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The Correlation of Urine Retinol Binding Protein-4 and Serum HbA_{1c} with Glomerular Filtration Rate in Type 1 (Insulin-Dependent) Diabetic Children: A Perspective on the Duration of Diabetes

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Abstract

Objective: To analyze the correlation between urine retinol binding protein-4 (RBP-4) and serum HbA_{1c} with glomerular filtration rate (GFR) in type 1 diabetic children. Methods: This was a cross-sectional observational analytic study. The subjects were type 1 diabetic children aged 2 - 14 years. Sample collection was conducted from October to November 2014. Exclusion criteria were patients with obesity, renal insufficiency that was not caused by diabetes, history of hepatic diseases, and history of blood cell disorders. We performed anamnesis, physical examination, and blood sampling for serum HbA_{1c} and serum cystatin-C, and urine sampling for RBP-4 on all subjects. Glomerular filtration rate was calculated from the concentration level of cystatin-C using Filler formula. Data analysis was performed by Spearman test to determine the correlation between urine RBP-4 and serum HbA_{1c} with GFR. The Fisher's exact test was used to determine the correlation between duration of diabetes and RBP-4, HbA_{1c}, and also GFR. Results: Twelve females (60%) and 8 males (40%) participated in the study. The mean age of the subjects with 95% CI was: 10.5 (2 - 14) years while the mean age of duration diabetes with 95% CI: 3.8 (0.5 - 10) years. Twelve (60%) subjects had <5 years duration of diabetes, while eight (40%) subjects had ≥5 years duration of diabetes. Twelve (60%) subjects had normal RBP-4 level, while eight (40%) subjects had elevated RBP-4 level. The mean level of HbA_{1c} with 95% CI: 8.9 (5.1 - 15.2)%. Thirteen (65%) subjects had poor metabolic. The mean GFR of the subjects with 95% CI: 99.3 (35.2 - 147.4) mL/1.73/m². Nineteen (95%) subjects had normal GFR, while 1 (5%) had renal insufficiency. The results of data analysis using Spearman test on the correlation between urine RBP-4 and serum

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HbA_{1c} with GFR were not significant. The result of correlation between duration of diabetes and urine RBP-4 was significant using Fischer's test. Conclusion: The results showed no correlation between urine RBP-4 and serum HbA_{1c} with GFR. Urine RBP-4 could be considered to assess renal function in type 1 diabetic patients with a duration of diabetes of more than 5 years.

Keywords

RBP-4, HbA_{1c}, Glomerular Filtration Rate, Diabetes, Children

1. Introduction

Diabetes mellitus (DM) is a world health problem affecting all age groups. Diabetic nephropathy (DN) is a serious major microvascular diabetic complication in type1 diabetic patients [1]. About 30% - 40% of diabetic patients develop an end-stage renal disease and require either dialysis or renal transplantation [2]. Recent studies demonstrated that there was a tubular component in renal complications of diabetes as shown by the detection of renal tubular enzymes and low molecular weight (LMW) proteins (e.g. RBP-4) in the urine. In fact, tubular involvement may precede glomerular involvement in DN [3] [4]. Declined GFR often occurs in type 1 diabetic patients with poor metabolic control, which is characterized by elevated serum HbA_{1c} level [5]. Glomerular filtration rate by measuring the level of cystatin-C would be better compared to creatinine which is commom for measuring GFR [6].

Retinol binding protein-4 is an LMW protein that filtrated by glomerulus and then re-absorbed by proximal tubules. Elevated level of RBP-4 occurs when tubules function decreases and re-absorption function of proximal tubules does not run properly [7]. The progression of nephropathy in type 1 diabetes has classically been described as a series of stages in relentlessly deteriorating course from normal renal function to end stage renal disease marked by increasing amounts of albuminuria. At the time of initial diagnosing, there are no significant renal histologic abnormalities, renal plasma flow and GFR. Within 3 years of duration diabetes, minimize changes of the renal will be seen [8] [9]. But some studies reported that renal changes will be happened at least 5 years duration of diabetes and tubular involvement may precede glomerular involvement in ND [10]-[12]. This is evidenced by the increase preceded RBP-4 before going on condition of albuminuria [4]. Poor metabolic control causes early renal function decrease which is characterized by declined GFR [5]. Therefore, We decided to analyze the correlation between urine RBP-4 and serum HbA_{1c} with GFR in type 1 diabetic children.

2. Materials and Methods

Subjects were patients with type 1 diabetic children aged between 2 - 14 years who visited the pediatric endocrinology division of Dr. Hasan Sadikin General Hospital, a tertiary level university teaching hospital with total of 554,000 inpatients and outpatients per year in West Java, Indonesia. The exclusion criteria were patients with obesity, renal disease that was not caused by diabetes, history of hepatic diseases, and history of blood cell disorders. This was a cross-sectional observational analytic study. Sample collection was conducted from October to November 2014 by inviting these patients to participate in this study. This study was approved by the ethical committee of the Faculty of Medicine, Universitas Padjadjaran/Dr. Hasan Sadikin General Hospital Bandung, Indonesia.

Urine RBP-4 were collected after informed consent the patient's parents were obtained in accordance with the standard protocol.This assay employs the quantitative sandwich enzymes immunoassay technique [13]. Serum HbA_{1c} was collected after informed consent. This assay employs chromatography technique [14]. Serum cystatin-C was used as a marker for GFR. Serum cystatin-C employs the particle enhanced turbidimetric immunoassay (PETIA) [15] and then the GFR value was gained through Filler formula [16].

Data were presented as means, standard deviations, and medians with range. Statistical parameters were calculated using SPSS™ version 20.0. Due to unnormally distributed data, the nonparametric Shapiro-Wilk test was used to test the significant differences between the groups. Data analysis was performed using Spearman's rho correlation to determine the correlation between urine RBP-4 and serum HbA_{1c} with GFR. The Fisher's exact test was used to determine the correlation between the duration of diabetes and RBP-4, HbA_{1c}, and also GFR. p Values < 0.05 were considered as significant.

3. Results

The study population consisted of 20 pediatric patients with type 1 DM. Subjects were recruited from patients who visited the pediatric endocrinology division of Dr. Hasan Sadikin General Hospital in Bandung, Indonesia. The 20 subjects consisted of 12 females (60%) and 8 males (40%). The mean age of the subjects with 95% CI: 10.5 (2 - 14) years while the mean age of duration of diabetes with 95% CI: 3.8 (0.5 - 10) years. Twelve (60%) subjects had had diabetes for <5 years, while eight (40%) subjects had had diabetes for ≥5 years. Twelve (60%) subjects had normal RBP-4 (<100 ng/mL), while eight (40%) subjects had elevated RBP-4 level (all of them with a duration of diabetes of ≥5 years). The mean level of HbA_{1c} with 95% CI: 8.9 (5.1 - 15.2)%. Thirteen (65%) subjects had poor metabolic (HbA_{1c} > 8%). The mean GFR of the subjects with 95% CI: 99.3 (35.2 - 147.4) mL/1.73/m^2. Nineteen (95%) subjects had normal GFR, while 1 (5%) had renal insufficiency (GFR < 80 mL/1.73/m^2). The clinical characteristics of the subjects are presented in **Table 1**, while biochemical parameters of the subjects are presented in **Table 2**.

Table 1. Characteristics of the subjects (n = 20).

Characteristics	n	
Sex		
Male	8	
Female	12	
Age (years)		
Mean (SD)		10.5 (3.3)
Median		12
Range		2 - 14
Duration of Diabetes (years)		
<5 years	12	
≥5 years	8	
Mean (SD)		3.8 (2.6)
Median		4
Range		5 - 10

SD, standard deviation.

Table 2. Biochemical parameters of the subjects.

Variables	n = 20
RBP-4 (ng/mL)	
Mean (SD)	163.6 (238.6)
Median	58.3
Range	2.4 - 881.9
HbA_{1c} (%)	
Mean (SD)	8.9 (3)
Median	9.1
Range	5.1 - 15.2
Cystatin-C (mg/L)	
Mean(SD)	0.7 (0.4)
Median	0.6
Range	0.3 - 2.3
GFR (mL/1.73/m^2)	
Mean (SD)	99.3 (21.1)
Median	100.5
Range	35.2 - 147.4

RBP-4, retinol binding protein-4; HbA_{1c}, glicosated hemoglobin; GFR, glomerular filtration rate; SD, standard deviation.

The results of data analysis with Spearman test on the correlation between urine RBP-4 and serum HbA1c with GFR were not significant (**Table 3**). The result of correlation based on Fischer's test shows that the duration of diabetes was only significant with urine RBP-4 (**Table 4**).

The results showed that the correlation of RBP-4 and HbA1c with GFR had p => 0.25. Therefore, this result cannot be proceed to multiple regression analysis because it does not meet the criteria for this analysis.

4. Discussion

In our work we studied the excretion of LMW protein RBP-4. Hong and Chia stated that the detection of renal tubular proteins and enzymes may precede glomerular involvement as several of these tubular proteins and enzymes are detectable even before the appearance of microalbumunuria [1]. Within 5 years of the onset of albuminuria in DN, approximately half of the individuals will have experienced a 50% reduction in the GFR and doubling of their serum creatinine [9]. Jung *et al.* stated that the urinary excretion of renal tubular LMW were recommended as a useful marker for detection of minor changes in proximal tubules function long before the elevation of other markers like proteinuria and rised in serum creatinine [12]. This study has the strength because the assessment of GFR was performed by serum cystatin-C. Previous studies had shown that cystatin-C is more superior to measure GFR than creatinine [6]. Metabolic control in type 1 DM with measurement serum HbA_{1c} level has a high risk of complication of DN if elevated level of serum HbA_{1c} was found [17].

This study reported that the correlation between RBP-4 and HbA_{1c} was not significant. This result is similar to Holm *et al.*'s statement that the correlation between RBP-4 and HbA_{1c} was not significant [18]. However, this study reported that, in the average most of the subjects had poor metabolic control. However, Olsen *et al.* stated that poor metabolic control will take more than 5 years to cause changes in renal function in type 1 DM [19]. In type 1 diabetes, hyperglycemia starts in the first decades of life and is usually the only recognized cause of nephropathy. On the contrary, in type 2 diabetes hyperglycemia starts after the forties, usually when the kidneys have already suffered from the long-term consequences of ageing and of other recognized promoters of chronic renal injury, such as arterial hypertension, obesity, dyslipidemia, and smoking [20]. Tubular dysfunction appears to be correlated with the duration of type 1 diabetes. This study records that duration of diabetes ≥5 years is associated to the rise of RBP-4 (p =< 0.01) (**Figure 1**), but not to HbA_{1c} and GFR (p = 0.69 and p = 0.4). This is similar to recent studies that renal changes need more than 5 years duration of diabetes [10]-[12]. Tubular involvement may precede glomerular involvement in DN [3] [4]. This study showed that duration of diabetes ≥5 years began to elevate RBP-4 value but it did not followed by declined GFR value. Most of the subjects still had normal GFR value. Actually, it is consistent to natural history of diabetic nephropathy in type 1 which needs at least 5 years to make changes in renal structure [21] [22].

This study showed that the correlation between urine RBP-4 and GFR was not significant. This results is similar to Fathy *et al.*'s statement that the correlation of urine RBP-4 and serum creatinine was not significant (p =

Table 3. Correlation between urine RBP-4 and serum HbA1c with GFR.

	r_s	p^* value
Correlation urine RBP-4 with GFR	0.18	0.45
Correlation serum HbA_{1c} with GFR	−0.06	0.82

*Spearman's rho Test. RBP-4, retinol binding protein-4; HbA_{1c}, glicosated hemoglobin; GFR, Glomerular filtration rate; rs, correlation coefficient.

Table 4. Duration of diabetes with urine RBP-4.

Duration of Diabetes	RBP-4		Total	p^* value
	<100 ng/mL	≥100 ng/mL		
<5 years	12	0	12	
≥5 years	0	8	8	
Total	12	8	20	<0.01

*Fisher's Exact Test. RBP-4, retinol binding protein-4.

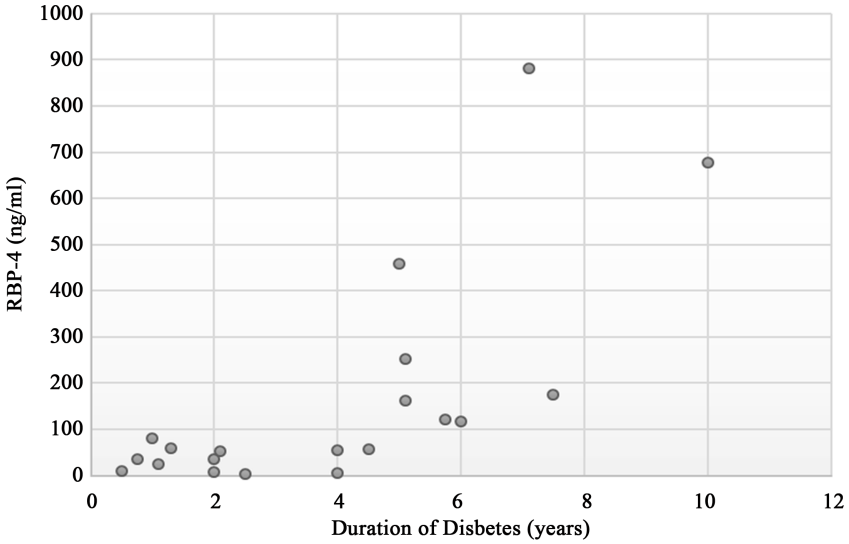

Figure 1. Urine concentrations of RBP-4 were significantly higher in type 1 diabetes children with duration of diabetes ≥5 years than <5 years (p =< 0.01). RBP-4, retinol binding protein-4.

0.32) [5]. This study, that used cystatin-C to measure GFR, showed that the correlation between urine RBP-4 and GFR was not significant (p = 0.45). The mean GFR of the subjects with 95% CI: 99.3 (35.2 - 147.4) mL/1.73/m^2. This result showed that most of the subjects has not declined GFR. This result is similar to recent studies demonstrated that, there is a tubular component in renal complications of diabetes as shown by the detection of renal tubular LMW proteins (RBP-4) in the urine. Tubular involvement may precede glomerular involvement in DN [3] [4]. This result showed there is a significant elevated RBP-4 in duration of diabetes ≥5 years than <5 years (p =< 0.01), but they do not have declined GFR. The early stage of DN is associated with greater than 18% - 26% increase in GFR. Hyperfiltration and the rise of glomerular filtration are believed to occur during the first five years of the disease [23]. At the time of initial diagnosis there are no significant renal histologic abnormalities, renal plasma flow, and GFR elevated. Within 3 - 5 years, histologic changes (increased measangial matrix and glomerular basement membrane thickening) of DN [8] [24]. In this study, according to Thomas *et al.* as normal reference for GFR aged 1 - 20 years is >80 ml/1.73/m^2 [25], GFR rises 24.1% of the baseline limit of the normal reference.

In our study, we could not find the correlation between urine RBP-4 and serum HbA_{1c} with GFR when using Spearman's rho test. The result of the correlation between duration of diabetes ≥5 years and increased urine RBP-4 was significant with Fischer's test. The weakness of this study is that duration of diabetes in most of subjects was less than 5 years and this study is not associated a series of stages in ND. We realized that our small sample size of this study could not be the benchmark to explain actual condition in general population. We suggested making further studies, to find out the correlation between RBP-4 and HbA_{1c} with GFR more than 5 years of diabetes duration.

5. Conclusion

In conclusion, no correlation was found between urine RBP-4 and serum HbA_{1c} with GFR in type 1 diabetic children. We found increased urine RBP-4 with ≥5 years duration of type 1 diabetes. However, due to our limitations, more studies are needed.

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