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Evaluation of Serum Soluble Cd40l as Marker of Nephropathy in Type 1 Diabetic Children and Adolescents

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ABSTRACT

Background: Diabetes is a prevalent disease, with increasing incidence in young individuals. Diabetic nephropathy (DN), a critical and potentially reversible complication if detected early, necessitates the identification of ideal early predictors. The interaction between CD40 and its soluble ligand (sCD40L) may play a key role in the vascular inflammation that initiates diabetic microangiopathy.

Objective: This study aimed to evaluate the potential of serum soluble CD40L for the early detection of kidney involvement in children and adolescents with type 1 diabetes. The goal was to enable timely intervention to prevent further complications and facilitate treatment if possible.

Patients and methods: Ninety children and adolescents (47 females, 43 males with mean age of 14.73 ± 1.7 years, mean diabetes duration of 7.2 ± 2.5 years and 60 with T1DM) were included. Participants underwent comprehensive evaluations, including medical history, clinical examination, microalbuminuria assessment (urine albumin/creatinine ratio), and serum sCD40L level measurements using ELISA.

Results: There was a highly significant increase in sCD40L levels in both the microalbuminuric and normo-albuminuric diabetic groups compared to the control group ($p < 0.001$). Notably, sCD40L levels were significantly higher in the microalbuminuric group compared to the normo-albuminuric and control groups (median 1.40 ± 0.9 ng/mL vs. 0.83 ± 0.09 ng/mL, $p < 0.001$). Additionally, the microalbuminuric group exhibited significantly increased levels of FBS, HbA1c, SGOT, and SGPT ($p < 0.001$, $p = 0.002$).

Conclusion: The increased serum sCD40L levels seen in children and adolescents with T1DM, especially those with microalbuminuria, and its positive correlation with the duration of diabetes, urinary albumin excretion, and glycemic control suggest that sCD40L may be involved in diabetic vasculopathy in this age group. This study emphasized sCD40L's potential as a promising marker for diabetic nephropathy.

Keywords: T1DM, SCD40L, DN, MA, Risk factors.

INTRODUCTION

Diabetic nephropathy (DN) stands as the primary etiological factor in the advancement of chronic kidney disease (CKD) and the subsequent development of end-stage renal disease (ESRD), thereby emphasizing the existing limitations and relative inadequacy of contemporary therapeutic interventions in effectively safeguarding individuals with diabetes mellitus from the insidious decline of their renal function. This highlights a critical area of unmet clinical need and underscores the imperative for the development of more effective strategies to prevent and manage this significant long-term complication of diabetes. The underlying mechanisms driving the progression of CKD in DN are intricate and not fully understood. Notably, various factors, with ischemic acute kidney injury being a significant contributor, can exacerbate DN and accelerate the progression of CKD to ESRD [1].

Microalbuminuria (MA) serves as a robust and clinically pertinent biomarker, signifying a heightened susceptibility in individuals with diabetes mellitus towards the future development of diabetic nephropathy and a progressive reduction in renal function [2].

Therefore, the successful implementation of proactive primary prevention strategies aimed at mitigating the burden of diabetic nephropathy is fundamentally reliant on the accurate identification and subsequent efficacious management of the etiological and pathophysiological factors that initiate the transition from a state of normal urinary albumin excretion to the early pathological stage of microalbuminuria, and subsequently, to the formation of established, clinically significant diabetic nephropathy [3].

Within this framework, the prompt commencement of therapeutic interventions, characterized by a comprehensive and sustained effort to achieve and maintain normoglycemic control, alongside the meticulous optimization of systemic blood pressure regulation within target ranges, and the judicious and evidence-based application of angiotensin-converting enzyme (ACE) inhibitors, represents a significant and promising clinical strategy. Such early and targeted interventions hold considerable potential for effectively impeding or even ameliorating the detrimental pathological cascade that leads from the initial manifestation of microalbuminuria (MA) to the more

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advanced and clinically consequential stages of diabetic nephropathy [4]. Thereby potentially preserving renal function and improving long-term outcomes in this at-risk population.

Notwithstanding advancements in the therapeutic management of DN, contemporary treatment modalities have not effectively curtailed the escalating prevalence of progressive CKD. The significant morbidity associated with CKD, and the relentless augmentation in the incidence of end-stage renal disease, necessitate the development and implementation of more efficacious strategies for the prevention and treatment of progressive renal dysfunction [1].

CD40, a protein spanning the cell membrane and classified within the tumor necrosis factor (TNF) receptor superfamily, is characterized by its expression across a broad spectrum of cell types. These include B lymphocytes, macrophages, monocytes, dendritic cells, and endothelial cells, indicating its potential involvement in a variety of immunological and inflammatory processes within different tissues and cellular compartments. The principal ligand for CD40 is CD40 ligand (CD40L), which can be expressed on the surface of activated platelets and also is secreted in a soluble form (sCD40L). Within the renal parenchyma, CD40 expression is upregulated following renal injury, and activation of this receptor triggers the infiltration of inflammatory cells into the kidney's interstitium [5].

Furthermore, activation of the CD40 receptor specifically on the proximal tubular epithelium of the kidney has been demonstrated in experimental models of renal injury to induce fibrosis and inflammation within the renal tissue [6]. This suggests a potential mechanistic role for the CD40/CD40L axis in the pathogenesis and progression of kidney damage in various disease states, including diabetic nephropathy.

AIM OF THE WORK

The primary objective of the present study was the early identification of renal involvement in children and adolescents with type 1 diabetes mellitus (T1DM) through the assessment of serum soluble CD40 ligand (sCD40L) levels. The ultimate objective was to facilitate timely interventions to prevent the progression of renal complications and to implement appropriate treatment strategies when indicated.

PATIENTS AND METHODS

Study Design: A cross-sectional, case control study.

Study Participants

- **Patient group:** Sixty children and adolescents with a confirmed diagnosis of type 1 diabetes mellitus (T1DM) for more than three years. Recruitment occurred prospectively from the Pediatric Department and Outpatient Clinic at the National Institute of Diabetes & Endocrinology (NIDE) between January 1st, 2016, and December 31st, 2016.
- **Control group:** Thirty apparently healthy children and adolescents without a diagnosis of type 1 diabetes, matched with the patient group for age and sex.

Data collection: Both study groups underwent a comprehensive evaluation that included: Detailed medical history. Thorough clinical examination. Precise anthropometric measurements. Relevant laboratory investigations.

Inclusion criteria: Type I diabetic children and adolescents diagnosed according to ADA criteria. Age: 4-18 years old. For the control group: Non diabetic children and adolescents. Age: 4-18 years old.

Exclusion criteria: Patients with non-diabetic renal diseases. Any acute stress conditions. Systemic inflammatory disorder or malignancy. Treatment regimens involving medications other than insulin and captopril. (Note: Captopril administration was specifically for patients in the microalbuminuria group as a treatment for their nephropathy and was not a general exclusion criterion).

METHODS

Each subject was subjected to the following:

- Full history taking. Thorough clinical examination as well as both general and systemic one with special emphasis blood pressure, **Growth assessment by performing anthropometric measurements** including height, weight and body mass index which was calculated using the equation: {BMI = wt (kg) / height (m^2)}.

Investigations included:

- **Assessment of glycemic control** by assessment of HbA**1**c using liquid chromatographic assay method.

- **Assessment of microalbuminuria:**

  Morning urine sample was processed and albumin in urine was measured by turbidimetric measure and creatinine by enzyme colourimetric measure and A/C ratio was calculated. A/C ratio between 30 mg/gm – 300 mg/gm is considered microalbuminuria or positive result and below this range is normoalbuminuria or negative result. If the microalbuminuria is positive, it is repeated and considered positive if it is positive for 2 times in a period of 3 months with negative urinary culture**.**

A) **Specific investigations:** Measurement of serum sCD40L levels was carried out leveraging a specific ELISA.

Ethical approval: Ethical approval for this research was granted from The Ethical Committee of the Faculty of Medicine for Girls, Al-Azhar University, and the Ethical Committee of the National Institute for Diabetes and Endocrinology (NIDE). Before commencing any study procedures, comprehensive written informed consents were carefully attained from the parents or legal guardians of all children and adolescents who participated in the research. This consent was obtained following a comprehensive explanation of the study's objectives, methodology, potential benefits for their children and the broader community, as well as any foreseeable risks associated with their children's involvement. Furthermore, in accordance with ethical guidelines for research involving minors, informed verbal assent was obtained from all children aged over 8 years, subsequent to providing them with a simplified and age-appropriate explanation of the study's purpose and anticipated benefits. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistical analysis

The acquired dataset was conducted utilizing MedCalc software, version 15.8. A comprehensive suite of statistical methodologies was implemented to ascertain the significance of identified disparities and correlations, encompassing one-way analysis of variance (ANOVA), the Chi-square (χ^2) test, multivariate linear regression analysis, receiver operating characteristic (ROC) curve analysis, and Kaplan-Meier survival curve analysis, as appropriate for the nature of the variables under investigation. The presentation of data and the selection of specific statistical methods were tailored to the distributional characteristics of each variable, distinguishing between parametric and non-parametric data. For all statistical tests, a p-value (P) of less than 0.05 was predetermined as the threshold for statistical significance. The statistical significance of the findings was determined based on the calculated p-values, with the following conventions applied: a p-value exceeding 0.05 was interpreted as statistically non-significant (NS), a p-value ≤ 0.05 was considered statistically significant (S), and a p-value ≤ 0.01 was deemed statistically highly significant (HS).

Descriptive statistics:

- **Parametric numerical data:** Expressed as the arithmetic mean, accompanied by the standard deviation (± SD) to quantify data dispersion around the mean, and the range (minimum to maximum values) to delineate the overall data spread.
- **Non-parametric numerical data:** Presented as the median (the central value in the ordered dataset) and the inter-quartile range (IQR), which represents the span between the 25th and 75th percentiles, indicating the variability of the central 50% of the data and offering robustness against outliers.
- **Non-numerical (Categorical) data:** Summarized by reporting the frequency (count) of observations within each distinct category and the corresponding percentage each category represents of the total sample.

Analytical statistics

To determine the statistical significance of differences among group means, the Student's t-test was deployed for comparisons including two groups, while the ANOVA test was leveraged for comparisons including more than two groups. The Chi-square test served to investigate the relationships between pairs of qualitative variables. Linear multivariate regression analysis was conducted to model and estimate the dependence of a quantitative outcome variable on a set of multiple independent variables. The discriminatory ability of quantitative diagnostic measures in classifying cases into two distinct groups was evaluated using the Receiver Operating Characteristic (ROC) curve, which provides insights into sensitivity and specificity. Finally, the Log-rank test, applied to Kaplan-Meier survival curves, was used to measure the statistical significance of differences in survival experiences between two study groups over a specified period.

RESULTS

Demographic data for the study subjects highlight their age and gender distribution. The adolescent age range (12-18 years) with a mean of 14.73±1.7 years is key to interpreting the study's findings. (Table 1) A near-equal gender split (47.8% male, 52.2% female) ensures a balanced cohort, minimizing gender-related biases. (Table 1) For studies involving comparative analyses, incorporating statistical measures like p-values for demographics would further strengthen academic rigor, confirming group comparability (Table 1).

Table (1): Demographic data of patients and control groups enrolled in the study

Variables		Study subjects (No 90)		
		Range	Mean ± SD	No (%)
Age (years)		12 – 18	14.73 ± 1.7	
Gender	Male			43 (47.8%)
	Female			47 (52.2%)

Basic clinical data for the study participants are summarized. The mean BMI of 21.41±4.74 suggests a healthy average weight, though the wide range indicates variability. (Table 2) Mean waist circumference (71.11±10.89 cm) and mean arterial pressure (85.52±9.35 mmHg) are generally within healthy limits. (Table 2) The average duration of diabetes mellitus (7.2±2.5 years) for the diabetic subgroup provides crucial context for disease chronicity (Table 2).

Table (2): Basic clinical data of patients and control groups enrolled in the study

Variables	Study subjects, No (90)	
	Range	Mean ± SD
BMI	15.15 – 33.73	21.41 ± 4.74
W.C (cm)	51 – 101	71.11 ± 10.89
MAP (mmHg)	65 – 110	85.52 ± 9.35
Duration of DM (years)*	3.5 – 14	7.2 ± 2.5

*Diabetes mellitus duration was assessed in 60 DM cases only, **W.C:** Waist circumference, **MAP:** Mean arterial pressure, **BMI:** Body mass index, **DM:** Diabetes mellitus.

Comprehensive laboratory data offer insights into the subjects' metabolic and inflammatory status. Elevated mean FBS (173.1±120.82 mg/dL) and HbA1c (7.72±2.62 mg/dL) indicate poor glycemic control, with significant individual variation.

(Table 3) Lipid profiles show a risk of dyslipidemia in some participants, despite average values. (Table 3) Crucially, evidence of microalbuminuria in a subset of individuals points to early renal involvement, particularly important in a diabetic population (Table 3).

Table (3): Basic laboratory data of patients and control groups enrolled in the study

Variables	Study subjects, No (90)
	Mean ± SD
Hb (g/dL)	12.72 ± 1.26
Platelets (10^3/µL)	262.21 ± 66.21
TLC (10^3/µL)	6.54 ± 1.75
FBS (mg/dL)	173.1 ± 20.82
HbA_1C (mg/dL)	7.72 ± 1.62
SGOT (U/L)	27.01 ± 2.64
SGPT (U/L)	24.61 ± 15.22
Total Cholesterol (mg/dl)	165.73 ± 46.5
TGs (mg/dl)	98.16 ± 66.44
HDL Cholesterol (mg/dl)	49.47 ± 11.21
LDL Cholesterol (mg/dl)	103.83 ± 33.62
Urea (mg/dl)	23.06 ± 6.71
Creatinine (mg/dl)	0.72 ± 0.13
Urinary microalbuminuria	29.25 ± 66.23
A/C ratio*	17.51 ± 20.46
sCD40L (ng/dl)	**0.899** ± **0.15**

Hb: Hemoglobin, **TLC:** Total leucocytic count, **FBS:** Fasting blood sugar, **HbA1c:** Glycosylated hemoglobin, **SGPT:** Alanine transaminase, **SGOP:** Aspartate transaminase **TGs:** Tri-glycerides, **LDL:** Low-density lipoprotein, **HDL:** High-density lipoprotein, **A/C:** Albumin/creatinine ratio, **sCD40L:** Soluble CD 40 legend.

Comparative study between the 3 groups revealed; highly significant decrease in Z-scores of BMI, height and weight in microalbuminuric DM group; compared to normo-albuminuric and control groups; with highly significant statistical difference ($p = 0.02$, $p < 0.001$ respectively) (Table 4).

Table (4): Comparison between the 3 groups as regards Z score for body weight, height and body mass index

Variable	Control Group (30)	Normo-albuminuric DM Group (30)	Microalbuminuric DM Group (30)	ANOVA test	
				Z•	P-value
BMI Z-score#	0.44 (-0.22 – 0.67)	-0.31 (-0.58 – 0.63)	-0.46 (-0.91 – 0.41)	7.82	= 0.02**
Height Z-score (cm)	0.6 (0.32 – 0.82)	0.049 (-0.72 – 0.43)	-0.005 (-1.58 – 0.54)	19.37	< 0.001**
Weight Z-score (kg)	0.42 (0.15 – 1.05)	-0.27 (-0.87 – 0.43)	-0.36 (-1.32 – 0.43)	16.03	< 0.001**

#Median (**IQR:** Interquartile range) for Z-scores.

The comparative analysis across the three study groups (microalbuminuric diabetic, normo-albuminuric diabetic, and control) revealed highly significant increase in both age and mean arterial pressure (MAP) within the microalbuminuric diabetic group when contrasted to the normo-albuminuric and control groups, demonstrating statistically significant differences ($p < 0.001$ and $p = 0.014$, respectively). When comparing solely the two diabetic groups, the microalbuminuric diabetic group exhibited a highly significant increase in the duration of their diabetes mellitus compared to the normo-albuminuric group ($p < 0.001$). Conversely, the comparative analysis across all three groups indicated a non-significant difference with respect to waist circumference (W.C.) ($p > 0.05$) (Table 5).

Table (5): Comparison between the 3 groups as regards some basic demographic and clinical data using one-way ANOVA test

Variable	Control group (30)	Normo-albuminuric DM group (30)	Microalbuminuric DM group (30)	ANOVA test	
	Mean ± SD	Mean ± SD	Mean ± SD	F-ratio	p value
Age (years)	14.23 ± 1.3	13.76 ± 1.27	16.2 ± 1.44	27.63	< 0.001**
W.C (cm)	72.8 ± 5.94	70.63 ± 12.28	69.9 ± 13.16	0.56	0.568
MAP (mmHg)	81.66 ± 7.11	86.5 ± 7.7	88.41 ± 11.56	4.46	=0.014**
Duration of DM (years)*	-----	5.41 ± 1.36	9 ± 2.06	0.00	< 0.001**

*Diabetes mellitus duration was assessed in 60 DM cases only. #ANOVA: analysis of variance. #logarithmic transformation was done to non-parametric data.

Table (6): Comparison between the 3 groups as regards gender using Chi square test

Variable		Control Group (30)	Normo-albuminuric DM group (30)	Microalbuminuric DM group (30)	p value
Gender	Female	18 (60%)	17 (56.7%)	12 (40%)	= 0.251
	Male	12 (40%)	13 (43.3%)	18 (60%)	

* Percentage of Column Total.

The comparative analysis across the three study groups (Microalbuminuric diabetic, normo-albuminuric diabetic, and control) demonstrated a highly significant decrease in both platelet count and total leukocyte count (TLC) in the microalbuminuric diabetic group when compared to both the normo-albuminuric diabetic and control groups. Furthermore, the normo-albuminuric diabetic group also exhibited a highly significant decrease in platelet count and TLC when compared to the control group ($p < 0.001$ and $p = 0.002$, respectively). Conversely, the same comparative analysis revealed a highly significant increase in fasting blood sugar (FBS), glycated hemoglobin (HbA1c), serum glutamic-oxaloacetic transaminase (SGOT), and serum glutamic-pyruvic transaminase (SGPT) in the microalbuminuric diabetic group when compared to both the normo-albuminuric diabetic and control groups. Similarly, the normo-albuminuric diabetic group also showed a highly significant increase in these metabolic and liver enzyme markers when compared to the control group ($p < 0.001$ and $p = 0.002$, respectively). Finally, the comparative analysis across all three study groups indicated a non-significant difference with respect to hemoglobin levels ($p > 0.05$) (Table 7).

Table (7): Comparison between the 3 groups as regards basic laboratory data using one-way ANOVA test

Variable	Control Group (30)	Normo-albuminuric DM group (30)	Microalbuminuric DM group (30)	ANOVA test	
	Mean ± SD	Mean ± SD	Mean ± SD	F-ratio	p value
Hb (g/dL)	13.03 ± 1.22	12.7 ± 1.05	12.44 ± 1.46	1.671	= 0.194
Platelets (10^3/µL)	309 ± 68.31	256.53 ± 44.2	221.1 ± 52.81	18.7	< 0.001**
TLC (10^3/µL)	7.39 ± 1.56	6.33 ± 1.84	5.89 ± 1.54	6.44	= 0.002**
FBS (mg/dL)	74.56 ± 10.08	199.76 ± 22.12	244.96 ± 16.88	24.45	< 0.001**
HbA_1C (mg/dL)	5.15 ± 0.23	7.98 ± 1.8	10.03 ± 2.32	61.93	< 0.001**
SGOT (U/L)	18.26 ± 3.05	26.33 ± 4.3	36.43 ± 5.91	9.51	< 0.001**
SGPT (U/L)	19.03 ± 4.39	22.5 ± 1.06	32.3 ± 2.19	6.94	= 0.002**

The comparative analysis across the three study groups (Microalbuminuric diabetic, normo-albuminuric diabetic, and control) demonstrated a highly significant increase in both serum creatinine and the soluble CD40 ligand (sCD40L) marker in both the microalbuminuric diabetic and normo-albuminuric diabetic groups when compared to the control group, with highly significant statistical differences observed ($p < 0.001$ for both). Furthermore, this analysis also revealed a highly significant increase in the sCD40L marker specifically in the microalbuminuric diabetic group when compared to both the normo-albuminuric diabetic group and the control group, again with highly significant statistical differences ($p < 0.001$ for both comparisons). The comparative study also showed a highly significant increase in urinary albumin levels and the albumin-to-creatinine ratio (A/C ratio) in the microalbuminuric diabetic group when compared to the normo-albuminuric diabetic group, with a highly significant statistical difference ($p < 0.001$). Finally, the comparative analysis across all three study groups indicated a non-significant difference with respect to serum urea levels ($p > 0.05$) (Table 8).

Table (8): Comparison between the 3 groups as regards renal laboratory data and sCD40L using one-way ANOVA test

Variable	Control group (30)	Normo-albuminuric DM Group (30)	Microalbuminuric DM group (30)	ANOVA test	
	Mean ± SD	Mean ± SD	Mean ± SD	F-ratio	p value
Urea (mg/dl)	23.53 ± 4.96	23.73 ± 3.62	21.93 ± 2.62	0.64	= 0.528
Creatinine (mg/dl)	0.64 ± 0.06	0.73 ± 0.12	0.78 ± 0.15	10.46	< 0.001**
Urinary microalbumin	10.53 ± 2.8	10.79 ± 2.07	96.31 ± 6.95	28.73	< 0.001**
A/C ratio*	10.32 ± 2.73	10.81 ± 2.88	144.52 ± 35.61	15.59	< 0.001**
sCD40L (ng/dl)	**0.56 ± 0.19**	**0.83 ± 0.09**	**1.40 ± 0.09**	**14.9**	**< 0.001****

Correlation studies: Correlation studies was performed to investigate the relationships between the soluble CD40 ligand (sCD40L) marker and a selection of fundamental clinical and laboratory independent variables. These associations were analyzed using multivariate regression analysis to determine the independent predictive value of these variables on sCD40L levels, while controlling for potential confounding factors. Multivariate regression analysis, employing a backward elimination method and incorporating several clinical and laboratory predictor variables, revealed that none of the examined factors demonstrated an independent statistically significant effect on the circulating levels of the sCD40L marker ($P > 0.05$). This suggests that within the context of this model and the included variables, the sCD40L levels were not independently predicted by any single clinical or laboratory parameter (Table 9).

Table (9): Multivariate regression model for the Factors affecting sCD40L marker in control group using backward method

Independent variables	Coefficient	Std. Error	$r_{partial}$	t	p value	Sig.
(Constant)	-1.1597					
Age	0.07368	0.03930	0.3394	1.875	0.0717	NS
BMI	---	---	---	---	---	NS
W.C	---	---	---	---	---	NS
MAP	---	---	---	---	---	NS
Hb	---	---	---	---	---	NS
Platelets	---	---	---	---	---	NS
TLC	---	---	---	---	---	NS
FBS	---	---	---	---	---	NS
HbA_1C	---	---	---	---	---	NS
SGOT	---	---	---	---	---	NS
SGPT	---	---	---	---	---	NS
Total Cholesterol	---	---	---	---	---	NS
TGs	---	---	---	---	---	NS
HDL Cholesterol	0.01160	0.006413	0.3288	1.809	0.0816	NS
LDL Cholesterol	---	---	---	---	---	NS
Urea	---	---	---	---	---	NS
Creatinine	---	---	---	---	---	NS

--- excluded from the model if (p value > 0.1)

Multivariate regression analysis, utilizing a backward elimination approach and incorporating various predictor variables, demonstrated that elevated levels of serum glutamic-pyruvic transaminase (SGPT), total cholesterol, and low-density lipoprotein cholesterol (LDL-C), as well as decreased levels of high-density lipoprotein cholesterol (HDL-C), independently contributed to an increase in the soluble CD40 ligand (sCD40L) level, with statistically significant associations observed for each of these lipid and liver enzyme parameters ($p < 0.05$ for each) (Table 10).

Table (10): Multivariate regression model for the Factors affecting sCD40L marker in normo-albuminuric DM group using backward method

Independent variables	Coefficient	Std. Error	$r_{partial}$	t	p value	Sig.
(Constant)	4.0225					
Age	---	---	---	---	---	NS
BMI	---	---	---	---	---	NS
W.C	---	---	---	---	---	NS
MAP	---	---	---	---	---	NS
Duration of DM	---	---	---	---	---	NS
Hb	---	---	---	---	---	NS
Platelets	---	---	---	---	---	NS
TLC	---	---	---	---	---	NS
FBS	---	---	---	---	---	NS
HbA_1C	---	---	---	---	---	NS
SGOT	---	---	---	---	---	NS
SGPT	0.03041	0.01064	0.4961	2.857	0.0085**	S
Total Cholesterol	---	---	---	---	---	NS
TGs	0.006671	0.002478	0.4741	2.692	0.0125*	S
HDL Cholesterol	-0.02642	0.009755	-0.4763	-2.709	0.012*	S
LDL Cholesterol	0.01317	0.005328	0.4431	2.472	0.0206*	S
Urea	---	---	---	---	---	NS
Creatinine	---	---	---	---	---	NS
Urinary albumin	---	---	---	---	---	NS
A/C ratio	---	---	---	---	---	NS

--- excluded from the model if (p value > 0.1).

Multivariate regression analysis, employing a backward elimination method and incorporating several predictor variables, revealed that increases in waist circumference (W.C.), mean arterial pressure (MAP), duration of diabetes mellitus (DM), fasting blood sugar (FBS), serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT), triglycerides (TGs), total cholesterol, low-density lipoprotein cholesterol (LDL-C), serum creatinine, and albumin-to-creatinine ratio (A/C ratio), along with decreases in platelet count and high-density lipoprotein cholesterol (HDL-C), all independently contributed to an increase in the soluble CD40 ligand (sCD40L) level, with statistically significant associations observed for each of these parameters ($p < 0.05$ for each) (Table 11).

Table (11): Multivariate regression model for the factors affecting sCD40L marker in microalbuminuric DM group using backward method

Independent variables	Coefficient	Std. Error	$r_{partial}$	t	p value	Sig.
(Constant)	7.885					
Age	---	---	---	---	---	NS
BMI	0.1231	0.07020	0.4125	1.754	0.0999	NS
W.C	0.07361	0.03012	0.5337	2.444	0.0274*	S
MAP	0.06488	0.01740	0.6936	3.729	0.002**	S
Duration of DM	0.1935	0.07200	0.5701	2.688	0.0169*	S
Hb	---	---	---	---	---	NS
Platelets	-0.005779	0.002301	-0.5441	-2.511	0.024*	S
TLC	---	---	---	---	---	NS
FBS	0.002902	0.001100	0.5628	2.637	0.0187*	S
HbA_1C	---	---	---	---	---	NS
SGOT	0.05036	0.01475	0.6614	3.415	0.0038**	S
SGPT	0.04443	0.01511	0.6048	2.941	0.0101*	S
Total Cholesterol	0.06639	0.01568	0.7379	4.234	0.0007**	S
TGs	0.006109	0.002645	0.5121	2.309	0.0356*	S
HDL Cholesterol	-0.03354	0.01368	-0.5348	-2.451	0.027*	S
LDL Cholesterol	0.06428	0.01617	0.7164	3.977	0.0012**	S
Urea	---	---	---	---	---	NS
Creatinine	2.2890	0.9847	0.5146	2.325	0.0345*	S
Urinary albumin	---	---	---	---	---	NS
A/C ratio	-0.002268	0.000563	0.7206	4.026	0.0011**	S

--- excluded from the model if (p value > 0.1).

ROC curve analysis: By doing Roc curve between the microalbuminuric and normoalbuminuric groups, the cutoff value of sCD40L marker was 0.9775. It revealed that sCD40L marker is highly sensitive and specific with excellent accuracy, sensitivity= 87.5% and specificity= 96.7%. So, the diabetic patients were split into 2 groups, the first are those with high sCD40L marker and the second are those with sCD40L marker (Table 12 and figure 1)).

Table (12): Roc-curve of sCD40L marker level to discriminate patients with normo-albuminuric DM from patients with microalbuminuric DM

Variable	AUC	SE	Best cut off point (Criterion)	Sensitivity	Specificity	p value	95% CI
sCD40L marker	0.966	0.0181	> 0.9775	87.5%	96.7 %	<0.0001**	0.902 to 0.993

ROC (Receiver operating characteristic), AUC= Area under curve, SE= Standard Error

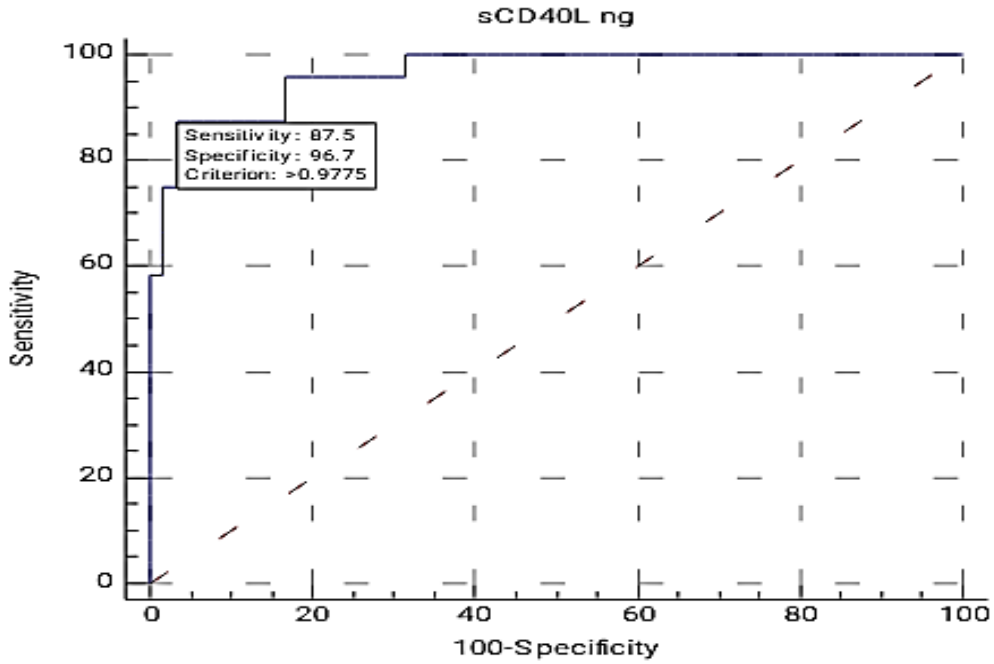

Figure (1): By using ROC-curve, sCD40L marker level at a cutoff point >0.9775 discriminated patients with normo-albuminuric DM from patients with microalbuminuric DM, with excellent accuracy, sensitivity= 87.5% and specificity= 96.7%.

Survival analysis: Survival analysis (regarding **microalbuminuria** and **duration of DM**) was conducted with "Kaplan-Meier survival analysis". Survival analysis indicated an increasing incidence of observed microalbuminuric diabetic mellitus (DM) events over the study period, with a calculated mean time to the development of microalbuminuria of 9 years (Figure 2).

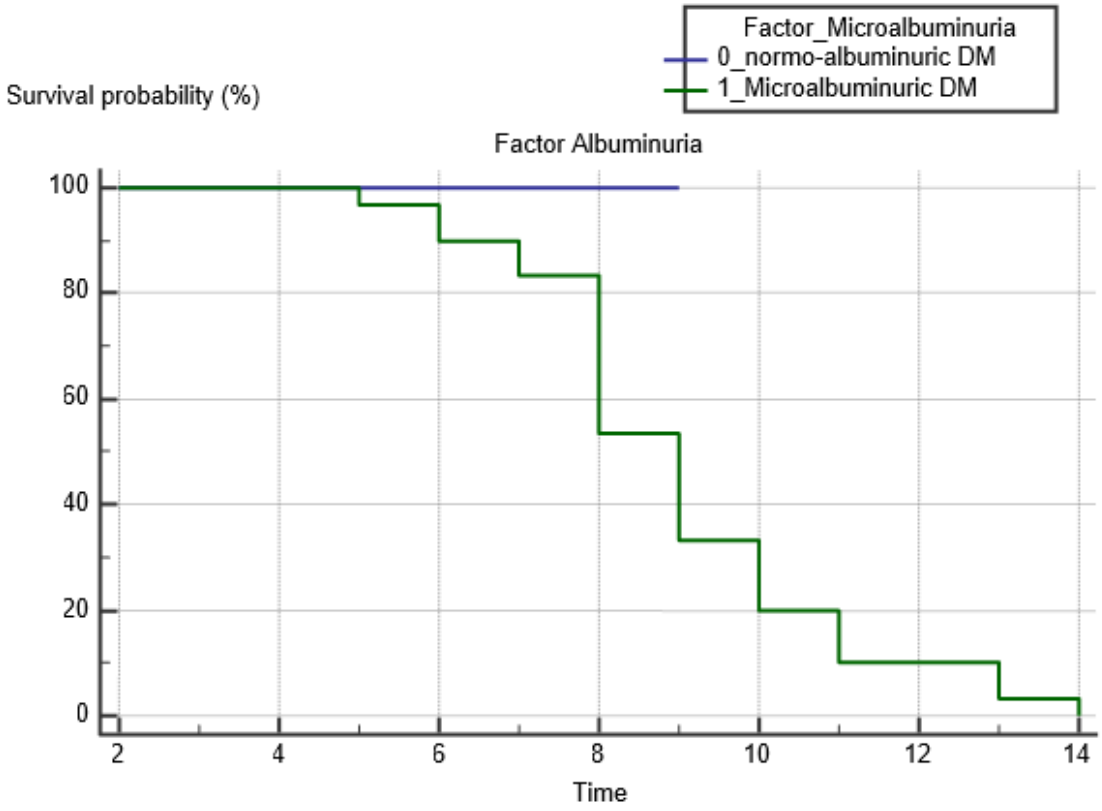

Figure (2): Kaplan-Meier survival curve showing decreased survival probability starting from 9th year of DM.

DISCUSSION

Our comparative analysis involved three groups—individuals with microalbuminuric diabetes mellitus, those with normo-albuminuric diabetes mellitus, and a healthy control group— that demonstrated a statistically highly significant elevation in the soluble CD40 ligand (sCD40L) marker in both diabetic groups when contrasted to the control group ($p < 0.001$ for each comparison). Moreover, sCD40L levels were found to be significantly higher in the microalbuminuric diabetic group specifically when compared to both the normo-albuminuric diabetic group and the control group (median, 1.40 ± 0.9 ng/mL vs. 0.83 ± 0.09 ng/mL; $p < 0.001$ respectively). The outcomes of our investigation are consistent with the findings documented by **El-Asrar *et al.*** [7], whose study revealed a statistically significant elevation in serum soluble CD40 ligand (sCD40L) levels across all patients with type 1 diabetes mellitus when compared to a control group (median, 750 pg/mL vs. 225 pg/mL; $p < 0.001$). Their research further demonstrated significantly increased sCD40L levels in both diabetic subgroups when individually compared to healthy control subjects ($p < 0.001$). Notably, patients exhibiting microvascular complications presented with markedly higher serum sCD40L concentrations (median, 13000 pg/mL; interquartile range [IQR], 1600–20000 pg/mL) in contrast to those without such complications (median, 450 pg/mL; IQR, 250–750 pg/mL) ($p < 0.001$), a significant difference that persisted even after adjusting for the confounding effects of age and disease duration ($p < 0.001$) [7]. Similarly, **Metwalley *et al.*** [8] indicated that children with type 1 diabetes mellitus (T1DM) had higher levels of soluble CD40 ligand (sCD40L) in their blood compared to healthy children. Importantly, they observed that sCD40L levels were significantly greater in T1DM patients who had microalbuminuria compared to those who did not. Furthermore, their research showed a strong and significant positive relationship between the amount of sCD40L in the blood and the presence of microalbuminuria.

In our study, we also observed significantly higher sCD40L levels in patients with elevated urinary albumin/creatinine ratio (microalbuminuria > 30 mg/g), indicating a significant positive correlation between sCD40L levels and microalbuminuria in these patients. Interestingly, sCD40L levels were also higher in the normoalbuminuric group (< 30 mg/g), suggesting that elevated sCD40L levels may precede the early signs of glomerular damage as indicated by the albumin/creatinine ratio. Thus, sCD40L levels may serve as an earlier marker of diabetic renal changes and the initiation of diabetic nephropathy. The outcomes of our investigation align with the observations reported by **Harding *et al.*** [9], whose research established a significant association between diabetes mellitus and elevated circulating levels of soluble CD40 ligand (sCD40L), as well as increased expression of CD40L on the surface of platelets. This consistent finding of elevated inflammatory markers, including sCD40L, across different studies suggests a potentially critical role for inflammation in the pathogenesis and progression of vascular complications that are frequently associated with diabetes mellitus [10]. Understanding the precise mechanisms by which these inflammatory markers contribute to diabetic vasculopathy may pave the way for the development of novel therapeutic strategies aimed at mitigating these debilitating long-term complications and improving the overall clinical management of individuals with diabetes. Further research focusing on the intricate interplay between inflammation and vascular damage in diabetes is warranted to fully elucidate these pathways. Furthermore, prior investigations conducted by **Varo *et al.*** [11], **Cipollone *et al.*** [12], **and Jinchuan *et al.*** [13] have consistently reported significantly elevated levels of soluble CD40 ligand (sCD40L) in individuals with type 1 diabetes mellitus when compared to healthy control subjects. Notably, analogous findings have been observed in patients with type 2 diabetes mellitus, thereby suggesting that an increase in sCD40L levels represents a significant characteristic feature associated with diabetes mellitus, regardless of the particular kind of disease [11].

The research conducted by **Gokulakrishnan *et al.*** [14] further demonstrated that patients diagnosed with metabolic syndrome exhibited significantly higher circulating levels of soluble CD40 ligand (sCD40L) when compared to individuals without diabetes who also presented with metabolic syndrome. This finding suggests that the presence of metabolic syndrome itself, even in the absence of diabetes, is associated with increased levels of this inflammatory marker.

Correspondingly, the research conducted by **Unek *et al.*** [15] revealed elevated soluble CD40 ligand (sCD40L) levels in individuals without a diagnosis of diabetes but who exhibited characteristics of pre-diabetes and metabolic syndrome. This observation implies that individuals with metabolic syndrome may experience a state of heightened inflammation, as indicated by increased sCD40L concentrations, irrespective of their capacity to regulate blood glucose. The consistent pattern of elevated sCD40L across a range of metabolic disorders highlights the potential utility of this marker as a broader indicator of systemic inflammation and heightened vascular risk, extending beyond its association with diabetes mellitus alone.

RECOMMENDATION

It is recommended to conduct additional prospective and longitudinal investigations involving larger cohorts of patients with type 1 diabetes. The future studies are essential to corroborate the current findings

and, importantly, to establish a robust and clinically applicable sCD40L cutoff value that can effectively identify individuals at risk of developing early diabetic complications. Such research endeavors are pivotal in ascertaining the true clinical utility of sCD40L as a predictive and diagnostic biomarker for both diabetic vasculopathy and nephropathy within this specific patient population, potentially leading to earlier interventions and improved patient outcomes.

CONCLUSION

The elevated serum sCD40L levels observed in children and adolescents with T1DM, especially those who exhibited microalbuminuria, coupled with its positive correlation with diabetes duration, urinary albumin excretion, and glycemic control, might underscore the involvement of sCD40L in the pathogenesis of diabetic vasculopathy within the pediatric age group. The findings of this study emphasize the potential utility of sCD40L as a prospective marker for the early detection and monitoring of diabetic nephropathy in this vulnerable population.

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