ISSN 2063-5346

ASSOCIATION BETWEEN INSULIN RESISTANCE AND ENDOTHELIAL DAMAGE: A BIOCHEMICAL STUDYWITH REFERENCE TO THE VARIOUSSTAGES OF DIABETIC NEPHROPATHY

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Article History: Received: 02.07.2023 Revised: 15.07.2023 Accepted: 23.07.2023

Abstract

Introduction and Aim: Endothelin-1 (ET-1) and Vascular Endothelial Growth Factor-A (VEGF-A) have been recognized as markers of endothelial dysfunction in diabetes mellitus. VEGF-A and ET-1 are vasoactive peptides that have a nexus with vascular manifestations including optic neuropathies and disturbances in retinal circulation. This study compared vascular dysregulatedmetrics,VEGF-A and ET-1 with insulin resistance and sensitivity, in the light of riskfactors for diabetic nephropathy in a graded manner, based on albuminuria.

Subjects and Methods: A total of 90 type 2 diabetics in the age group 35 -50 years were enrolled as three groups, comprising 30 each, based on albuminuria. 30 healthy age and gender matched subjects constituted the control group.VEGF-A, ET-1 and Insulin were quantitated by ELISA. Microalbumin was estimated by turbilatex method. Routine biochemistry analyses wascarried out on fully automated analyzer. Stringent quality control was promulgated . The study was begun following approval accorded by the competent authority.

Results: The dysregulated metricspositivelycorrelated with HOMA-IR (Insulin resistance)andalbumin-creatinine ratio.; VEGF-A, ET-1 negatively correlatedwith HOMA-β (Beta cell mass), QUICKI (Insulin sensitivity)and eGFR.Thus, VEGF-A and ET-1 might find clinical utility as indicators of imminent vascular problems.

Conclusion:VEGF-A and ET-1, with reference to theindicators of insulin resistance, insulin sensitivity and Beta cell mass would acquire significance, in the light of albuminuria, as biomarkers in angiopathy related to diabetic nephropathy

Key words:HOMA-IR, HOMA –β, QUICKI, eGFR, VEGF-A,endothelin-1,diabetic nephropathy.

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DOI:10.48047/ecb/2023.12.9.201

Introduction

Diabetic nephropathy is frequently observed microvascular complication of both types of diabetes.[1] It contributes immensely to the medical burden and affects over 75 million people globally.[2,3] The major hotspot is Chinaas exemplified by 38.8%.[4] and in India, 34.4 % diabeticsare affected by nephropathy.[5]The parenchymal, mesenchymal, and endothelial cells undergo extensive damagethat is attributed to hyperglycemia and its metabolic sequelae.[6]The documented evidences cite the accumulation of sorbitol, besides the Advanced Glycation End-Products, referred to as AGEs.[7]

It must be noted that the innermost layer of all blood vessels is essentially constituted by the endothelial cells (ECs). ECsrulevascular permeability and other typical events that include leukocyte adhesion, tone, and vascular homeostasis, in general[8]From anatomical considerations, the kidney and gut harbor fenestrated endothelium[9] Reduction in surface fenestrated endothelium holds the key in cardinal cellular developments, as linked to insulin resistance[10]. Endothelial dysfunction is linked to the onset and progression of vascular issues [11,12]. Endothelial failure has been envisaged primarily as one that is linked to glomerular enlargement and mesangial expansion. Other typical events are also observed[13].

Endothelin-1 and Vascular Endothelial Growth Factor (VEGF), which are both generated in abnormal quantities are synonymous with angiogenesis and vaso permeability[14].Literature is available to quote the fact that Endothelin-1 and circulating VEGF-A contribute to the glomerular capillary hyperpermeability of macromolecules[15].This in all probability would largely denote the etiology of diabetic albuminuria.

In the current study, we have made a sincere effort to decipher the relationship between various phases of diabetic nephropathy and the endothelial dysfunction markers VEGF-A and Endothelin-1, in the light of albuminuria.

Methodology

Subjects And Methods

For this study, 90type 2 diabetics of both genders were enrolled, keeping in view the fact that they had a history of diabetes mellitus lasting five years or more and between the ages of 35 and 50. They were maintained on standard care with oral antidiabetic (hypoglycemic) medications. The study commenced only following the accord provided by the competent authority. The Helsinki declaration was complied with.

The study groups were segregated appropriately: thirty patients with normoalbuminuria (<30 mg/g creatinine), and an equal number each of patients with microalbuminuria (30–299 mg/g creatinine), and macroalbuminuria (≥300 mg/g creatinine). Patients on insulin as well as such of those subjects who had a history of tobacco and alcohol consumption were promptly excluded. Also, subjects with urinary tract infections, characteristic inflammation,organ and endocrine (metabolic) disorders, stroke and peripheral vascular disease were excluded. The controls comprised thirtyage and gender matched healthy volunteers.

Control and Test groups

Group-1- Thirty healthy age, gender matched subjects constituted controls

Group-2 - Thirty type-2 diabetic patients with normoalbuminuria (<30 mg/g creatinine)

Group-3 - Thirty type-2 diabetic patients with microalbuminuria(30–299 mg/g creatinine)

Group-4 - Thirty type-2 diabetic patients with macroalbuminuria (≥300 mg/g creatinine)

Biochemical analysis: Following an overnight fast, eight ml of venous blood was withdrawn from the subjects (based on informed consent), under standard aseptic conditions. In order to quantitate endothelin and VEGF-A in the serum, aliquots of venous blood samples weremaintained atminus80 °C and blood glucose in the fasting or post absorptive state (FBS) was measured immediately using an autoanalyzer. In order to enable the quantitation of microalbumin and creatinine, the first-morning samples of urine were collected in sterile containers. Fasting blood glucose was estimated by enzymatic method using Glucose oxidase-Peroxidase. Microalbumin was assessed by turbilatex method and urinary creatinine was estimated by Jaffe's kinetic method. The biochemical analyses were performed on ERBA EM-200 fully automated analyzer, whereas fasting insulin, endothelin and VEGF-A were estimated using ELISA kits that were provided by M/S Diametra, Spello, Italy, Boster Biotech Ltd, USA and Sincere Biotech Co. Ltd., China respectively. LISA SCAN (ERBA) ELISA reader was employed for the purpose of enabling the estimation ofthe above-mentionedanalytes.

Insulin Resistance

Homeostatic Model Assessment For Insulin Resistance (HOMA-IR)

A surrogate indicator for insulin resistance (IR), namely, HOMA -IR was calculated based on the fasting glucose and insulin values using the formula.

HOMA – IR = Fasting insulin (µU/ml) X fasting glucose (mg/dL)/405

HOMA –BETA

HOMA-beta=(360 × fasting insulin (µU/mL))/(fasting glucose (mg/dl)-63).

Quantitative Insulin-Sensitivity Check Index(QUICKI)

It is estimated according to the formula by **Katz et al.**

QUICKI = 1/[log(Fasting Insulin in µU/ml) + log (Fasting Glucose in mg/dl)]

eGFR

It is calculated by CKD-EPI (Chronic Kidney Disease Epidemiology) formula[16].

Table - I: Computation of eGFR

	Serum Creatinine (mg/dl)	**eGFR**
Females	**≤0.7**	$\mathbf{144 \times (Scr/0.7)^{-329} \times (0.993)^{age}}$
	>0.7	$\mathbf{144 \times (Scr/0.7)^{-1.209} \times (0.993)^{age}}$
Males	**≤0.9**	$\mathbf{141 \times (Scr/0.9)^{-0.411} \times (0.993)^{age}}$
	>0.9	$\mathbf{141 \times (Scr/0.9)^{-1.209} \times (0.993)^{age}}$

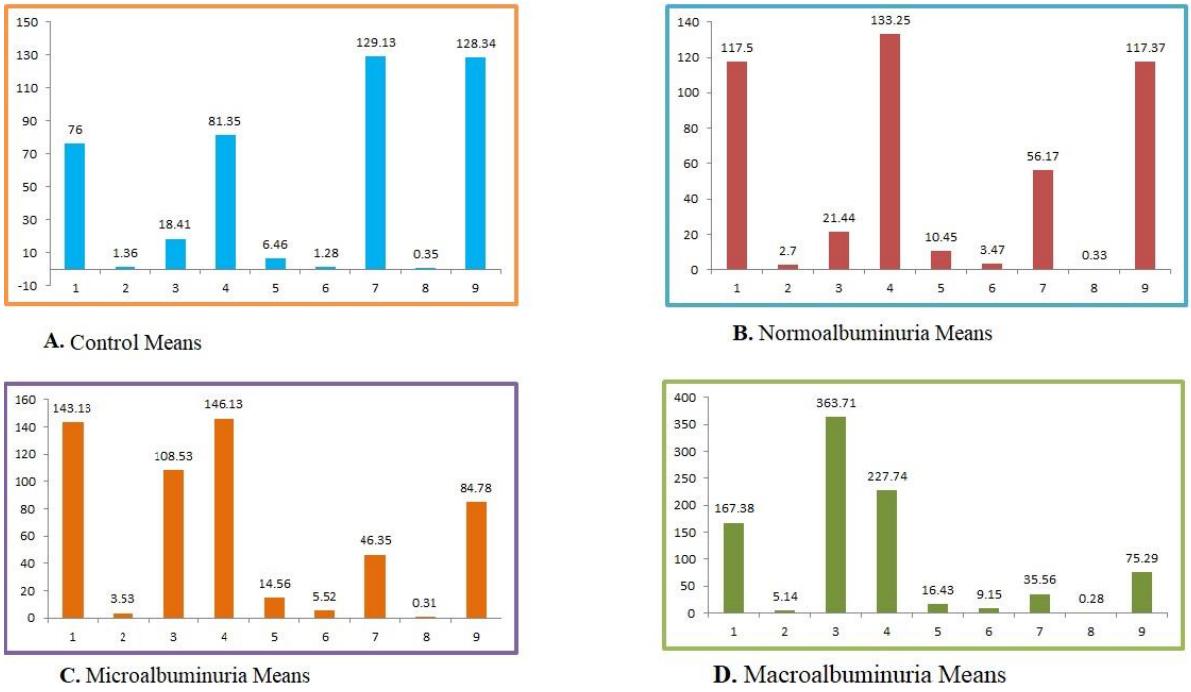
Scr: serum creatinine

Statistical analysiswas carried out by SPSS software, version-22.

Results

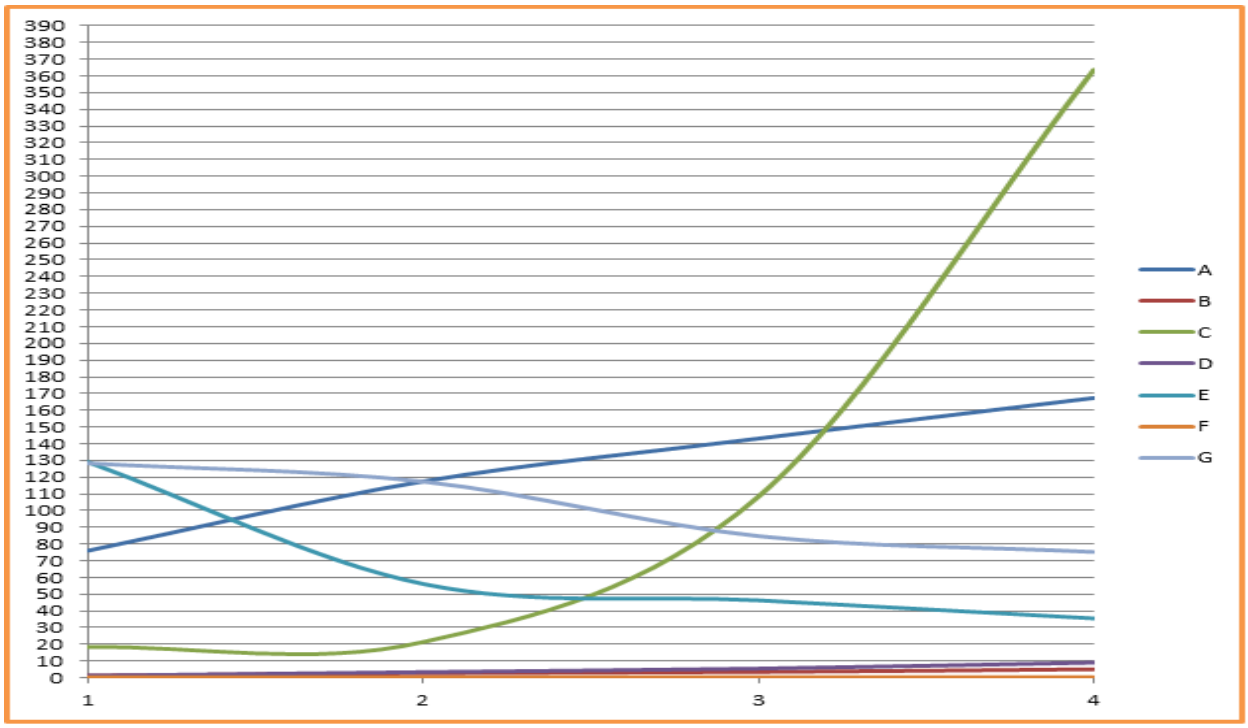
Figures- 1 & 2 depict the findings related to the means of the different groups in terms of the characteristic biochemical parameters.

Figure 1: Histogram depicting the observed Mean values



1→VEGF pg/ml; 2→Endothelin pg/ml; 3→Urinary Albumin Creatinine Ratio (UACR) ; 4→FBS; 5→Insulin µ IU/ml : 6→HOMA-IR ; 7→HOMA-β ; 8→QUICKI ; 9→e-GFR.

Figure 2: Diagram depicting the observed mean values



A→VEGF pg/ml ; B→Endothelin pg/ml; C→Albumin Creatinine Ratio (UACR) ; D→HOMA-IR ; E→HOMA-β ; F→QUICKI ; F→e-GFR.

1→Control; 2→Normoalbuminuria; 3→Microalbuminuria ; 4→Macroalbuminuria.

Table -IIcompares all of the groups in order to determine the statistical significance of the various factors. When compared to controls, normoalbuminuria, microalbuminuria, macroalbuminuria, normoalbuminuria vs. microalbuminuria, and microalbuminuria vs. macroalbuminuria, **Table-II** shows endothelin, VEGF-A, and other biochemical parameters with varying degrees of statistical significance.

Table-II-Comparison of Groups for obtaining 'p' values

S.No	Parameters	1 vs. 2	1 vs. 3	1 vs. 4	2 vs. 3	2 vs. 4	3 vs. 4	All Groups For the purpose of computing ANOVA
1	**VEGF-A pg/ml**	0.00005	0.00002	0.00001	0.02490	0.00001	0.00012	0.00012
2	**Endothelin pg/ml**	0.00026	0.00001	0.00001	0.00451	0.00001	0.00001	0.00037
3	**UACR**	0.00122	0.00015	0.00023	0.00011	0.00072	0.00025	0.00043
4	**FBS mg/dl**	0.00015	0.00041	0.00013	0.00101	0.00051	0.00026	0.00012
5	**Insulin µIU/ml**	0.00373	0.00032	0.00012	0.02254	0.00016	0.19507	0.00027
6	**HOMA-IR**	0.00178	0.00015	0.00010	0.01394	0.00001	0.00010	0.00013
7	**HOMA-β**	0.00186	0.00062	0.00053	0.00296	0.00031	0.43836	0.00014
8	**QUICKI**	0.00015	0.00011	0.00012	0.007603	0.00044	0.00010	0.00021
9	**eGFR**	0.03916	0.04521	0.02085	0.05544	0.05736	0.03788	0.00980

1 – Control; 2 – Normoalbuminuric T2 DM ; 3 – Microalbuminuric T2 DM;

4 – Macroalbuminuric T2 DM

P<0.05 – Significant and P<0.001 – highly significant

Table-III: Correlation between VEGF-A and key biochemical parameters

Correlation coefficient(r)	UACR	HOMA-IR	HOMA-BETA	QUICKI	eGFR
VEGF-A	0.823**	0.864**	- 0.734**	- 0.846**	- 0.527**
Endothelin- 1 (ET-1)	0.847**	0.943**	- 0.736**	- 0.921**	- 0.512**

**Correlation is significant at the 0.01 level (2-tailed)

Table-IIIconfirm the diagnostic significance of VEGF-A and Endothelin in the context of impending nephropathy. Furthermore,thetwo proteins strongly correlate with albumin/creatinine ratio. Additionally, there was a strong association between VEGF-A, Endothelin, and insulin sensitivity and resistance, as determined by the established markers.

Discussion

We had attempted to evaluate the relationship between the endothelial dysfunction markers VEGF-A and Endothelin-1 with reference to the insulin

resistance and insulin sensitivity metrics ,namelyHOMA-IR, HOMA- β, and QUICKI in nephropathy observed in T2DM patients, but with respect to gradation based on UACR. In the entire study population, HOMA-IR, HOMA-β, VEGF-A, and Endothelin-1 were the risk factors for diabetic nephropathy. However, we discovered that the magnitude of HOMA-IR, VEGF-A, and Endothelin-1 were the risk factors for diabetic nephropathy in the micro- and macroalbuminuric nephropathy but not in the normoalbuminuric nephropathy. It may please be recalled that the subjects weresegregated into three groups on the basis of albumin-creatinine ratio (UACR).

Our findings depict a strong correlation among the levels of insulin resistance, VEGF-A, and endothelin-1 with the decline in diabetic kidney function.Studies have shown as to how HOMA-IR, VEGF-A, and Endothelin-1 could contribute to diabetic nephropathy[17]However, it is not unequivocal. Higher HOMA-IR levels may be linked to an increased risk of microalbuminuria, according to a few studies[18] In T2DM patients with early-stage renal illnesses, elevated HOMA-IR values were observed[19] Studies by **Zhang A et al**. and **Mohajan et al**. in 2017 and 2020 respectively suggested that VEGF-A and Endothelin-1 were useful in predicting the progression of renal impairment in people with diabetes[20].

We investigated the functions of VEGF-A, Endothelin-1, and insulin resistance in the early stage (normal albuminuria), moderate stage (microalbuminuria), and severe stage (macroalbuminuria) of nephropathy in T2DM patients. When it comes to the onset of proteinuria in Type 2 Diabetes, VEGF-A and Endothelin-1 are key players[21] However, nephropathy is more common in the diabetic population in China and India than to Western nations. An increased risk of microvascular problems and cardiovascular events have been linked to insulin resistance in diabetes patients.[22] Additionally, because of the complex mechanism and molecular cross -talk of insulin resistance, a higher total mortality rate has been documented, in comparison to diabetics who did not possess the condition[23].

According to our findings, the early stages of diabetic nephropathy showed higher evidence of the linkages between insulin resistance, VEGF-A, and endothelin-1. In T2DM patients with low proteinuria, our research revealed that endothelial dysfunction and insulin resistance were risk factors for diabetic nephropathy; progressive diabetic nephropathy was associated with a pronounced endothelial dysfunction[24].

A previous report highlighted the association between insulin resistance (HOMA-IR) and kidney dysfunction and that HOMA-IR levels were higher in all three stages in diabetic nephropathy namely Normoalbuminuria, Microalbuminuria, and macroalbuminuria. HOMA-IR was correlated with eGFR and UACR[26].

An indicator of insulin secretory capacity, the homeostasis model assessment of β -cell function (HOMA- β) is derived from fasting plasma glucose and insulin concentrations[26]. It has been used in the evaluation ofβ -cell failure in T2DM brought about by low-grade inflammation and oxidative stress in the pancreas[27], as well as to predict the onset of diabetes in non-diabetics. According to theevidences generated in the present study, β -cell dysfunction is a risk factor for renal illness. Decreased HOMA- β values in diabetic nephropathy was noted, besides eGFR and UACR that had inverse relationships with HOMA- β, as per a previous report[28]. These findings suggest that HOMA- β could contribute to the elevated risk of proteinuria independently of diabetic nephropathy, despite the strong relationship between pancreatic β -cell failure and diabetic nephropathy.

Fasting blood sugar and insulin levels are transformed mathematically to create the quantitative insulin-sensitivity check index (QUICKI)[29]. The lowest decile of insulin sensitivity is also known as insulin resistance, and measuring reduced insulin sensitivity is important, especially in people who have risk factors for developing insulin resistance[30].Our findings indicate that insulin resistance enhances the risk of kidney disease. In diabetic nephropathy, QUICKI readings were lower. QUICKI is considered as an indirect marker of insulin resistance, and studies have indicated that it plays a role in the development of diabetic nephropathy. eGFR and UACR demonstrate inverse associations with QUICKI.Inflammation, oxidative stress, and enhanced lipotoxicity, which culminate in the development of microangiopathy, are potential mechanistic avenues thatlink insulin resistance to diabetic nephropathy, despite the fact that the mechanism behind the linkage is yet to be comprehensively elucidated. Numerous studies have revealed that dyslipidemia plays a significant part in the development of renal damage in people with diabetes[31]. Despite the significant association between Insulin receptor sensitivity and diabetic nephropathy, these results suggest that QUICKI could contribute to the enhanced risk of proteinuria,independent of diabetic nephropathy.

Vascular endothelial growth factor (VEGF-A) is an important player in vasculogenesis and neoangiogenesis and promotes cell proliferation. Other actions include inhibition of apoptosis,enhancedinflammation[32]
Endothelial dysfunction is influenced by insulin resistance, which is nothing but a form of systemic low-grade inflammation[33]. The type-2 diabetes mellitus patients exhibited higher VEGF-A levels and a higher likelihood of renal dysfunction[34]. By activating the VEGFR2-nephrin-nck-actin signaling cascade in podocytes, chronic hyperglycemia-induced excess podocyte VEGF-A and low endothelial nitric oxide promote the onset and progression of diabetic nephropathy.The abnormal cross-talk between the VEGF-A and NO pathways is propelled by the elevated oxidative stress[35] this results in podocyte permeability.

Three isotypes of endothelin ET-1, ET-2, and ET-3 are regarded as powerful vasoactive peptides that have recently been linked to diabetes[36].In our study, elevated ET-1 levels were discovered in all three stages of diabetic patients. This finding, in conjunction with the highly correlated HOMA-IR and HOMA- parameters measuring insulin resistance and beta cell dysfunction as well as the QUICKI insulin receptor sensitive index, demonstrate the high risk of nephropathy associated with type-2 diabetes and endothelial dysfunction[37].It must be stated that ET-1 is a crucial biomarker for endothelial dysfunction, which is probably well connected to the free radicals observed in diabetes.[38]Our work is one among the few instances to compare the various phases of nephropathy in type -2 diabetes mellitus with insulin resistance, VEGF-A, and ET-1

Conclusion

In conclusion, statistically significant levels of VEGF-A, ET-1, and insulin resistance indicated a higher risk for diabetic nephropathy in people with type-2 diabetes mellitus. These correlations were not evident in people with normal albuminuria, but were observed in the subjects with micro and macro albuminuria

Significance of the present study

Recent studies pertaining to the cardinal aspects of diabetic nephropathy (DN), with reference to the use of pharmaceutical agents and considerations in pharmacology have been essentially targeted at the faithful interpretation of the nodal molecular mechanisms involved in the onset and progression of DN and ways and means to effectively address the same.

A point of great relevance is the role of such therapeutic preparations in the prevention of disease onset as well as progression. In the current scenario, a few therapeutic drugs for DN are available. However, the armamentarium largely comprises conventional anti-hypertensives, besides antidiabetic formulations. Having said that, itremains to be seen as to whether such modalities would confer optimal benefits. We need to appreciate the fact that there exists a possibility ofintroducing drugs that could envisage benefits beyond the horizons of glycemic control, insulin resistance and blood pressure. It is in thiscontext that our present study could acquire relevance in pharmacology and pharma industry, where therapeutic formulations could be preparedand targeted at VEGF –A and ET-1 as a whole, in addition to the standard care that is promulgated for not only controlling DM but preventing the catastrophe of diabetic nephropathy.

Novelty of the study

1. The two cardinal markers of chronic kidney disease are urinary albumin- Creatinine ratio and estimated glomerular filtration rate (eGFR). Though studies performed by earlier workers point to the assessment of urine albumin excretion annually so as to monitor renal damage in patients with T2DM, ambiguity still exists in its clinical applications
2. Recent evidences suggest that combined changes in albuminuria and eGFR over prolonged duration of diabetes are strongly associated with future risk of kidney failure in patients with type 2 diabetes
3. Our study has endeavoured to integrate the markers of endothelial dysfunction, namely ET-1 and VEGF-A in the light of insulin resistance and insulin sensitivity, but as a function of UACR and e-GFR. This we feel might signal the advent of a novel and concerted approach that would enable us to intensely and closely monitor the progression to end-stake kidney disease(ESKD)

Limitations of the study

The products of oxidative stress and inflammatory indicators represent the primary causes of endothelial dysfunction in diabetes mellitus, but we did not measure inflammatory indexes. Further research is needed to determine the relationship between the specific risk variables and severity of diabetic nephropathy.

Acknowledgement

The authors would like to acknowledge the support of Prof.Anand Acharya, Dean, Konaseema Institute of Medical Sciences, Amalapuram, Prof. K.S.Reddy, Dean Research, Sri BalajiVidyapeeth, Pondicherry campus and Prof. A.K. Das, Dean Academics, Sri BalajiVidyapeeth,Pondicherry campus for extrinsic motivation.

Conflict of interest: The authors declare that the present study was not funded by any agency and is not bound by any conflict of interest whatsoever.

Ethics approval

The study was begun only following the approval of the Research Advisory Committee at the institute where the first author had registered himself as a doctoral candidate and also essentially following clearance that was duly obtained from the Institutional Human Ethics Committee (IHEC),with reference to the workplace of the first author.

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