (OR 1.9; 95% CI 0.8-1.3) and higher insulin dose (OR 62.6; 95% CI 2.3-1678.5). Markers of insulin resistance such as higher body mass index (BMI) which was statistically significantly related to MA ($\rho = 0.241$, $p<0.05$) and higher dehydroepiandrosterone sulfate (DHEA-S) which was significantly higher in patients with MA (7.82 μmol/L vs. 5.02 μmol/L, $p<0.01$), were also identified as predictors but did not remain significant by multivariate analysis, possibly because of a small sample of subjects with persistent MA.

Conclusion In addition to poor glycemic control and higher night-time systolic blood pressure, markers of insulin resistance (higher insulin dose, higher BMI and higher DHEA-S) contribute to the increased risk of MA.

Keywords: microalbuminuria; type 1 diabetes mellitus; adolescents

INTRODUCTION

Microalbuminuria (MA) is the earliest stage of clinical nephropathy, and its persistence predicts the progression to overt nephropathy and cardiovascular disease [1, 2]. In adult inception cohorts, the cumulative incidence of MA is around 30% after 20 years of type 1 diabetes mellitus (T1DM) [3].

In people with childhood-onset T1DM, MA is often detected during puberty and its prevalence has been reported to be between 4 and 20% [4, 5]. Risk of MA is only partially related to increases in glycated hemoglobin (HbA1c) during puberty, and puberty itself is an independent risk factor. This supports the concept that endocrine changes of puberty (abnormalities of the growth hormone/insulin-like-growth factor (IGF)-I axis, hyperandrogenism) can lead to early initiation or acceleration of diabetic kidney damage [5, 6].

OBJECTIVE

The aim of our cross-section study was to assess the prevalence of MA and its connection between metabolic risk factors and pubertal hormones in adolescents with T1DM.

METHODS

Using a cross-section study involving 100 adolescents (49 female and 51 male) of mean age 14.90±2.18 (11.0-19.4) years with T1DM, with mean disease duration of 5.99±3.64 (2.0-18.0) years, we evaluated the presence of MA. The patients were treated at the University Children's Hospital of Belgrade, Serbia. All the patients were selected based on the following criteria: the duration of T1DM of over two years, patients aged over 11 years with preserved global renal function and who were not on angiotensin-converting enzyme inhibitors renoprotective therapy. They were classified into two groups according to the presence of MA: microalbuminuric groups and normoalbuminuric groups. Adolescents were seen four times a year or more if required and assessed at the end of the year. Assessments consisted of measurements of HbA1c, body mass index (BMI), total cholesterol, triglycerides and calculations of insulin doses during the first visit to the hospital, updated at each following visit.

We also assessed patients' Tanner stage of puberty and their hormonal parameters during the first visit. IGF-I, DHEA-S and sex hormone binding globulin (SHBG) were measured using

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a solid-phase chemiluminescent immunometric assay on a gamma scintillation counter. Testosterone was measured by microparticle enzyme immunoassay using an Axsym system Abbot apparatus.

Albumin-to-creatinine ratio (ACR) in three morning first-void urine samples detected MA in the period below six months. MA was defined as an ACR ≥2.5 mg/mmol in male subjects and ≥3.5 mg/mmol in female subjects in two of three first morning urine samples. Albumin was measured by a particle-enhanced turbidimetric inhibition immunoassay method with Dimension RxL Max. Creatinine was measured using Jaffe method on a Dimension RxL Max.

Ambulatory blood pressure monitoring using auscultation method of Schiller BR 102 plus was performed after obtaining the second or third morning urine samples to assess MA.

Statistical analysis

Data are presented as the mean ± SD. When analyzing difference in variables between the groups, the Student's *t*-test or a χ^2 test was used. For variables not normally distributed, Mann-Whitney *U*- nonparametric test was used. Using Spearman correlation coefficient (ρ), correlation between the total cholesterol and ACR and between triglycerides and ACR, were evaluated. All variables significant in univariate analysis were used in multivariate logistic regression. A p value <0.05 was considered significant. SPSS version 12.0 was used for analyses. The study was approved by the local Ethical Committee.

RESULTS

Anthropometric, metabolic and hormonal parameters were assessed across two groups: microalbuminuric and normoalbuminuric groups (Table 1). Sixteen patients (16.0%) met the criteria for MA (6 female and 10 male subjects), with mean ACR 9.6±6.7 mg/mmol. All of the adolescents with MA were either in puberty Tanner stage II (at entry), III and IV (at exit) (4, 25%) or completed pubertal development (12, 75%).

Patients with MA had longer diabetes duration than patients with normoalbuminuria (7.7±3.5 vs. 5.7±3.6 years, respectively; p=0.040), their HbA1c was higher (11.0±2.0 vs. 8.2±1.0 %, p=0.000) and their insulin dose was higher (1.3±0.3 vs. 1.0±0.3 units/kg/day, p=0.001) but did not differ with regard to ages (15.2±2.0 vs. 14.8±2.2, p=0.551) or BMI (21.4±3.4 vs. 21.7±3.7 kg/m^2, p=0.751).

Total cholesterol (5.5±1.4 vs. 4.4±1.1 mmol/l, p=0.001) and triglycerides (1.8±0.9 vs. 1.1±1.2 mmol/l, p=0.000) were significantly higher in patients with MA than in normoalbuminuric patients. Also total cholesterol and triglycerides were statistically significantly related to ACR (total cholesterol: ρ=0.218, p=0.020, triglycerides: ρ=0.356, p=0.001).

We observed a significantly higher mean values of night-time systolic blood pressure (SBP) (110.7±8.6 vs. 103.5±11.6 mmHg, p=0.020) and night-time diastolic blood pressure (DBP) (64.6±5.1 vs. 57.8±9.3 mmHg, p=0.006) in patients with MA in comparison to normoalbuminuric patients. There was no statistically significant difference with respect to all other blood pressure values.

Table 1. Anthropometric, metabolic and hormonal parameters of patients with and without microalbuminuria (X±SD)

Characteristics		Normoalbuminuric patients (n=84)	Microalbuminuric patients (n=16)	p
Gender (male) (%)		41/84 (80.4)	10/16 (19.6)	NS
Pubertal Tanner stage I/II-IV/V (%)		4.8/27.4/67.8	0/25/75	NS
Ages (years)		14.8±2.2	15.2±2.0	NS
Diabetes duration (years)		5.7±3.6	7.7±3.5	0.04
ACR (mg/mmol)		1.4±1.0	9.6±6.7	<0.001
HbA1c (%)		8.2±1.0	11.0±2.0	<0.001
Insulin dose (units/kg/day)		1.0±0.3	1.3±0.3	0.001
BMI (kg/m^2)		21.7±3.7	21.4±3.4	NS
Total cholesterol (mmol/l)		4.4±1.1	5.5±1.4	0.001
Triglycerides (mmol/l)		1.1±1.2	1.8±0.9	<0.001
SBP day-time (mmHg)		115.1±10.3	118.0±9.3	NS
DBP day-time (mmHg)		68.6±11.3	69.8±3.2	NS
SBP night-time (mmHg)		103.5±11.6	110.7±8.6	0.02
DBP night-time (mmHg)		57.8±9.3	64.6±5.1	0.006
IGF-I (ng/ml)	Male	307.6±136.7	301.1±163.6	NS
	Female	326.8±144.4	231.8±91.9	NS
Testosterone (ng/ml)	Male	5.3±3.2	6.4±3.3	NS
	Female	0.5±0.4	0.5±0.1	NS
SHBG (nmol/l)	Male	80.9±132.7	48.4±37.2	NS
	Female	57.8±34.5	53.9±28.9	NS
DHEA-S (μmol/l)	Male	4.64±2.5	7.9±3.8	0.008
	Female	5.38±4.9	7.7±4.5	NS

X – mean value; SD – standard deviation; NS – non significant; ACR – albumin-creatinine ratio; HbA1c – glycated hemoglobin; BMI – Body Mass Index; SBP – systolic blood pressure; DBP – diastolic blood pressure; IGF-I – insulin-like-growth factor I; SHBG – sex hormone binding globulin; DHEA-S – dehydroepiandrosterone sulfate

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Table 2. Univariate logistic regression for microalbuminuria

Characteristics	p	OR	95% CI
Gender	0.319	1.75	0.58–5.24
Ages (years)	0.547	1.08	0.84–1.38
Puberty	0.610	1.27	0.50–3.20
Diabetes duration (years)	0.045	1.14	1.00–1.31
HbA1c (%)	0.000**	3.87	2.11–7.10
BMI (kg/m^2)	0.748	0.98	0.84–1.13
Total cholesterol (mmol/L)	0.004**	1.95	1.24–3.05
Triglycerides (mmol/L)	0.116	1.46	0.91–2.34
SBP day-time (mmHg)	0.300	1.03	0.98–1.08
SBP night-time (mmHg)	0.023*	1.06	1.01–1.12
DBP day-time (mmHg)	0.684	1.01	0.96–1.06
DBP night-time (mmHg)	0.012*	11.97	1.01–1.14
Insulin dose (units/kg/day)	0.003**	17.79	2.63–120.61
IGF1 (ng/ml)	0.390	1.00	0.99–1.00
Testosterone (ng/ml)	0.167	1.11	0.96–1.28
DHEA-S (μmol/L)	0.041*	1.15	1.01–1.32

* p<0.05; ** p<0.01
OR – odds ratio; CI – confidence interval

Table 3. Multivariate logistic regression for microlabuminuria

Characteristics	p	OR	95% CI
HbA1c (%)	0.000**	4.60	2.11–10.00
SBP night-time (mmHg)	0.034*	1.08	0.84–1.3
Insulin dose (units/kg/day)	0.014*	62.62	2.34–1678.48

DHEA-S was significantly higher in patients with MA (7.8±4.0 vs. 5.0±3.9 μmol/l, p=0.003), and DHEA-S was significantly higher in males with MA (7.9±3.8 vs. 4.6±2.5 μmol/l, p=0.008) but not females (7.7±4.5 vs. 5.4±4.9μmol/l, p=0.123). There were no significant difference in serum IGF-I, testosterone and SHBG between the two groups overall and when divided into sexes.

On univariate analysis, the variables significant for developing MA were: higher HbA1c (p=0.000), higher total cholesterol (p=0.004), higher night-time SBP (p=0.023), higher night-time DBP (p=0.012), higher insulin dose (p=0.003) and higher DHEA-S (p=0.041) (Table 2). Using multivariate logistic regression the variables which remained associated with microalbuminuria were: higher HbA1c (odds ratio – OR 4.6;95% confidence interval – 95% CI 2.1-10.0; p=0.000), higher night-time SBP (OR 1.9; 95% CI 0.8-1.3; p=0.034) and higher insulin dose (OR 62.6; 95% CI 2.3-1678.5; p=0.014) (Table 3).

DISCUSSION

In this study, the prevalence of MA of 16% was similar to prevalence rates of 8-18% that have been reported in cross-sectional studies [6, 7]. In our cross-sectional analysis of those with and without MA, subjects with MA presented significant differences: longer diabetes duration, higher HbA1c, higher insulin dose, higher total cholesterol and triglycerides, higher night-time SBP and DBP and higher DHEA-S.

Nowadays, it is clear that pre-pubertal duration of T1DM and glycemic control have a significant effect, even though this may only become evident at puberty [6, 7]. Children with an early onset of diabetes show a silent period, followed by an acceleration of albumin excretion during puberty, whereas the rate of development of MA in those diagnosed during puberty is relatively constant [8]. Our adolescents with MA had longer diabetes duration (7.7±3.5 vs. 5.7±3.6 years) and poorer glycemic control (HbA1c: 11.0±2.0 vs. 8.2±1.0 %) than normoalbuminuric adolescents. Poor glycemic control is a common finding among adolescents with T1DM, and it is closely linked to the development of MA and progression to macroalbuminuria.

Dyslipidemia is prevalent in children and adolescents with diabetes. Recently, it was shown that 34.4% of subjects with T1DM had elevated total cholesterol levels, 25% had elevated low-density lipoprotein (LDL) cholesterol and 15.6% had elevated triglycerides [9]. In our report, microalbuminuric adolescents had significantly higher total cholesterol (5.5±1.4 vs. 4.4±1.1 mmol/l) and triglycerides (1.8±0.9 vs. 1.1±1.2 mmol/l) than normoalbuminuric adolescents. Also, these parameters were significantly related to ACR (total cholesterol: ρ=0.218, p=0.020, triglycerides: ρ=0.356, p=0.001) and evidence that increased total cholesterol and triglycerides may be a surrogate marker for MA in adolescents with T1DM. There are few longitudinal studies looking the relationship with renal disease [10, 11].

Elevated blood pressure has been detected in adolescents with T1DM, and it could also be associated with the risk of developing MA. Our microalbuminuric adolescents differed from the normoalbuminuric subjects in having higher night-time SBP (110.7±8.6 vs. 103.5±11.6 mmHg) and higher night-time DBP (64.6±5.1 vs. 57.8±9.3 mmHg). An early increase in night-time BP may have a key role in the development of diabetic nephropathy. For instance, systemic pressure overload, initially restricted to systolic pressure during sleep, when transmitted to the glomerular circulation, may cause intrarenal hemodynamic changes, leading to MA, structural renal damage, or both [12, 13]. A preliminary report from the International Diabetic Nephropathy Study Group [14] indicates that early renal morphometric abnormalities in patients with diabetes are associated with increased night-time blood pressure (in the normal range) but not HbA1c.

Puberty is associated with a decrease in insulin sensitivity, and adolescents with T1DM are more insulin resistant when compared with healthy controls [1, 15]. Pubertal onset is associated with acceleration in the urine albumin excretion rate compared with years during prepuberty, and this may be predictive of MA. Perturbations in the GH-IGF-I axis and changes in sex steroid levels have been related to an increased risk of developing MA [1]. Our microalbuminuric patients had lower IGF-I (but not significantly lower) than normoalbuminuric patients. In T1DM, relative portal insulinopenia (caused by failure to administer insulin directly into the portal vein) results in impaired hepatic generation of IGF-I, leading to a lack of negative feedback drive for GH hyper secretion, over and above that seen in normal puberty. Thus, circulating GH levels are increased while circulating IGF-I levels remain low. The integrity of GH pathways in tissues other than the liver are thought to remain intact, and both GH

hypersecretion and local paracrine IGF-I production have been implicated in the path physiology of diabetic nephropathy [16, 17]. The significantly higher DHEA-S levels found in our adolescents with MA when compared with matched controls without MA could also contribute to renal disease, as suggested by experimental studies where sex steroids have been implicated in the pathogenesis of diabetic kidney disease [17, 18]. These data suggest that the development of diabetic microvascular complications may be associated with abnormalities in hormonal variables related to pubertal development: abnormalities of the GH-IGF-I axis, hyperandrogenism and low SHBG (reflecting enhanced tissue effect of androgenes) [4, 19]. Our findings did not show any significant differences in serum testosterone and SHBG levels between the two groups when stratified for sex except DHEA-S which was significantly higher in males but not in females with MA.

In our study the risk factors identified for persistent MA were poorer glycemic control, higher night-time SBP and one of the markers of insulin resistance: higher insulin dose. Other markers of insulin resistance, including hypercholesterolemia and higher DHEA-S were also identified as predictors but did not remain significant in multivariate analysis, possibly because of our low rate of persistent microalbuminuria.

CONCLUSION

In summary, the development of microalbuminuria at puberty may reflect not only poor glycemic control but also changes in the GH-IGF-I axis and hyperandrogenism. Changes in pubertal hormonal variables differ in those MA and these differences may relate to disease progression.

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Утицај метаболичких и хормонских параметара на микроалбуминурију код адолесцената са дијабетес мелитусом тип 1

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КРАТАК САДРЖАЈ

Увод Преваленција микроалбуминурије (МА), најзначајнијег предиктора дијабетичке нефропатије код особа са дијабетес мелитусом тип 1 (ДМ1), повећава се у пубертету, периоду појачане резистенције на инсулин.

Циљ рада Циљ рада је био да се утврде преваленција МА и повезаност МА с метаболичким и хормонским параметрима током пубертета код адолесцената са ДМ1.

Методе рада Студија пресека је обухватила 100 адолесцената оба пола, просечног узраста од 14,90±2,18 година и с просечним трајањем ДМ1 од 5,99±3,64 година, код којих је испитивано постојање МА. Свим испитаницима је одређиван однос албумина и креатинина у два-три узорка прве јутарње мокраће сакупљене у периоду до шест месеци. Испитаници чије су вредности односа албумина и креатинина биле 2,5–25 *mg/mmol* (мушки пол), односно 3,5–25 *mg/mmol* (женски пол), у два-три узорка мокраће означени су да имају перзистентну МА.

Резултати МА се развила код 16 испитаника (16,0%). У моделу мултиваријантне логистичке регресије као независни предиктори МА издвојене су следеће варијабле: повећан ниво *HbA1c* (*RR*=4,6; *95%CI*=2,1–10,0), повећан ноћни систолни крвни притисак (*RR*=1,9; *95%CI*=0,8–1,3) и већа доза инсулина (*RR*=62,6; *95%CI*=2,3–1678,5). Параметри инсулинске резистенције, као што су повећан индекс телесне масе (ИТМ), који је статистички значајно повезан са МА (ρ=0,241; $p<0,05$), и повећан ниво дехидроепиандростерон-сулфата (ДХЕАС), који је био статистички значајно већи код испитаника са МА (7,82 *μmol/l* prema 5,02 *μmol/l*; $p<0,01$), такође су означени као предиктори МА, али нису остали статистички значајни у моделу мултиваријантне логистичке регресије вероватно због малог узорка испитаника са МА.

Закључак Лоша метаболичка контрола и повећан ноћни систолни крвни притисак заједно с параметрима инсулинске резистенције (веће дозе инсулина, повећан ИТМ и повећан ДХЕАС) доприносе повећању ризика за настанак МА.

Кључне речи: микроалбуминурија; дијабетес мелитус тип 1; адолесценти

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