Endothelial Dysfunction Markers as Predictive of Nephropathy in Pediatric Type 1 Diabetes

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Abstract: Recent studies found a correlation between endothelial dysfunction and the development of diabetic nephropathy. Soluble intracellular adhesion molecule-1(sICAM) and nitric oxide metabolites (NOx) are known markers for endothelial dysfunction. This study aims at validation of the correlation between sICAM and NOx levels with the degree of microalbuminuria in pediatric type-1 diabetic patients. Thirty children with Type 1 diabetes (T1D) were enrolled for the study and compared to thirty healthy age and gender matched non-diabetic controls. In both groups, the levels of sICAM1, HBA1cpercentage, nitric oxide metabolites, and spot collection of urinary albumin / mg creatinine were measured. The results revealed that children with T1D had significantly higher glycated hemoglobin (HbA1c %) values versus the control group (P<0.05). The mean serum sICAM-1 concentration was significantly higher in T1D children versus the control group (P<0.05). 0n the other hand, the mean serum nitric oxide metabolite (NOx) showed a significant reduction (P< 0.05) in the T1D. The mean urinary albumin / mg creatinine was significantly higher (P< 0.05) in diabetic children than in control group. Correlation analysis showed a significant (P< 0.05) positive correlation between the levels of sICAM-1 & microalbuminuria and a negative correlation between microalbuminuria and NOx level. It could be concluded that sICAM1and NO may play important roles in the pathophysiology and progression of endothelial dysfunction and its related disorders as diabetic nephropathy in T1D which should be considered in the monitoring the disease as well as for developing effective preventive and therapeutic interventions to prevent or retard overt diabetic nephropathy.
Keywords: T1D, sICAM1, NOx, endothelial dysfunction, nephropathy

INTRODUCTION

Type 1 Diabetes (T1D) is common in KSA and it accounts for about 5-10% of all cases of diabetes [1]. It is accepted that the etiology of T1D is related to genetic predisposition and commonly precipitated by virus or food factor leading to a progressive chronic autoimmune process [2]. However, other factors are found as the adhesion molecules, which bind the cells to each other or to the extracellular matrix, which may play a role during the prediabetic state, as they are important for the activation of the lymphocytes, the migration of cells to the sites of inflammation, and the adhesion of the lymphocyte to lymph node endothelium [3]. High levels of soluble adhesion molecules have been reported to associate active immune processes and sICAM may play an important role in the initiation of the inflammatory process [4]. Elevated levels of sICAM molecule are indicative of endothelial dysfunction, which may play a role in the pathogenesis of diabetic nephropathy [4-6].

Endothelial dysfunction is associated with diminished activity of the endothelial nitric oxide synthase (eNOS) and in turn decreased levels of plasma nitric oxide (NO) and the nitric oxide metabolite (NOx) [6]. Microalbuminuria has been linked to the identification of incipient diabetic kidney disease and as a predictor of outcome in patients with renal disease [7]. The albumin/creatinine ratio has been shown to be a valid screening tool for diabetic nephropathy as the traditionally used 24-hour albumin excretion [8]. This study aims at validation of the correlation between sICAM & NOx levels and the degree of microalbuminuria in pediatric type-1 diabetic patients.

MATERIAL AND METHODS

This was a prospective controlled clinical study conducted at King Abdul Aziz Specialist Hospital in Taif, Saudi Arabia from January 2015 to December 2017. Thirty children with Type 1 diabetes (T1D) were enrolled for the study and compared to thirty healthy age and gender matched non-diabetic controls. The

study was conducted after approval of the ethical committee of the hospital and informed consents were obtained from the parents of all the enrolled children.

The children included in this study were then classified into two groups:

T1D group; they were 18 males and 12 females and their ages ranged between 4 – 15 years with mean age of 9.05 ± 2.14 years. The study included patients under insulin treatment and those under oral hypoglycemic medications were excluded. Children with acute or chronic diabetic complications were also excluded.

Control group; this group included thirty apparently healthy children (15 males and 15 females). Their ages ranged between 2 –15 years with a mean age of 9.12 ±2.2 years. They had no family history of diabetes, or any other autoimmune disease.

Both groups were subjected to the following: Full medical history and thorough physical examination with measurement of BMI, in addition to the measurement of sICAM1, nitric oxide metabolites, and spot collection of urinary albumin / mg creatinine. Fasting plasma glucose and fasting glycated hemoglobin (HbA1c) were also assayed.

STATISTICAL ANALYSIS

SPSS program version 20 (SPSS Inc., Chicago, IL, USA) were used and the results were expressed as mean ± standard deviation The student's t-test was used to differentiate between two groups and Pearson and Spearman's correlation tests were used to correlate between each parameter and different variants in the same group to differentiate between positive and negative correlations and to find significant differences. $P<0.05$ was considered as statistically significant.

RESULTS

The results revealed that children with T1D had median duration of the disease of 62 months. They had significantly higher glycated hemoglobin (HbA1c %) values versus the control group ($P<0.05$). The mean serum sICAM-1 concentration was significantly higher in T1D children versus the control group. 0n the other hand, the mean serum nitric oxide metabolite (NOx) showed a significant reduction ($P< 0.05$) in the T1D. The mean urinary albumin / mg creatinine was significantly higher ($P< 0.05$) in diabetic children than in control group. Comparing the mean urinary albumin/mcg creatinine with the level of sICAM-1 and NOx above and below the median of each and the correlation analysis showed a significant ($P< 0.05$) positive correlation between the levels of sICAM-1 & microalbuminuria and a negative correlation between microalbuminuria and NOx level. The HbA1cpercentage was also correlated positively with sICAM-1 and NOx levels. Tables 1 & 2 show the results in details.

Table-1: Comparison between T1D group and control group

Parameters	T1D group (n=30)	Control group (n=30)	p- value
age	9.05 ± 2.14 years	9.12 ±2.2 years	NS
Sex(M/F)	(18/12)	(15/15)	NS
BMI (kg/m^2)	26.66 ± 2.87	18.43 ±1.96	S
Fasting glucose (mg/dl)	122.5 ±8.90	80.2 ±12.60	NSN S
HbA1c%	7.6±1.28	4.99±1.07	S
Mean urinary albumin/mg creatinine	67.6 ± 14.6 mcg	18.7 ± 4.6 mcg	S
Mean serum sICAM-1 concentration	307.54±75.92 ng/ml	231.69±54.67 ng/ml	S
Mean serum NOx	2.41±0.54 mmol/L	5.1±0.98 mmol/L	S

NS; Non Significant, S; Significant

Table-2: Correlating microalbuminuria with the levels of sICAM-1 and NOx

	Below the median sICAM-1	Above the median sICAM-1	P value
Mean urinary albumin/mg creatinine	47.6 ± 16.6 mcg	87.6 ± 17.5 mcg	< 0.05
	Below the median NOx level	Above the median NOx level	P value
Mean urinary albumin/mg creatinine	84.7 ± 18.6 mcg	49.3± 12.1 mcg	<0.05

Non-significant = P > 0.05, Significant = P < 0.05

Table-3: Correlating the variation in the levels of sICAM-1 and NOx with HbA1c%

	Below median of HbA1c%	Above median of HbA1c%	P Value
Mean sICAM level	269.18±66.44 ng/ml	337.33±84.11 ng/ml	< 0.05
Mean NOx level	3.31±0.76 mmol/L	2.35±0.13 mmol/L	< 0.05

Non significant = P > 0.05, Significant = P < 0.05

DISCUSSION

Markers of endothelial dysfunction and inflammatory activity have been associated with onset and progression of microalbuminuria as proved in several large cohort follow-up studies in both diabetic and non-diabetic populations [7, 8]. The present study proved a correlation between the degree of microalbuminuria with the sICAM-1 and NOx levels, reflecting the role of endothelial dysfunction in the progress of the urinary albumin and in turn the kidney disease. Similar results were obtained by von Scholten and his colleagues , they proved in their study that elevation of the markers of endothelial dysfunction was correlated with the progression of the degree of microalbuminuria which supports the hypothesis that endothelial dysfunction is involved in the development of diabetic nephropathy [3].

Lin *et al.* found in their study that increased risk of progressive nephropathy in type 1 diabetes can be predicted and correlated with higher baseline sICAM-1 levels concluding that it may represent an early risk marker that reflects the important role of vascular endothelial dysfunction in this long-term complication [4]. Similar results were obtained in our study where a significant positive correlation between the levels of sICAM-1 & microalbuminuria were detected. In the Pittsburgh Epidemiology of Diabetes Complications longitudinal follow-up study of type 1 diabetic subjects they proved that sICAM predicts incident microalbuminuria which was strongly predictive of subsequent cardiovascular disease events [5].

Previous studies suggested that insulin-mediated stimulation of blood flow, which is mediated by nitric oxide, becomes defective in diabetic patients [9, 10] and they found that this defect is the cornerstone in the pathogenesis of diabetic nephropathy [10]. Tessari *et al.* verified in their study that, in diabetic patients with nephropathy, whole-body NOx synthesis is decreased with impairment of the fractional conversion of arginine to NOx [11]. Several studies have shown that endothelial dysfunction was correlated to the autoimmune process and the associated elevation in inflammatory mediators in addition to formation of anti-endothelial antibodies [12-14]. The findings of the present study verified lower NOx levels in T1D children reflecting the reduced bioactivity of endothelial nitric oxide synthase and NO in diabetic children compared to the control group .This was in accordance with the Kuboki *et al.*, study [15] and Correa and Alfieri [16] and Lo *et al.*, [17], who found that diabetic patients have significantly lower circulating NO level; however, they claimed low nitric oxide levels to the absence of the stimulatory effect of insulin on eNOS. Type 1diabetes is more prevalent in children and the development of endothelial dysfunction would eventually progress to cardiovascular and kidney diseases. Balarini *et al.* [18], revealed in their study on the apolipoprotein E knockout mice that the drugs which restore the bioactivity of NO as sildenafil improved endothelial dysfunction.

However, the potential effect of this drug in prevention or retarding the progress of nephropathy must be widely investigated, in addition; further prospective studies in T1D children with changes in markers of endothelial dysfunction are needed to enhance the knowledge in this area.

CONCLUSION

It could be concluded that sICAM1and NO may play important roles in the pathophysiology and progression of endothelial dysfunction and its related disorders as diabetic nephropathy in T1D which should be considered in the monitoring the disease as well as for developing effective preventive and therapeutic interventions to prevent or retard overt diabetic nephropathy.

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