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ORIGINAL ARTICLE

Microalbuminuria in Children with Type 1 Diabetes Mellitus: 10-Year Data Analysis in Amirkola Children Hospital, Babol, Iran

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ABSTRACT

Background: Given the global rising incidence of type 1 diabetes (T1DM), this research was conducted to assess the demographic and laboratory findings of children with T1DM, based on the status of microalbuminuria.

Methods: This cross-sectional study compared the demographic and laboratory characteristics of all children under the age of 18 years who were diagnosed with T1DM at least for two years and have been referred to the referral Children's Hospital in Babol, Iran. Age, sex, duration of diabetes, body mass index (BMI) and average of three measures of hemoglobin A1c were compared in two groups with and without microalbuminuria. Urinary albumin excretion of 30 to 300 mg/24 hours was considered as microalbuminuria.

Results: In the current study of 97 patients, 75 (77.3%) had microalbuminuria; while 22 (22.7%) did not experience microalbuminuria. There was no statistical difference in sex, age, BMI, duration and HbA1c distribution between patients with microalbuminuria and without microalbuminuria. In the univariate model, odds of microalbuminuria increase by 2.6 percent by each unit increased in HbA1c; however, this association was not statistically significant (odds ratio (OR): 1.026, confidence interval (CI) 95%: 0.779-1.325). No statistically significant association was also found between microalbuminuria status and sex, age, BMI, or duration.

Conclusion: Although the age, sex, duration of diabetes, BMI and hemoglobin A1c did not have statistically significant difference between the two groups, regular and periodic monitoring of glycemic control status and screening of protein excretion in urine, in order to identify early diabetic nephropathy is recommended in children with T1DM.

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Introduction

Diabetes is one of the most common chronic disorders in population ≤18 years old (1, 2). Unlike adults, where type 2 diabetes is more prevalent, type 1 diabetes mellitus (T1DM) is the most common form of this disease in children and adolescents, and two-thirds and even more proportion of disease in this age-group is from this type (3, 4). The worldwide epidemiology of T1DM represents a significant increase in the incidence of T1DM in children, its annual incidence is estimated to be 1 in every 1,000 children; and 1.5 million deaths occur due to short- and long-term complications of diabetes in the world, every year (5).

In a study in Iranian population, the annual incidence of T1DM for entire population, the population of girls and boys, was reported as 6.7%, 6.9% and 6.3%, respectively (3). In the study in Iran, increasing in age-standardized incidence and the prevalence rate of T1DM were noticed; while the mortality rate due to this disease has decreased, and the number of years of life lost due to disability has remained unchanged during the past 30 years in Iran (6). One of important microvascular complications of T1DM is diabetic nephropathy. The prevention and prompt treatment of diabetic nephropathy is highly emphasized because of its related morbidity and mortality (7-9). Microalbuminuria is usually the first clinical sign of kidney dysfunction in patients with diabetes, occurs due to vascular complications and endothelial damage in this population (10-12).

Several mechanisms have been introduced for occurrence of microalbuminuria including an increase in glomerular filtration rate (GFR), a damage in glomerular basal membrane and the mesangial proliferation, which promotes abnormal amounts of albumin to be passed from the pores of this membrane, and to be excreted through urine (13, 14). Microalbuminuria refers to a condition when the concentration of albumin is in the range of 30-300 mg in 24-hour urine (15, 16). The prevalence of microalbuminuria in children with T1DM differs based on the years passed since the onset of diabetes; its occurrence after 5 to 10 years following disease diagnosis that has been reported as 12-25% (17). Some previous studies reported the incidence of microalbuminuria even after 2 years of diabetes diagnosis in children (17, 18). In Iran, although some studies investigated the factors affecting on microalbuminuria in the adult population with diabetes (19); however, the studies examined the factors influencing on microalbuminuria in children with diabetes are very limited (20, 21). This research was conducted to compare the demographic and laboratory features of children with T1DM with and without microalbuminuria in north region of Iran.

Materials and Methods

This cross-sectional study was carried out during the last 10 years to compare the demographic and laboratory findings of children under the age of 18 who had been diagnosed with T1DM and were referred to Children's Hospital in Babol, northern Iran,. The research population was consisted of children under 18 years of age who were at least 2 years with T1DM. The diagnosis of T1DM was confirmed according to the standard diagnostic criteria of the American Diabetes Association (22). The minimum required sample size for this study was estimated with a type I error rate of 0.05 and a power of 80%. A census sampling method was employed, and all eligible individuals were included in the study. The findings of the children were compared in two groups of case (with microalbuminuria) and control (without microalbuminuria). Inclusion criteria were being age of ≤ 18; 2, pssing of at least 2 years since diagnosis with T1DM and absence of any previous history of kidney disease that led to proteinuria (such as urinary tract infections, glomerulonephritis, and nephrotic syndrome). Exclusion criteria were incompleteness of hospital information on patient' files and patients whose 24-hour urine collection was not done correctly.

All the laboratory examinations in this research were necessary for periodic monitoring and control of diabetes in patients, and no additional blood sampling was performed. The research protocol was approved by the Ethics Committee of Babol University of Medical Sciences, Iran with approval of IR.MUBABOL.REC.1401.236. Baseline characteristics including age, gender, and the time of T1DM diagnosis were collected with direct interview. T1DM diagnosis was confirmed by a pediatric endocrinologist taking into account the standard diagnostic criteria of diabetes; and presence of one of the criteria of having HbA1c equal to or greater than 6.5% with symptoms of hyperglycemia; fasting plasma glucose equal to or more than 126 mg/dL; two-hour plasma glucose equal to or more than 200 mg/dL (after eating 75 grams of glucose dissolved in water); and presence of hyperglycemic symptoms (polyuria, polydipsia and weight loss) along with glucose equal to or more than 200 mg/dL in a random blood sample at any time of the day (22).

All children underwent physical examination by a pediatric specialist. The children's weight was measured with a Balas digital scale manufactured in Iran (with an accuracy of 5 grams) and their height was measured with a caliper (with an accuracy of 1 mm). Body mass index was calculated using

the weight (kilograms) divided by the square of height (in meters). HbA1c was assessed with the Diazyme laboratory kit made in the United States by taking 2 mL of blood sample and using an enzymatic method in the laboratory. All laboratory measurements were conducted at a certain time (8 am). The average of three times HbA1c was considered for data analysis.

The main outcome of the study was microalbuminuria. In order to diagnose microalbuminuria, a 24-hour urine sample of the child was taken on two occasions with an interval of three months to determine the amount of urinary albumin. The amount of urinary albumin 30-300 mg per 24 hours was considered as microalbuminuria. The 24-hour urine sample was tested without any additives or preservatives. The sample was kept at a temperature of 2-8°C (refrigerator), and microalbuminuria was examined by immunoturbidimetry with a Hitachi autoanalyzer and using the Audit or Iranian Pars Azmoon kits. All children were on basal bolus treatment protocol and were taken glargine and aspart insulins. In order to achieve the research objectives, the minimum sample size was calculated as at least 25 samples in the case group and 75 patients in the control group.

The collected data were analyzed using SPSS software (Version 22, Chicago, IL, USA). Independent-samples t-test and Chi-square test were used for quantitative and qualitative data analysis. The Kolmogorov-Smirnov test was used to determine the normal distribution of data (such as age, BMI, duration and HbA1c). Logistic and linear regression was used to determine the association of different variables with microalbuminuria in two univariate and multivariate analysis. The multivariate model was constructed using a hybrid approach. Initially, the key variable, HbA1c, was entered using the "Enter" method. Subsequently, the remaining independent variables (sex, age, BMI, and duration of diabetes) were evaluated using "Backward Elimination". A *p* value less than or equal to 0.05 was considered statistically significant.

Results

In the current study of 97 patients, 75 (77.3%) had microalbuminuria, while 22 (22.7%) did not. There was no statistical difference in relation to sex, age, BMI, duration and HbA1c distribution between patients with microalbuminuria and without microalbuminuria ($p>0.05$) (Table 1). Univariate and multivariate logistic regression for association between microalbuminuria status and clinical and demographic characteristics were shown in Table 2. In the univariate model, odds of microalbuminuria increased by 2.6 percent by each

Table 1: Baseline clinical and demographic characteristics of patients regarding microalbuminuria status.

Variable		Without microalbuminuria	With microalbuminuria	*P* value
Sex, %[1]	Male	31 (41.30%)	12 (54.50%)	0.273
	Female	44 (58.70%)	10 (45.50%)	
Age (year)[2]		12.24±2.81	12.25±2.70	0.988
BMI (kg/m^2)[2]		19.43±3.61	19.03±3.21	0.638
Duration (day)[2]		3.22±2.20	4.08±3.22	0.251
HbA1c (%)[1]		9.28±1.78	9.35±1.57	0.857

BMI: Body mass index; kg: Kilogram; m: Meter; HbA1c: Hemoglobin A1c. [1]Using the Chi-square test for categorical variables and the values were presented as frequency (percentage). [2]Using independent samples T-test for parametric variables and the values were presented as mean±SD.

Table 2: Univariate and multivariate logistic regression regarding association of microalbuminuria status with clinical and demographic characteristics.

Variable	OR	*P* value	95% CI for OR		OR	*P* value	95% CI for OR	
			Lower	Upper			Lower	Upper
HbA1c	1.026	0.855	0.779	1.352	1.026	0.855	0.779	1.352
Sex (Ref: Male)	0.587	0.275	0.226	1.529	-	-	-	-
Age (year)	1.001	0.988	0.843	1.190	-	-	-	-
BMI (kg/m^2)	0.966	0.634	0.838	1.114	-	-	-	-
Duration (day)	1.142	0.158	0.950	1.374	-	-	-	-

HbA1c: Hemoglobin A1c; BMI: Body mass index; kg: Kilogram; m: Meter; OR: Odds ratio; CI: Confidence interval. These values were obtained from logistic regression. The final model was constructed using a hybrid approach. Initially, the key variable, HbA1c, was entered using the "Enter" method. Subsequently, the remaining independent variables (sex, age, BMI, and duration of diabetes) were evaluated using "Backward Elimination".

unit increase in HbA1c; however, this association was not statistically significant [odds ratio (OR): 1.026, confidence interval (CI) 95%: 0.779-1.325, p=0.855). No statistically significant association was found between microalbuminuria status and sex, age, BMI, or duration. In the multivariate model, following the forced entry of HbA1c, no other variables met the criteria for entry.

Univariate and multivariate linear regression for association between microalbuminuria status with clinical and demographic characteristics was shown in Table 3. In the univariate model, microalbuminuria increased by 0.900 by each unit increase in HbA1c; however, this association was not statistically significant (β: 0.900, CI 95%: -2.077 to 3.877, p=0.550). No statistically significant association was found between microalbuminuria and sex, age, BMI, or duration. In the multivariate model, following the forced entry of HbA1c, no other variables met the criteria for entry. Also, the scatter plot of microalbuminuria and HbA1c and duration were shown in Figure 1 and 2.

Discussion

This study was carried out to compare demographic and laboratory findings of children diagnosed with T1DM, with and without microalbuminuria. Comparing the average HbA1c between the two study groups, the average HbA1c in the groups with and without microalbuminuria was 9.35% and 9.28%, respectively. Contrary to our findings, in Yang *et al.*'s study in Korea, HbA1c (hazard ratio (HR): 1.27, p=0.005) and diastolic blood pressure (HR: 1.06, p<0.001) had a statistically significant association with the occurrence of diabetic nephropathy (23). In the study of Bhattarai *et al.* in Nepal, the average level of hemoglobin A1c in people who had microalbuminuria was 7.67, which is lower than that of the current study (24). The standard care guideline for diabetes in children and adolescents, the level of HbA1c below 8.5% (69 mmol/mol) was recommended as a criterion for stable diabetes (25). Considering this guideline, in both groups with and without microalbuminuria of our research, the glycemic control of patients needed to be improved.

Table 3: Univariate and multivariate linear regression regarding association of microalbuminuria status with clinical and demographic characteristics.

Variable	β	*P* value	95% CI for β		β	*P* value	95% CI for β	
			Lower	Upper			Lower	Upper
HbA1c	0.900	0.550	-2.077	3.877	0.900	0.550	0.900	0.550
Sex (Ref: Male)	-3.287	0.528	-13.581	7.008	-	-	-3.287	0.528
Age (year)	-0.534	0.569	-2.391	1.323	-	-	-0.534	0.569
BMI (kg/m^2)	-0.465	0.530	-1.930	1.000	-	-	-0.465	0.530
Duration (day)	-0.228	0.828	-2.308	1.853	-	-	-0.228	0.828

Abbreviation: HbA1c: Hemoglobin A1c; BMI: Body mass index; kg: Kilogram; m: Meter; β: Beta; CI: Confidence interval. These values were obtained from linear regression. The final model was constructed using a hybrid approach. Initially, the key variable, HbA1c, was entered using the "Enter" method. Subsequently, the remaining independent variables (sex, age, BMI, and duration of diabetes) were evaluated using "Backward Elimination".

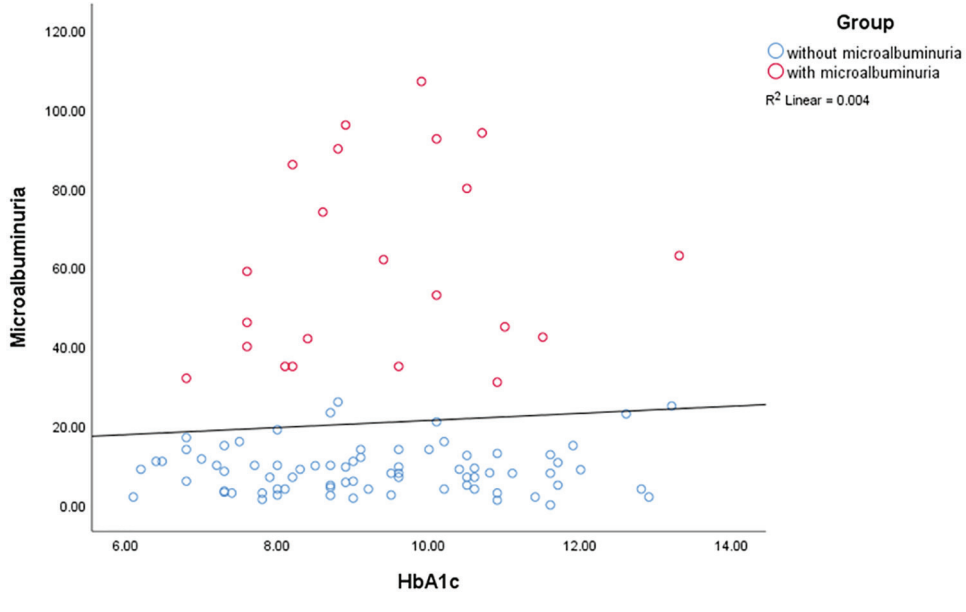

Figure 1: Scatter plot of microalbuminuria and HbA1c.

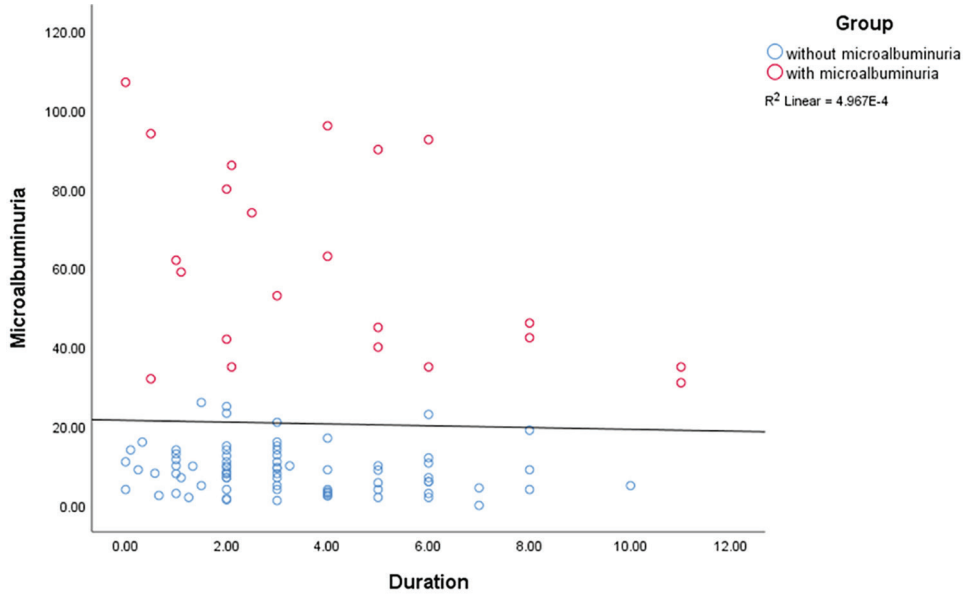

Figure 2: Scatter plot of microalbuminuria and duration.

Comparing the average age of children with T1DM with and without microalbuminuria, our finding showed that mean age in the group with microalbuminuria was 12.25 years and in the group without microalbuminuria was 12.24 years; and these two groups did not show a significant difference from a statistical point of view. Similar to our study, in the study by Mohammed *et al.* in Iraq, the age of patients with T1DM did not have a significant association with microalbuminuria (26). Contrary to our finding, in the study of Zabeen *et al.* in Bangladesh, age had a significant association with microalbuminuria (17). Given the study population which was selected from different age groups of children and adults, it was difficult to compare the findings of various studies.

The finding of the present study showed not statistically significant association between microalbuminuria and BMI. In Mohammed *et al.*'s study in Iraq, similar to our study, the body mass profile of patients with T1DM did not have a significant association with microalbuminuria (26). In Nguyen *et al.*'s study, it was pointed out that the impact of weight gain on kidney function in patients with diabetes was influenced by other covariates such as raised blood pressure and fasting blood glucose. In such a way, the high BMI did not show a significant effect on estimated amount of GFR and microalbuminuria after adjusting the covariates of hypertension and fasting blood sugar (27).

Obesity and being overweight can interfere with the optimal glycemic control of patients with diabetes. Being overweight in patients with T1DM leads to an increase in the risk of some serious complications such as metabolic syndrome, cardiovascular or kidney diseases, and as a result, can increase the mortality, and reduce the life expectancy of these patients. In addition, higher BMI, and subsequent higher insulin resistance, might lead to a more complicated condition, which is called double diabetes (28). Our finding showed that the duration of diabetes in patients with microalbuminuria was longer than that of children without microalbuminuria, but this difference was not statistically significant. Duration of diabetes can be a significant predictor of diabetic nephropathy (29). Some evidence demonstrated that children with T1DM are at an increased risk of kidney involvement. Some proportion of this risk occurs at the beginning of T1DM; up to 65% of children with T1DM may suffer from acute kidney injury at initial phases of the disease, and this may lead to chronic kidney disease in the future (30).

The important limitation of this study is the limited number of patients with T1DM during a 10-year time point. In addition, some other risk factors of diabetic nephropathy, such as the medications the patients were taking, were not considered in the study design. Another limitation of the study was the inability to measure several additional biomarkers, such as interleukins, tumor necrosis factor-alpha, and neutrophil gelatinase-associated lipocalin (31); which are among the newer markers used in the screening of microalbuminuria.

Conclusion

Although the HbA1c, sex, age, BMI and duration of diabetes did not have statistically significant difference between the two groups with and without microalbuminuria, regular and periodic monitoring of glycemic control status and screening of protein excretion in urine, in order to identify early diabetic nephropathy is recommended in children with

T1DM. In this regard, it is suggested that future studies be conducted in a multicenter fashion, with a larger sample size, and incorporate prospective follow-up of patients in a cohort study design to evaluate the incidence of diabetic nephropathy over time in the pediatric population with T1DM.

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Authors' Contribution

Study concept and design were performed by S.S.M, M.P and M.A. Analysis and interpretation of data were performed by H.G.A. Drafting of the manuscript was performed by F.M, H.E and S.S.M.

Conflict of Interest

No competing interests are declared.

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