Circulating Soluble ACE2 Activity in Children and Adolescents with Type 1 Diabetes Mellitus: Potential Biomarker for early detection of Diabetic Vascular Complications

Eman M. Sherif [1], Rahma A. Elmohymen Ahmed [1], Nesma A. Safwt [2], Eman M. Elsayed [1], Dalia N. Mohamed Toaima [1]

Pediatrics [1] and Clinical Pathology [2] Departments, Faculty of Medicine, Ain Shams University, Cairo, Egypt.

Corresponding author: Dalia N. Mohamed Toaim

Tel no: 01028220905, E-mail: dtoaima@yahoo.com.

ABSTRACT

Background: Given the serious health consequences including vascular complications among type 1 diabetes mellitus (T1DM), circulating ACE2 activity could be used as biomarker for early detection of vascular complication. **Objectives: To** assess serum levels of circulating ACE 2 among children and adolescents with type 1 diabetes mellitus (T1DM) and evaluate the relation of circulating ACE2 levels to glycemic control and diabetic vascular complications. **Patients and methods:** This cross-sectional study included 50 children and adolescents with type 1 diabetes mellitus divided equally into 2 groups according to presence or absence of vascular complications. The cases were recruited from attendees of the Pediatrics and Adolescents Diabetes Unit (PADU), Children's Hospital, Ain Shams University, Cairo, Egypt. Cases were compared to 25 healthy age and sex-matched controls. Demographic and anthropometric data, medical history, lipid profile, urinary albumin/creatinine ratio and mean glycated hemoglobin & circulating ACE 2 level were assessted . by Enzyme linked immunosorbent assay (ELISA) technique and Echocardiography was performed by specialized paediatric echocardiographer. **Results:** Our results revealed significantly higher level of circulating ACE2 in children and adolescents with T1DM compared to healthy control group ($p<0.001$). Circulating ACE2 level was higher among cases who had neuropathy ($p<0.001$) and nephropathy ($p=0.008$), compared to patients without neuropathy and nephropathy, respectively, but ACE2 level did not correlate to HbA1c, lipid profile or albumin/creatinine ratio. Furthermore, it showed significant correlation to right ventricular enlargement and carotid intima-media thickness (CIMT) among complicated cases indicating possible role in cardiovascular and vascular remodeling. **Conclusion:** Children and adolescents with T1DM have elevated levels of circulating ACE2, particularly when associated with neuropathy or nephropathy, irrespective of their glycemic control. Further research is warranted into the clinical utility of ACE2 as a predictive marker for early detection and management of diabetes-related complications.

Keywords: Type 1 Diabetes, Circulating ACE2, neuropathy, nephropathy, CIMT

INTRODUCTION

The renin-angiotensin system (RAS) plays a crucial role in regulating renal hemodynamics and transport functions under both normal and pathological conditions ***(Simões et al., 2021)***. Over-activation of the renin–angiotensin system (RAS) is considered to directly cause vascular injury in diabetes ***(Hollenberg et al., 2003)***. Angiotensin-converting enzyme 2 (ACE2), a counterpart of ACE, facilitates the transformation of Angiotensin II (Ang II) into Ang1-7, which promotes vasodilation and exhibits antifibrotic, antiproliferative, and anti-inflammatory properties. Due to its ability to counteract the effects of Ang II, ACE2 has been suggested as a potential biomarker for kidney disease. Its enzymatic activity has been extensively examined in various tissues, including the kidneys and heart, under both normal and disease conditions ***(Fountain et al., 2022)***. ACE2 has been identified within human atherosclerotic plaques, with its expression observed in various cell types involved in the lesion, including endothelial cells and macrophages ***(Sluimer et al., 2008)*** ACE2 is an enzyme known for its crucial role in the regulation of the renin-angiotensin system (RAS) and plays a pivotal role in maintaining cardiovascular homeostasis. In recent years, its association with various vascular pathologies, including those seen in Type 1 diabetes, has garnered attention ***(Oudit & Pfeffer, 2020)***. In individuals with T1DM, chronic hyperglycemia and metabolic imbalances can disrupt the normal homeostatic balance, leading to alterations in circulating ACE2 levels ***(Vargas et al., 2022).*** Evidence indicates that higher circulating ACE2 levels may correlate with markers of vascular damage, such as increased carotid intima-media thickness or microalbuminuria ***(Hussain et al., 2021).*** **The aim of our current study** was to assess serum levels of circulating ACE 2 among children and adolescents with type 1 diabetes mellitus (T1DM) compared to healthy controls and evaluate its relation to glycemic control and diabetic vascular complications to assess its potential role as biomarker for early detection of vascular damage in T1DM.

PATIENTS AND METHODS

- **Ethical consideration:** This study was approved by Ain Shams University Research Ethics Committee (REC). All patients and/or caregivers were provided with a clear explanation of the study's purpose and potential benefits. Informed consent was obtained from them in straightforward Arabic before their participation in the research.
- Data from medical records were collected and used for private and confidential research purposes.
- The patients and parents had the right to withdraw at any time.
- No conflicts of interest were associated with this study or its publication.
- There was no financial support or sponsorship.

Sample size: Using PASS 11 for sample size calculation, setting power at 99%, alpha error at 5% and after reviewing previous study results **(Soro-Paavonen et al., 2012)** showed that the ACE activity was higher among diabetic patients with microalbuminuria than those without microalbuminuria (30.2 ‡ 1.5 ng.E/ml versus 27.0 ‡ 0.5 ng.E/ml respectively); based on that, a sample size of at least 25 diabetic patients with complication and 25 diabetic patients without complication and 25 healthy controls will be sufficient to achieve study objective.

Inclusion criteria: 5 to 18 years old patients of both sexes, who were previously diagnosed with T1DM according to ISPAD guidelines ***(2022).***

Exclusion criteria: We excluded other forms of diabetes such as type 2 diabetes, secondary diabetes or monogenic diabetes, as well as patients with liver dysfunction, urinary tract disorders, other autoimmune diseases or those with recurrent or recent infection.

Study procedure: This comparative case control study included 75 child and adolescent .recruited from Pediatrics and Adolescents Diabetes Unit (PADU) and general outpatient clinic. Children's Hospital, Ain Shams University during the period from March 2023 to August 2023.

Group A (patient group): Including 50 T1DM patients regularly attending PADU. The patient group was divided equally into two subgroups (25 patients in each group) according to presence or absence of microvascular and macrovascular complications e.g neuropathy , nephropathy and retinopathy.

Group B (control group): 25 pediatric healthy controls who were age and sex-matching attending the general outpatient clinic for general check-up.

Patient evaluation:

All patients were subjected to:

1-**Medical history** including age of onset and duration of diabetes, insulin therapy and history of associated co-morbidities and any vascular complications.

2-**Clinical examination** including General examination for anthropometric measurements (weight, height, weight for height, height for age and BMI in (SD-Z score) ***(El Omda et al., 2023)*** and vital data as blood pressure as well as Local examination including abdominal, chest, heart examination to exclude any underlying illness. Neurological examination with stress on assessment of superficial & deep sensation. Tanner staging ***(Marshall et al., 1969 and Marshall et al., 1970).*** Physical development was evaluated using a scale that measured external primary and secondary sexual features, such as breast development, penile size, testicular volume, and pubic hair growth. Diabetic retinopathy was assessed by direct ophthalmoscopy collected from patient files.

3- **Laboratory data** including Fasting lipid profile from patient files. Assessment of mean HbA1c % in our study period by immunoturbidimetric method. Urinary albumin excretion (UAE) was evaluated using the albumin/creatinine ratio from early morning fasting urine samples. Microalbuminuria was defined as a UAE of 30–299 mg/g creatinine, while macroalbuminuria was

identified at levels of 300 mg/g creatinine or higher, based on patient records ***(Molitch et al., 2004).*** Measurement of circulating ACE 2 level was assessed by Enzyme linked immunosorbent assay (ELISA) technique ***(Hayrapetyan et al., 2023).***

4- Echocardiography was performed using GE Vivid E95 Ultrasound Machine with probe M5Sc by specialized paediatric echocardiographer and Carotid intima-media thickness (CIMT) was measured to detect early vascular changes e.g. subclinical atherosclerosis.

Statistical analysis: The data collected underwent revision, coding, and organization before being analyzed using SPSS version 27. For parametric numerical variables, descriptive statistics included the mean, standard deviation (±SD), and range. Non-parametric numerical data were summarized using the median and interquartile range (IQR), while categorical data were presented as frequencies and percentages. The statistical analysis involved various tests to evaluate differences and relationships between variables. To analyze the data, various statistical methods were utilized. The Student t-test was used to compare the means of two groups for parametric variables, while the Mann-Whitney U test evaluated differences in non-parametric data. Associations between categorical variables were assessed using the Chi-square test, and Fisher's exact test was applied when more than 20% of expected cell frequencies were below five. Correlations between quantitative variables were examined using Spearman's rho, with the correlation coefficient (r) indicating the strength and direction of the relationship. The Receiver Operating Characteristic (ROC) curve was employed to evaluate the sensitivity and specificity of diagnostic measures distinguishing between two groups.

ESULTS

The following tables and figures present our results:

Table (1): Socio-demographic data and clinical characteristics among cases and control group.

				Test of significance		
		Controls (N= 25)	Cases (N= 50)	value	p-value	Sig.
Gender	Male (N (%))	16 (64%)	33 (66%)	X^2= 0.029	0.864	NS
	Female (N (%))	9 (36%)	17 (34%)			
Age (year)	Mean ± SD	9.04 ± 2.62	9.07 ± 2.57	*t*= -0.047	0.962	NS
	Range	(5 - 15)	(6 - 16)			
	Range	(18 - 60)	(18 - 65)			
WT z-score	Median (IQR)	-0.48 (-0.97 - 0.26)	0.27 (-0.63 - 1.1)	*z*= -2.349	0.019	**S**
	Range	(-79 - 1.05)	(-94 - 3.9)			
	Range	(104 - 165)	(104 - 170)			
HT z-score	Median (IQR)	-1.55 (-2.08 - -0.95)	-0.53 (-1.85 - 0.29)	*z*= -2.158	0.031	**S**
	Range	(-3.34 - 0.85)	(-3.8 - 5)			
Height for age z-score	Median (IQR)	-1 (-1.5 - 0)	0 (-1 - 1)	*z*= -1.789	0.074	NS
	Range	(-3 - 1)	(-3 - 3)			
	Range	(15.5 - 23.4)	(11.8 - 32)			
BMI z-score	Median (IQR)	0.61 (0.18 - 0.9)	0.7 (-0.22 - 1.67)	*z*= -0.528	0.597	NS
	Range	(-0.34 - 2.22)	(-3.54 - 3.9)			
Clinical findings:						
Blood pressure percentile	Median (IQR)	50 (50 - 50)	50 (50 - 50)	*z*= 0.000	1.000	NS
	Range	(50 - 50)	(5 - 95)			
Tanner staging	I	4 (16%)	7 (14%)	FE	0.954	NS
	II	11 (44%)	23 (46%)			
	III	5 (20%)	11 (22%)			
	IV	2 (8%)	2 (4%)			
	V	3 (12%)	7 (14%)			

WT: Weight, HT: Height, BMI: Body mass index, SBP: Systolic blood pressure, DBS: Diastolic blood pressure, X2: Chi-Square test of significance, t: Student t-test of significance, z: Mann-Whitney test of significance, FE: Fisher's Exact test of significance, P: P-value, NS: Non-significant p-value >0.05, S: Significant P-value < 0.05.

Table (1) shows that cases have statistically significant higher WT z-score (p=0.019) and higher HT. z-score (p=0.013) compared to control group. There was no significant difference between cases and controls regarding age, gender, weight, height, height for age z-score, BMI, BMI z-score, SBP, DBP and blood pressure percentile.

Table (2): Comparison between Laboratory investigations of cases and control group.

		Controls (N= 25)	Cases (N= 50)	value	p-value	Sig.
TGs (mg/dl)	Mean ± SD	56.48 ± 14.07	73.91 ± 23.47	***t*= -4.004**	<0.001	**S**
	Range	(36 - 84)	(34 - 134)			
Total Cholesterol (mg/dl)	Mean ± SD	116.52 ± 32.61	142.4 ± 33.86	***t*= -3.158**	0.002	**S**
	Range	(20 - 146)	(15 - 214)			
HDL (mg/dl)	Mean ± SD	72.34 ± 7.84	65.52 ± 26.67	***t*= 1.668**	0.1	NS
	Range	(55 - 85)	(35 - 131)			
LDL (mg/dl)	Mean ± SD	79.96 ± 11.47	87.4 ± 34.32	***t*= -1.385**	0.171	NS
	Range	(47 - 98)	(20 - 183.2)			
ACE2 (ng/ml)	Median (IQR)	1.95 (1.23 - 2.4)	7.2 (4.6 - 11.86)	***z*= -6.339**	<0.001	**S**
	Range	(0.49 - 4.43)	(1.76 - 91.12)			

TGs: Total triglycerides, HDL: High density lipoprotein, LDL: Low density lipoprotein, ACE2: angiotensin converting enzyme2, P: P-value, NS: Non-significant p-value >0.05, S: Significant P-value < 0.05, t: Student t-test of significance, z: Mann-Whitney test of significance.

Table (2) shows that cases have statistically significant higher levels of TGs (p<0.001), total cholesterol (p=0.002) and ACE2 (p<0.001) compared to control group.

Table (3): Echo findings of cases and control group.

		Gro	up	Student t-test		Sig.
		Controls (N= 25)	Cases (N= 50)	*t*	p-value	
LA/AO (cm)	Mean ± SD	0.9 ± 0.23	0.94 ± 0.24	-0.719	0.474	NS
	Range	(0.67 - 1.2)	(0.67 - 1.4)			
EF (%)	Mean ± SD	71.64 ± 2.84	71.34 ± 3.15	0.401	0.690	NS
	Range	(65 - 76)	(66 - 78)			
LV EDD (cm)	Mean ± SD	3.34 ± 0.3	3.54 ± 0.45	-2.309	0.024	**S**
	Range	(2.9 - 3.9)	(2.73 - 4.58)			
LV ESD (cm)	Mean ± SD	2.3 ± 0.35	2.41 ± 0.34	-1.305	0.196	NS
	Range	(1.8 - 2.7)	(1.7 - 2.9)			
RV EDD (cm)	Mean ± SD	2.88 ± 0.14	2.94 ± 0.13	-1.939	0.056	NS
	Range	(2.8 - 3.2)	(2.8 - 3.5)			
RV ESD (cm)	Mean ± SD	2.53 ± 0.15	2.38 ± 0.23	3.248	0.002	**S**
	Range	(2.2 - 2.7)	(2 - 2.7)			
E/A ratio (cm)	Mean ± SD	1.84 ± 0.18	1.82 ± 0.17	0.391	0.697	NS
	Range	(1.5 - 2)	(1.4 - 2.1)			
RV MPI (mm)	Mean ± SD	0.29 ± 0.02	0.29 ± 0.02	-1.696	0.094	NS
	Range	(0.26 - 0.31)	(0.26 - 0.33)			
LV MPI (mm)	Mean ± SD	0.31 ± 0.02	0.32 ± 0.02	-0.438	0.663	NS
	Range	(0.3 - 0.36)	(0.3 - 0.36)			
TAPSE (mm)	Mean ± SD	18.24 ± 1.33	17.72 ± 1.97	1.349	0.182	NS
	Range	(16 - 20)	(12 - 21)			
MAPSE (mm)	Mean ± SD	16.24 ± 1.3	16.82 ± 1.32	-1.799	0.076	NS
	Range	(15 - 19)	(15 - 20)			
CIMT (cm)	Mean ± SD	0.62 ± 0.03	0.64 ± 0.04	-1.397	0.167	NS
	Range	(0.6 - 0.68)	(0.6 - 0.78)			

Cardiac and vascular assessments included the **LA/AO ratio** (left atrium to aortic root), **EF%** (ejection fraction), and ventricular measurements (**LV EDD/ESD** and **RV EDD/ESD**) for diastolic and systolic diameters. Diastolic function was evaluated using the **E/A ratio**, while global ventricular performance was assessed through **RV MPI** and **LV MPI**. Systolic function indicators included **TAPSE** (tricuspid) and **MAPSE** (mitral), with vascular health measured by **CIMT**. Statistical significance was determined by **P-values**, where **P < 0.05** indicated significance (**S**) and **P > 0.05** was non-significant (**NS**).

Table (3) shows that cases have a statistically significant increase in LV EDD (p=0.024) and decrease in RV ESD (p=0.002) compared to control group.

Table (4): Laboratory investigations between cases who had complications and who had no complications.

Cases group (N= 50)		Cases group		Test of significance		
		Cases not complicated (N= 25)	Cases complicated (N= 25)	value	p-value	Sig.
HbA1C (mean)	Mean ± SD	8.72 ± 1.43	9.46 ± 2.32	***t*= -1.366**	0.178	NS
	Range	(6.7 - 11.9)	(6.2 - 17.6)			
TGs (mg/dl)	Mean ± SD	73.54 ± 18.94	74.28 ± 27.67	***t*= -0.111**	0.912	NS
	Range	(34 - 101)	(35 - 134)			
Total Cholesterol (mg/dl)	Mean ± SD	139.52 ± 40.48	145.28 ± 26.18	***t*= -0.597**	0.553	NS
	Range	(15 - 214)	(116 - 205)			
HDL (mg/dl)	Mean ± SD	73.63 ± 27.59	57.42 ± 23.54	***t*= 2.236**	0.030	S
	Range	(35 - 126)	(35 - 131)			
LDL (mg/dl)	Mean ± SD	74.03 ± 33.93	100.77 ± 29.68	***t*= -2.966**	0.005	S
	Range	(20 - 153)	(50 - 183.2)			
Albumin/creatinine ratio (mg/g)	Median (IQR)	9 (7 - 13)	31 (16 - 32)	***z*= -4.756**	<0.001	S
	Range	(2.3 - 22.8)	(7.83 - 50)			
ACE2 (ng/ml)	Median (IQR)	5.08 (2.95 - 6.46)	11.5 (7.73 - 19.52)	***z*= -4.007**	<0.001	S
	Range	(1.76 - 35.06)	(4.13 - 91.12)			

HbA1c: Glycated hemoglobin, TGs: Total triglycerides, HDL: High density lipoprotein, LDL: Low density lipoprotein, ACE2: angiotensin converting enzyme2, P: P-value, NS: Non-significant p-value >0.05, S: Significant P-value < 0.05, t: Student t-test of significance, z: Mann-Whitney test of significance.

Table (4) When comparing between complicated and non-complicated cases as regards laboratory data, we found that complicated cases had statistically significant higher level of LDL (p=0.005). albumin/creatinine ratio (p<0.001) and ACE2 (p<0.001) and lower level of HDL (p=0.030) compared to non-complicated cases.

Table (5): Correlation between ACE2 and Echo findings among complicated case group.

Cases complicated group (N= 25)	ACE2 (ng/ml)		
	Spearman's rho	p-value	Sig.
LA/AO (cm)	-0.209	0.316	NS
EF (%)	-0.116	0.582	NS
LV EDD (cm)	-0.250	0.229	NS
LV ESD (cm)	-0.154	0.464	NS
RV EDD (cm)	0.656	<0.001	**S**
RV ESD (cm)	0.031	0.883	NS
E/A ratio (cm)	-0.100	0.634	NS
RV MPI (mm)	-0.268	0.196	NS
LV MPI (mm)	-0.205	0.326	NS
TAPSE (mm)	-0.360	0.077	NS
MAPSE (mm)	0.339	0.098	NS
CIMT (cm)	0.645	<0.001	**S**

Cardiac and vascular assessments included the **LA/AO ratio** (left atrium to aortic root), **EF%** (ejection fraction), and ventricular measurements (**LV EDD/ESD** and **RV EDD/ESD**) for diastolic and systolic diameters. Diastolic function was evaluated using the **E/A ratio**, while global ventricular performance was assessed through **RV MPI** and **LV MPI**. Systolic function indicators included **TAPSE** (tricuspid) and **MAPSE** (mitral), with vascular health measured by **CIMT**. Statistical significance was determined by **P-values**, where **P < 0.05** indicated significance (**S**) and **P > 0.05** was non-significant (**NS**).

Table (5) and figure (1) show positive correlation between ACE2 and CIMT ($p<0.001$) and RV EDD ($p<0.001$), among cases who have complications and no correlation with the rest of the measured Echo parameters.

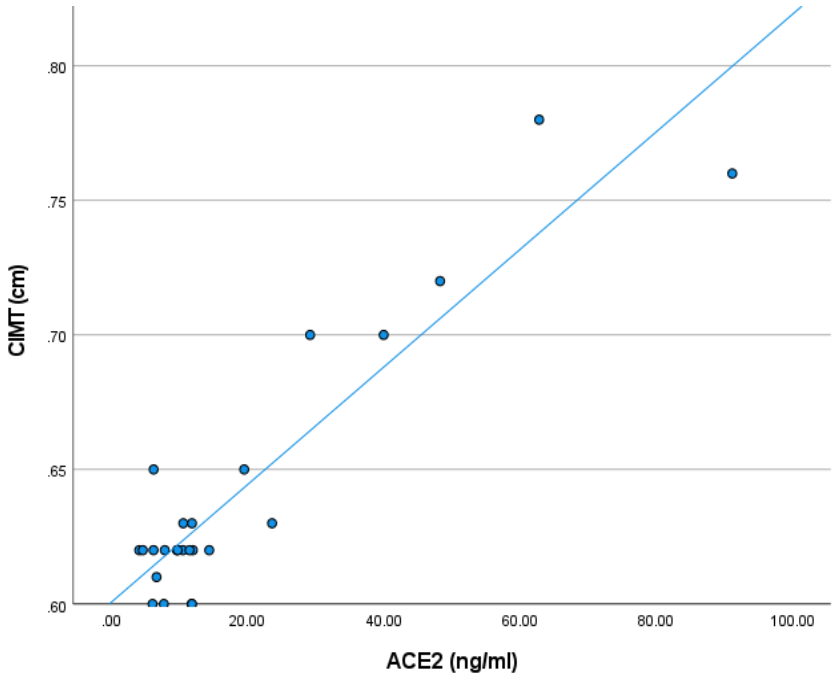

Figure (1): Positive correlation between ACE2 and CIMT among cases who had complications.

Table (6): Comparison between cases with and without neuropathy and nephropathy regarding ACE2 level.

Cases group (N= 50)		ACE2 (ng/ml)	Mann-Whitney test		
		Median (IQR)	*z*	p-value	Sig.
Neuropathy	No (N= 27)	5.11 (2.95 - 8.17)	-3.689	<0.001	S
	Yes (N= 23)	11.5 (7.73 - 23.61)			
Nephropathy	No (N= 36)	5.98 (3.71 - 11.47)	-2.669	0.008	S
	Yes (N= 14)	11.04 (7.87 - 19.52)			

P: P-value, NS: Non-significant p-value >0.05, S: Significant P-value < 0.05, Z: Mann-Whitney test of significance

Table (6) and figures (2,3) show significantly higher level of ACE2 among cases who have neuropathy (p<0.001) and nephropathy (p=0.008) compared to cases who had no neuropathy or nephropathy.

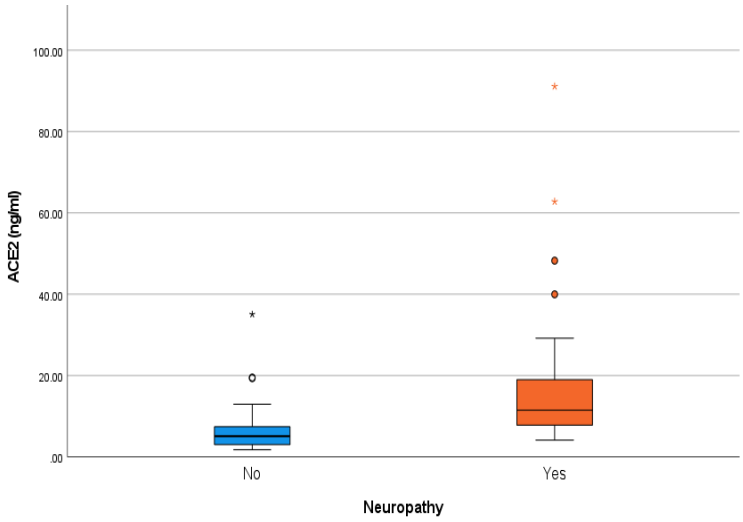

Figure (2): Comparison between cases with and without neuropathy as regarding to ACE2 level.

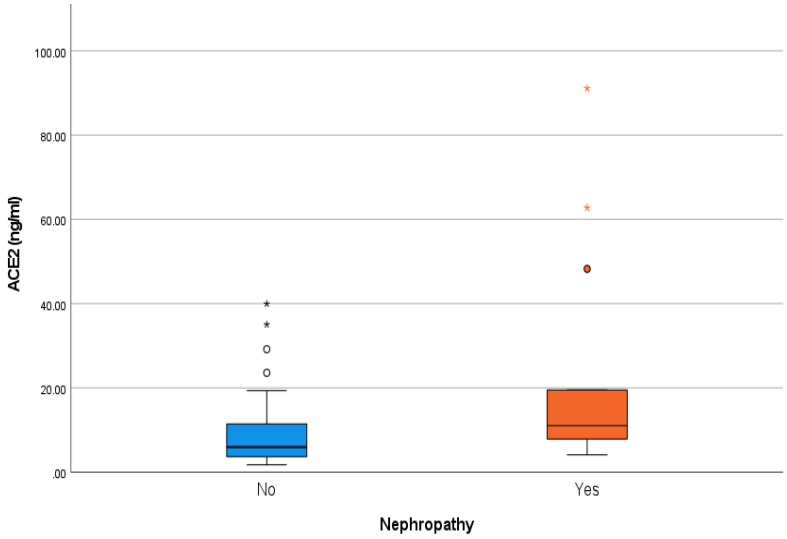

Figure (3): Comparison between cases with and without nephropathy as regarding to ACE2 level.

Table (7): ROC curve analysis for ACE2 to predict neuropathy within cases group.

AUC	95% CI	Sig.	Cut-off value	Sensitivity	Specificity	+PV	-PV
0.805	0.669 - 0.903	<0.001	>5.873	91.3%	62.96%	67.7	89.5

AUC: Area Under curve, CI: Confidence interval, P: P-value, S: Significant P-value < 0.05.

Table (7) shows ROC analysis done to assess the performance of serum ACE2 to detect diabetic neuropathy in cases with type I DM; AUC was 0.805 (95% confidence interval: 0.669-0.903), p<0.001. At a cutoff point > 5.873 ng/ml, the sensitivity was 91.3% and specificity was 62.96%.

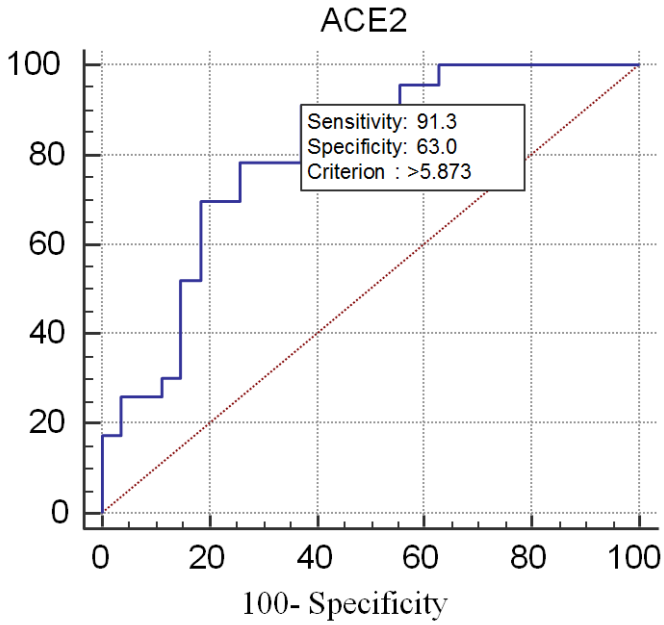

Figure (4): ROC curve analysis for ACE2 to predict neuropathy within cases group

Table (8): ROC curve analysis for ACE2 to predict nephropathy within cases group.

AUC	95% CI	Sig.	Cut-off value	Sensitivity	Specificity	+PV	-PV
0.745	0.603 - 0.888	0.001	>6.664	85.71%	63.89%	48	92

AUC: Area Under curve, CI: Confidence interval, P: P-value, S: Significant P-value < 0.05.

Table (8) shows ROC analysis done to assess the performance of serum ACE2 to detect diabetic nephropathy in cases with type I DM; AUC was 0.0.745 (95% confidence interval: 0.603-0.888), p=0.001. At a cutoff point > 6.664 ng/ml, the sensitivity was 85.71% and specificity was 63.89%.

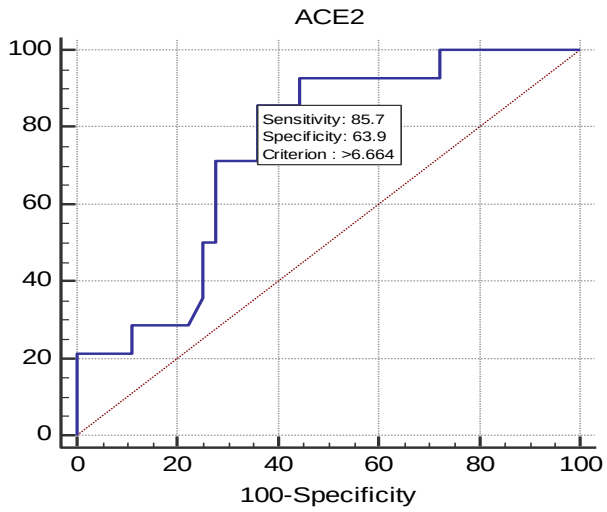

Figure (5): ROC curve analysis for ACE2 to predict nephropathy within cases group

DISCUSSION

Our study revealed no statistically significant differences in gender distribution, age, BMI, BMI z-scores, systolic blood pressure (SBP), diastolic blood pressure (DBP), or Tanner staging between children with Type 1 Diabetes Mellitus and healthy control indicating similar baseline characteristics. However, significant differences were observed in weight and height z-scores, with cases having higher weight z-scores (p=0.019) and height z-scores (p=0.031) compared to controls. These findings suggest that while general growth parameters like BMI remain comparable, subtle differences in weight and height z-scores may reflect underlying variations in growth patterns or metabolic status influenced by Type 1 Diabetes Mellitus. Overall, the groups are well-matched demographically, allowing for a focused assessment of other study parameters.

The socio-demographic findings in this study align with evidence from similar research highlighting growth pattern variations in children with Type 1 Diabetes Mellitus (T1DM). ***Sherif et al. (2014)*** reported that anthropometric measures, including weight and height, were closely linked to diabetes management and complications, with increased BMI and weight z-scores associated with disease progression. The absence of significant differences in BMI and blood pressure between cases and controls in this study is consistent with their findings, which noted that systolic and diastolic blood pressures in T1DM patients were often within normal ranges but correlated with vascular parameters such as carotid intima-media thickness (CIMT). These studies collectively support the observed demographic similarities and subtle growth differences in T1DM cases, underscoring the importance of further exploration into how metabolic changes influence growth trajectories in diabetic children.

The comparison of laboratory investigations between cases and controls revealed significant differences in several parameters. Cases have significantly higher triglyceride (TGs) levels and total cholesterol levels,

indicating a dyslipidemic profile associated with Type 1 Diabetes Mellitus. However, there were no statistically significant differences in HDL or LDL cholesterol levels, despite trends suggesting slightly lower HDL and higher LDL in cases. These findings highlight the metabolic and biochemical changes in diabetic children that may contribute to their increased risk of vascular complications.

The observed dyslipidemic profile in children with Type 1 Diabetes Mellitus (T1DM) is consistent with previous studies highlighting the metabolic disturbances associated with diabetes. ***Makita et al., (1999)*** emphasized the role of glycation in promoting LDL uptake into macrophages, contributing to atherosclerosis, which aligns with the trends of higher LDL levels in T1D. The elevated triglyceride and total cholesterol levels observed in this study are further supported by findings from ***Gül et al. (2010),*** who reported similar lipid abnormalities correlating with vascular risk in T1DM patients. Furthermore, the significantly higher circulating ACE2 levels in cases compared to controls are consistent with ***Soro-Paavonen et al. (2012)***, who identified increased ACE2 activity in T1DM patients with vascular complications, highlighting its potential as a biomarker for vascular risk. These studies collectively support the metabolic and vascular changes observed in diabetic children and underscore the importance of monitoring dyslipidemia and ACE2 levels as early indicators of complications.

The echocardiographic findings between cases and controls showed some significant differences, while most parameters remained comparable. Notably, the left ventricular end-diastolic diameter (LV EDD) was significantly higher in cases compared to controls (p=0.024), indicating potential early cardiac remodeling associated with diabetes. Additionally, the right ventricular end-systolic diameter (RV ESD) was significantly lower in cases (p=0.002), which may reflect subtle differences in right ventricular function. However, other parameters, such as left atrium to aorta ratio (LA/AO), ejection fraction (EF), E/A ratio, myocardial performance index (MPI), and tissue Doppler measures like TAPSE and MAPSE, did not differ significantly, suggesting preserved overall cardiac function. Similarly, carotid intima-media thickness (CIMT) was comparable between the groups, indicating no significant early vascular changes in the studied population. These findings suggest that while cardiac remodeling might begin early in diabetic children, functional impairments are not yet evident.

The echocardiographic findings in this study align with previous research highlighting early cardiac remodeling in children with Type 1 Diabetes Mellitus (T1DM). ***Margeirsdottir et al. (2010)*** reported subtle structural changes in the hearts of diabetic children despite preserved systolic function, suggesting that diabetes-induced cardiac remodeling may begin before functional decline occurs. Similarly, ***Gül et al. (2010)*** found that increased left ventricular dimensions in T1DM patients were associated with early myocardial changes, consistent with the observed increase in left ventricular end-diastolic diameter (LV EDD) in this study. The significantly lower right ventricular end-systolic diameter (RV ESD) may reflect early myocardial adaptation, although the absence of significant differences in parameters such as ejection fraction (EF) and myocardial performance index (MPI) supports the notion of preserved global cardiac function. Additionally, studies such as ***Jarvisalo et al. (2002)*** emphasize the predictive role of carotid intima-media thickness (CIMT) in vascular changes; however, its

comparability between cases and controls in this study suggests that major vascular remodeling has not yet occurred. These findings collectively indicate that while early cardiac structural changes may be present in diabetic children, functional impairments remain minimal, reinforcing the need for long-term monitoring of cardiovascular health in T1DM.

In the complicated cases group, the correlation analysis between ACE2 levels and echocardiographic findings revealed significant positive associations with right ventricular end-diastolic diameter (RV EDD) and carotid intima-media thickness (CIMT), suggesting that higher ACE2 levels may be linked to right ventricular dilation and early vascular changes in children with Type 1 Diabetes Mellitus and complications. No significant correlations were observed with other echocardiographic parameters, including left atrium-to-aorta ratio (LA/AO), ejection fraction (EF), left and right ventricular systolic diameters (LV ESD, RV ESD), E/A ratio, or myocardial performance indices (RV MPI, LV MPI). While mitral annular plane systolic excursion (MAPSE) showed a moderate positive correlation and tricuspid annular plane systolic excursion (TAPSE) exhibited a negative correlation, neither reached statistical significance. These findings suggest that in diabetic children with complications, ACE2 levels may be associated with structural and vascular alterations, particularly in the right ventricle and carotid arteries, potentially indicating early cardiovascular remodeling.

The observed correlations between ACE2 levels, right ventricular end-diastolic diameter (RV EDD), and carotid intima-media thickness (CIMT) in complicated Type 1 Diabetes Mellitus (T1DM) cases align with prior studies linking ACE2 to cardiovascular remodeling in diabetes. ***Soro-Paavonen et al., (2012,*** found increased ACE2 activity in T1DM patients with vascular complications, supporting its role in vascular changes such as CIMT thickening. Similarly, ***Gül et al., (2010)*** demonstrated a significant association between CIMT and diabetic complications, reinforcing its utility as an early marker of atherosclerosis. The strong correlation between ACE2 and RV EDD suggests potential right ventricular dilation, which aligns with findings from ***Epelman et al., (2009)*** that ACE2 is upregulated in myocardial stress conditions. These findings collectively highlight ACE2 as a potential biomarker for structural and vascular alterations in diabetic children with complications.

The analysis of the relationship between ACE2 levels and gender, neuropathy, and nephropathy within the cases group revealed significant associations with diabetic complications but not with gender. There was no significant difference in ACE2 levels between males (median: 7.73 ng/ml, IQR: 4.3–11.99) and females (median: 6.66 ng/ml, IQR: 5.53–11.07; p=0.894), indicating that ACE2 expression is not influenced by sex in this cohort. However, ACE2 levels were significantly higher in cases with neuropathy (median: 11.5 ng/ml, IQR: 7.73–23.61) compared to those without (median: 5.11 ng/ml, IQR: 2.95–8.17; $p<0.001$), suggesting a potential role of ACE2 in diabetic nerve complications. Similarly, cases with nephropathy had significantly elevated ACE2 levels (median: 11.04 ng/ml, IQR: 7.87–19.52) compared to those without nephropathy (median: 5.98 ng/ml, IQR: 3.71–11.47; p=0.008), highlighting a possible link between ACE2 and kidney dysfunction in diabetic children. These findings suggest that ACE2 may serve as a potential biomarker for identifying diabetic neuropathy and nephropathy, warranting further investigation into its clinical significance. The observed associations

between ACE2 levels and diabetic complications, particularly neuropathy and nephropathy, align with prior research suggesting that ACE2 plays a role in diabetes-related vascular and renal dysfunction. ***Soro-Paavonen et al. (2012)*** reported increased ACE2 activity in T1DM patients with vascular complications, supporting its involvement in endothelial dysfunction and microvascular changes, which are key mechanisms underlying diabetic neuropathy and nephropathy. Similarly, ***Kalousová et al. (2006)*** found elevated soluble ACE2 levels in patients with impaired renal function, reinforcing its potential as a biomarker for nephropathy. The significant elevation of ACE2 in neuropathic cases is consistent with findings from ***Suzuki et al. (2006),*** who demonstrated that the expression of advanced glycation end products (AGEs) and their receptors (RAGE) is strongly linked to diabetic nerve damage.

The ROC curve analysis for ACE2 in predicting neuropathy within the cases group demonstrated a strong diagnostic performance, with an area under the curve (AUC) of 0.805 (95% CI: 0.669–0.903, $p < 0.001$), indicating good discriminatory ability. The optimal cut-off value for ACE2 was identified as >5.873 ng/ml, which achieved a high sensitivity of 91.3%, meaning it correctly identified most cases with neuropathy. The specificity was moderate at 62.96%, indicating some false positives. The positive predictive value (+PV) was 67.7%, suggesting that a patient with ACE2 levels above this threshold has a fair probability of having neuropathy, while the negative predictive value (-PV) was high at 89.5%, indicating that ACE2 levels below the cut-off strongly predict the absence of neuropathy. These findings suggest that ACE2 could be a useful biomarker for early detection of diabetic neuropathy, allowing for timely intervention and management in children with Type 1 Diabetes Mellitus. The strong diagnostic performance of ACE2 in predicting diabetic neuropathy, as indicated by the ROC curve analysis ($p < 0.001$), aligns with previous studies highlighting ACE2 as a potential biomarker for diabetes-related complications. ***Soro-Paavonen et al. (2012)*** reported elevated ACE2 activity in patients with vascular complications, suggesting its role in endothelial dysfunction, which is a key contributor to diabetic neuropathy.

Similarly, the ROC curve analysis for ACE2 in predicting nephropathy within the cases group demonstrated a strong diagnostic performance, with an area under the curve (AUC) of 0.745 (95% CI: 0.603–0.888, $p = 0.001$), indicating good discriminatory ability. The optimal cut-off value for ACE2 was identified as >6.664 ng/ml, which achieved a high sensitivity of 85.71%, meaning it correctly identified most cases with nephropathy. The specificity was moderate at 63.89%, indicating some false positives. The positive predictive value (+PV) was 48%, suggesting that a patient with ACE2 levels above this threshold has a fair probability of having nephropathy, while the negative predictive value (-PV) was high at 92%, indicating that ACE2 levels below the cut-off strongly predict the absence of neuropathy. ***Kalousová et al. (2006)*** found elevated soluble ACE2 levels in patients with impaired renal function, reinforcing its potential as a biomarker for nephropathy.

CONCLUSION

Children and adolescents with Type 1 Diabetes Mellitus have elevated levels of circulating ACE2, particularly when associated with neuropathy and nephropathy. The echocardiographic finding of significant increase in the left ventricular end-diastolic diameter (LV EDD) in our cohort of type 1 diabetic patients, indicates potential early cardiac remodeling associated with diabetes. The observed correlations between ACE2 levels, right ventricular end-diastolic diameter (RV EDD), and carotid intima-media thickness (CIMT) in T1DM patients with vascular complications reinforcing its utility as an early marker of cardiac and early vascular changes, such as subclinical atherosclerosis,

Understanding the dynamics of ACE2 in T1D could open new avenues for therapeutic interventions, aiming to reduce the risk of vascular complications and improve outcomes for patients with

RECOMMENDATIONS

Given the significant association of ACE2 with neuropathy and nephropathy, assessment of circulating ACE2 levels in children and adolescents with Type 1 Diabetes Mellitus may aid in early detection of vascular complications.

Further studies should explore the clinical application of ACE2 as a reliable biomarker for diabetic complications and its potential role in monitoring disease progression and treatment response with a focus on defining standardized cut-off values for risk stratification.

Routine echocardiographic evaluations should be considered for diabetic children, especially those with elevated ACE2 levels, to identify early cardiovascular involvement and subclinical atherosclerosis.

LIMITATIONS

Collection data from old files and clinical examination including tanner staging.

Collection of blood sample from patients to assess ACE2 and arranging appointment for Echocardiography.

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