Endoglin: a novel predictor of vascular complications in type 1 diabetic children and adolescents

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Received 24 August 2017
Accepted 16 November 2017

Journal of The Arab Society for Medical Research 2017, 12:73–78

Background/aim
The incidence of developing complications in type 1 diabetic children and adolescents is increased along with increased development of vascular complications. Endoglin is a cell-surface coreceptor for the transforming growth factor β1 it is highly expressed in the endothelial cell having a crucial role in angiogenesis, indicating endothelial dysfunction and diabetic complications and pathologies. The present study aims to assess serum endoglin levels in relation to vascular and endothelial dysfunction associated with type 1 diabetes in a group of children and adolescents.
Patients and methods
This study was conducted on 30 diabetic children and adolescents, aged 6–16 years, in addition to 30 healthy children served as a control group of matched age. Clinical examination and anthropometric assessment were done for all children. In addition, endoglin, metabolic lipid parameters, glycated hemoglobin, and urinary albumin were determined, in addition to ophthalmologic examination for diabetic retinopathy.
Results
The levels of serum endoglin, systolic blood pressure *Z*-score, diastolic blood pressure *Z*-score, cholesterol, low-density lipoprotein, high-density lipoprotein, and triglycerides in patients were significantly higher than in controls. Endoglin levels were higher in cases with albuminuria and retinopathy compared with other cases. Significant correlations were detected between endoglin and systolic blood pressure *Z*-score, diastolic blood pressure *Z*-score, cholesterol, triglycerides, low-density lipoprotein, glycated hemoglobin, and albuminuria.
Conclusion
We conclude that endoglin can serve as a predictor of cardiovascular risk in pediatric population and other diabetes-associated complications such as retinopathy and albuminuria.

Keywords:
albuminuria, diastolic blood pressure *Z*-score, endoglin, glycated hemoglobin, retinopathy

J Arab Soc Med Res 12:73–78

1687-4293

Introduction

Long-term complications of type 1 diabetes are secondary to the damaging effects of metabolic consequences of insulin deficiency on tissues of different body organs. Maintaining good control of diabetes by preventing prolonged hyperglycemia is important to prevent complications from developing it in later life [1]. Incidence of developing complications in diabetic children depends on metabolic control, pubertal status, genetic susceptibility, sex, and lifestyle (e.g. diet, exercise, etc.) [1].

Hyperglycemia causes subclinical alterations due to premature atherosclerosis and endothelial dysfunction (ED) representing the initial step toward microvascular complications and the development of cardiovascular diseases [1]. In ED, the endothelium loses its physiological properties and shifts toward a pro-inflammatory prothrombotic state [2].

Diabetic nephropathy occurs in 30% of people with type 1 diabetes mellitus mostly in postadolescents. Albumin excretion rate increases until frank albuminuria occurs which may lead to renal failure. Blood pressure rises with increased albumin excretion rate, and hypertension accelerates the progression of renal failure [3].

Having diabetic nephropathy also increases the risk of significant diabetic retinopathy. Estimated 80% of people with type 1 diabetes mellitus develop retinopathy. Although diabetic retinopathy is rare in diabetic children, prevalence and severity of

retinopathy increase with age and are greatest in patients with uncontrolled diabetics [3].

Several biomarkers have been used to assess alterations secondary to diabetes; nitric oxide is one of the early indicators of ED. Changes in the amount and/or bioavailability of nitric oxide molecule are considered as an early finding of ED. Another molecule, which has been implicated in the regulation of endothelial function, is endoglin (Eng) [4].

Eng or CD105, a cell-surface coreceptor for transforming growth factor (TGF)-β1 and TGF-β3 isoforms, is highly expressed in the endothelial cell, it modulates actions of TGF-β1 and TGF-β3. Eng has a crucial role in angiogenesis; therefore, it is an important protein for tumor growth, survival, and metastasis of cancer cells [4].

Increased soluble Eng levels could be related to ED, consequently an indicator of cardiovascular damage in diabetes-associated vascular alterations. Increased circulating concentration of soluble Eng was reported in the early stages of atherosclerosis due to damage of endothelial cells and then Eng concentration decreases in the later stages, which suggests a potential role of soluble Eng in acute heart failure [5].

Eng is suggested to serve as an indicator of diabetes-associated vascular complications such as hypertension, ED, and cardiovascular risks in adults [6]; yet there is not enough studies focusing on Eng concentrations in diabetic children and adolescents (type 1) and its implication on long-term complications.

Thus current study aims to assess serum Eng levels in relation to vascular and ED associated with type 1 diabetes in a group of children and adolescents compared with a control group of matched age.

Patients and methods

Patient inclusion and exclusion criteria

This study was conducted on 30 diabetic children and adolescents with type 1 diabetes mellitus; their age ranged from 6 to 16 years attending the Endocrinology and Diabetology Unit, Pediatric Hospital, Ain Shams University. Another 30 apparently healthy children of the same age were recruited as the control group. Diabetic patients group was subsequently divided into two subgroups according to their glycated hemoglobin (HbA1c) level: controlled (<8%) and uncontrolled (>8%) diabetic groups.

Participants were excluded for any of the following reasons: presence of other chronic or inflammatory disease, medications or hormones (other than insulin), presence of known renal disease, and systemic disease or acute infection at the time of testing.

Ethical approval

This study was approved by the local Ethics Committee of National Research Centre, Cairo, Egypt. After taking a written informed consent from the parents of children enrolled in the study, a detailed medical history and physical examination were carried out.

Anthropometric measurements

Anthropometric evaluation were carried out on all children. Children were weighed (kg) using a calibrated Seca scale to the nearest 0.1 kg (Seca, Hamburg, Germany), whereas height (cm) was measured using a Seca 225 stadiometer to the nearest 0.1 cm. BMI was calculated by weight (kg)/height (m^2). The BMI *Z*-score was assessed using the new WHO reference [7]. Blood pressure was measured by a sphygmomanometer (Accoson, London, UK) as the mean of two measurements. *Z*-scores were determined using the German reference [8].

Blood sampling

Three milliliter of fasting (8 h) venous blood samples were taken from each child participating in the study and was divided into two parts: the first part was added to a tube containing EDTA for HbA1c determination by cation-exchange resin and the second part was put in a serum separator tube. The separated serum was stored at −20°C for determination of Eng, total cholesterol, and triglyceride (TG), high-density lipoprotein (HDL) and low-density lipoprotein (LDL).

Biochemical measurements

HbA1c was determined by the cation-exchange resin [9], using kits of Pariksha Biotech Neochem (Balanagar, Hyderabad, Telangana, India). Total cholesterol, TG, and HDL were determined using colorimetric techniques on Synchron Cx7 (Beckman Instruments Inc., Brea, California, USA). LDL cholesterol was measured by the Friedewald formula [10]. For random urinary albumin measurement, an early morning midstream specimen was used. The cloudy samples were centrifuged before use and the clear supernatant was stored at −20°C until analysis. Albumin concentrations were measured in urine using a Minineph Microalbumin kit based on the nephlometry method on a Minineph nephelometer (AD200) (The Binding Site, Birmingham, UK)

[11]. We compared albumin in the sample against its creatinine concentration (measured by Jaffe reaction) on a Synchron Cx7 autoanalyzer, and the albumin/creatinine ratio was calculated [12]. Serum Eng was measured by quantitative sandwich enzyme immunoassay technique supplied from the R&D Systems Inc. (Minneapolis, Minnesota, USA) [13].

Ophthalmologic examination

Indirect ophthalmoscopy was done after complete pupillary dilatation by application of 1% tropicamide eye drops. Classification of retinopathy was based on the findings of the worst eye of each subject. Retinopathy was classified as grade I (generalized arteriolar constriction), grade II (irregularly located, tight constrictions), grade III (retinal edema, cotton-wool spots, large spot hemorrhages, hard exudates including a macular star), and grade IV (same as grade III but with swelling of the optic disk and silver wiring) according to the method of Shrote and Diagavane [14].

Statistical analysis

Data entry was carried on Excel sheet and statistical analysis was done using the SPSS software program, version 18.0 (SPSS Inc., PASW Statistics for Windows, Version 18.0. Chicago, USA). χ^2 was done for qualitative data that were presented by numbers and percentages. *t*-Test was done for comparison between two means. Simple linear correlation (Pearson's correlation) for quantitative data was also done. *P* value was considered statistically significant when *P* was less than 0.05 and considered statistically highly significant when its value was less than 0.01.

Table 1 Anthropometric, clinical, and laboratory data in diabetic and nondiabetic groups

Items	Diabetic (*N*=30)	Nondiabetic (*N*=30)	*P*
Age	10.13±3.37	10.4±3.36	0.76
Weight *Z*-score	0.0537±1.117	0.35±0.99	0.144
Height *Z*-score	0.552±1.399	0.47±1.33	0.815
BMI *Z*-score	0.56±1.112	0.341±0.934	0.413
SBP *Z*-score	0.913±0.766	0.116±0.928	0.001*
DBP *Z*-score	0.955±0.64	0.35±0.551	0.000*
HbA1c (g/dl)	8.91±1.516	5.43±1.37	<0.001*
Cholesterol (mg/dl)	234.7±35.27	139.2±16.51	0.000*
TG (mg/dl)	156.8±18.94	72.53±7.126	0.000*
LDL (mg/dl)	175.7±25.51	72.44±18.25	0.000*
HDL (mg/dl)	27.62±6.879	52.32±4.4	0.000*
Endoglin (ng/ml)	10.64±2.298	5.153±1.045	0.000*
Albuminuria (mg/g)	32.46±60.08	7.936±5.035	0.030*

All data are expressed as mean±SD; DBP, diastolic blood pressure; HbAlc, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SBP, systolic blood pressure; TG, triglyceride. **P* value <0.05 is significant.

Table 2 Anthropometric, clinical, and laboratory data in controlled and uncontrolled diabetic subgroups

Item	Controlled diabetic (*N*=10)	Uncontrolled diabetic (*N*=20)	*P*
Weight *Z*-score	0.609±0.656	0.385±1.164	0.019*
Height *Z*-score	0.78±1.28	0.439±1.47	0.539
BMI *Z*-score	0.008±0.591	0.845±1.216	0.046*
SBP *Z*-score	0.698±0.975	1.02±0.639	0.285
DBP *Z*-score	0.605±0.543	1.126±0.632	0.034*
Cholesterol (mg/dl)	211.5±34.16	246.3±30.34	0.008*
TG (mg/dl)	144.5±19.95	162.9±15.48	0.009*
LDL (mg/dl)	158±25.98	184.67±20.59	0.005*
HDL (mg/dl)	24.66±4.976	29.11±7.317	0.1
Endoglin (ng/ml)	9.24±2.1	9.9±3.62	0.015*
Albuminuria (mg/g)	10.17±7.54	44.36±70.98	0.046*

All data are expressed as mean±SD; DBP, diastolic blood pressure; HbAlc, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SBP, systolic blood pressure; TG, triglyceride. **P* value <0.05 is significant.

Results

The data presented in Table 1 shows the anthropometric, clinical, and laboratory data of children and adolescents included in the study, confirming the similar age distribution. In the diabetic group (cases), we had 13 men and 17 women, while in the nondiabetic (control) group we had 11 men and 19 women with a total of 30 children in each group. The mean values of systolic blood pressure (SBP) *Z*-score, diastolic blood pressure (DBP) *Z*-score, cholesterol, TG, LDL, HDL, Eng, and albuminuria were all significantly higher in diabetic children in comparison with the control. Meanwhile, the mean value of BMI *Z*-score, weight *Z*-score, and height *Z*-score were different between the two groups. Table 2 describes the anthropometric, clinical, and laboratory data of controlled and uncontrolled diabetic children and adolescents included in the cases group; there was statistically significant difference between mean values of BMI *Z*-score, weight *Z*-score, DBP *Z*-score, cholesterol, TG, LDL, and Eng.

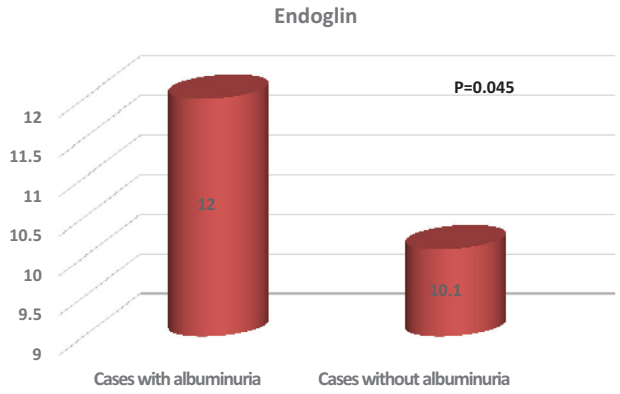
Data in Table 3 shows that in the diabetic group there are significant positive correlations between Eng and SBP *Z*-score, DBP *Z*-score, cholesterol, TG, LDL, HbA1c, and albuminuria. Moreover, there is significant negative correlation between Eng and BMI *Z*-score in the diabetic group as well. Figures 1 and 2 show that Eng levels were higher in cases with albuminuria and retinopathy (respectively) compared with other cases, whereas Fig. 3 shows the correlation between serum Eng and HbA1c.

Table 3 Pearson's correlation between endoglin and anthropometric, clinical, and laboratory data in the diabetic group

Item	Endoglin in diabetic (*N*=30)	
	r	*P*
BMI *Z*-score	−0.095	0.791
SBP *Z*-score	0.058	0.761
DBP *Z*-score	0.395	0.045*
HbA1c	0.413	0.023*
Cholesterol	0.537	0.002**
TG	0.534	0.002**
LDL	0.583	0.001**
HDL	0.298	0.11
Albuminuria	0.279	0.039*

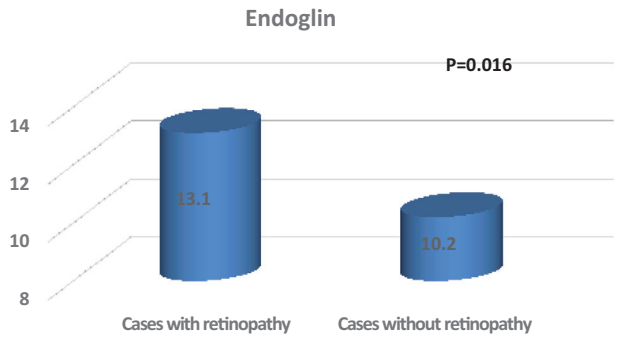
DBP, diastolic blood pressure; HbAlc, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SBP, systolic blood pressure; TG, triglyceride; *$P<0.05$, significant correlation; **$P<0.01$, significant correlation.

Figure 1



Endoglin levels in albuminuric and nonalbuminuric cases.

Figure 2

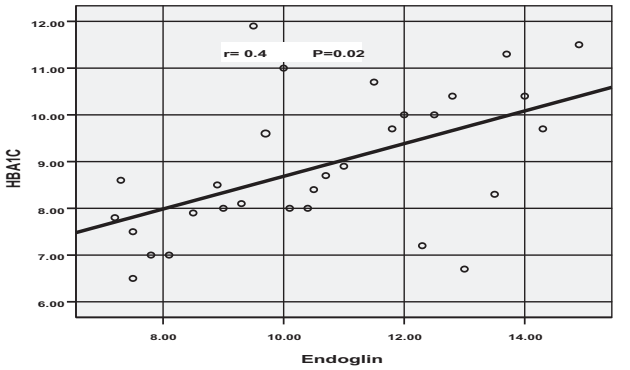


Endoglin levels in diabetic cases with and without retinopathy.

Discussion

The relevance of Eng in vascular biology is reflected by the fact that it is a regulator of vascular tone and in a considerable number of studies, it has been reported that impaired endothelial function in diabetic patients is related to elevated serum Eng level yet

Figure 3



Correlation between serum endoglin and glycated hemoglobin (HbA1c).

no enough studies investigated this cross-linkage in children and adolescents; thus the present study summarizes the relation of Eng and microvascular complication in a group of diabetic children and adolescents in comparison to normal healthy children.

The current study findings show that serum Eng levels are significantly higher in diabetic children when compared with the control group of nondiabetic children. SBP and DBP was also higher in diabetic children who had impaired lipid profile and albuminuria when compared with the healthy group, which indicate that Eng is overexpressed in diabetic children and adolescents with diabetes-associated pathologies. Results fit what Blázquez-Medela *et al.* [15] and El-Naggar and El-Srogy [6] found in adult diabetic patients when compared with the healthy nondiabetic control group. Moreover, comparing the two subgroups of diabetics according to their diabetes level of control using HbA1c 8% as a cutoff [controlled (<8%) and uncontrolled (>8%) diabetic group]. we found that Eng is significantly higher in uncontrolled diabetic patients. Weight, BMI *Z*-score, SBP *Z*-score, DBP *Z*-score, cholesterol, TGs, LDL, and HDL are all showing significant statistical difference between groups, which confirm a strong connection between serum Eng level, hyperglycemia, and indicators of long-term diabetes complications.

This comes in agreement with the results of the first study of Eng in humans conducted in 2010 [13] that showed the emerging role of Eng as an indicator of diabetes-associated hypertension and cardiovascular risk.

Eng is a homodimeric transmembrane glycoprotein mostly expressed on proliferating endothelial cells

and has a regulatory role in TGF-β signaling, first detected in endothelial cells, smooth muscle cells, activated macrophages, and in fibroblasts. Eng proved in limited number of studies to increase in vessels during various pathological situations such as vascular injury and hypoxia [16].

These facts are proved by the results of this study as positive correlations with high significance between serum Eng and DBP *Z*-score, HbA1c, cholesterol, TG, LDL, and albuminuria which support the association of Eng with ED and angiogenesis supposing that Eng might be used as a predictor of complications in type 1 diabetic patients. These results confirm data documented by El-Wakeel *et al.*'s [17] study, which proved that DBP was correlated with diabetes duration and HbA1c levels. The results shed more light to the effect of impaired control of hyperglycemia on the premature development of macrovascular complications in pediatric population of diabetic patients.

In addition, Eng levels of retinopathy patients and patients with albuminuria were found to be higher than patients without retinopathy or albuminuria with high statistical significance.This matches what Kovacs *et al.* [18] had reported of higher Eng concentrations in proliferative retinopathy patients than other diabetic patients without retinopathy in their study patients, yet not reached any statistical significance. Also current results come in agreement with El-Naggar and El-Srogy [6] who found higher Eng levels in adult type 2 diabetic patients with retinopathy, proteinuria, and renal impairment than in patients with proteinuria alone.

The role of Eng in microvascular complication is emphasized by this study that showed elevated serum levels of Eng in correlation to retinopathy, DBP, and albuminuria.

Positive correlation of serum Eng levels and HbA1c found by results of the present study proves the strong association of poor diabetes control and development of microvascular and cardiovascular complications, the same was reported by Emeksiz *et al.* [1].

In the light of data documented by the results of the current study, we put forward the evidence that high serum Eng levels in type 1 diabetic children and adolescents might be collateral to deterioration in ED before evident subclinical structural vascular alterations.

Conclusion

This study is an innovative and relevant research in children and adolescent population showing that serum Eng levels can be served as a predictor of diabetes-associated pathologies such as hypertension, ED, and cardiovascular risk.

Much research should be done to follow the steps of previous studies for fully understanding the molecular mechanisms that underlie the role of Eng in these pathologies, which can provide new strategies for better diagnosis of target organ damage in early preventable phases.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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