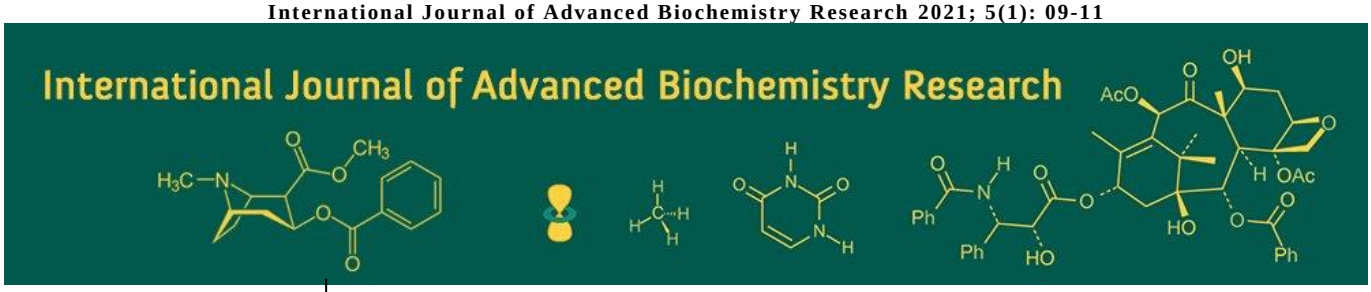

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Study of significance of serum cystatin-C and vascular endothelial growth factor as an early marker of diabetic nephropathy in type 2 diabetes patients

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Abstract
Aim: To determine the usefulness of serum cystatin-C along with vascular endothelial growth factor (VEGF) as an early marker of diabetic nephropathy.
Method: The study includes diabetic patients visiting the Medicine OPD and was divided into groups according to the duration of diabetes. Group 1 with diabetes between 5-10 years, group 2 with diabetes between 10-15 years, group 3 with diabetes more than 15 years and control group.
Results: Serum cystatin-C and VEGF levels raised significantly in group 2 patients (10-15 years duration) and increases further with the progression of the disease.
Conclusion: Both cystatin-C and vascular endothelial growth factors can be a useful marker of early diabetic nephropathy in patients with type 2 diabetes.

Keywords: Cystatin-C, VEGF, type 2 diabetes, nephropathy

Introduction
Diabetic nephropathy is the major microvascular complication of diabetes and the leading cause of end-stage renal disease globally, causing high morbidity and mortality in patients with diabetes [1]. In the United States, diabetic nephropathy is the most common cause of chronic renal failure and accounts for 43.9% of patients enrolled in long-term dialysis programs [2]. Diabetic patients will be reached to advance and irreversible nephropathy if not treated early and adequately [3].
Cystatin-C is a low molecular weight protein (13.36 KD) produced by all nucleated cells and exhibits a stable production rate. Due to its small size and basic pH (~9.0) it is freely filtered by glomerulus. Cystatin-C does not return to the bloodstream nor secreted by renal tubules, so it is suggested to be closer to the "ideal" endogenous marker [4]. Vascular endothelial growth factor is a multifunctional glycoprotein with a variety of effects on angiogenesis, endothelial cell proliferation and differentiation, vascular permeability and endothelial dependent vasodilation. Hyperglycemia increases VEGF expression acts to cause endothelial injury in diabetes. VEGF stimulates the formation of α3 chain of collagen, which is an important component of the glomerular basement membrane and increased formation of collagen leads to the thickening of glomerular membrane [5].
Creatinine is the most widely used biomarker of kidney function but non-specific as its levels can vary with age, sex, muscle mass, diet and tubular creatinine secretion [6]. There are also limitations in using albuminuria as a marker of diabetic nephropathy as many patients experience GFR loss without deterioration in albuminuria [7] suggesting a search for new biomarkers for screening of diabetic nephropathy. The overall aim of the study was to evaluate serum cystatin-C and vascular endothelial growth factor (VEGF) levels in control and diabetic groups in order to assess markers for early detection of diabetic nephropathy.

Material and Method
The present study was conducted in the Department of Biochemistry, in association with the Department of Medicine, S.M.S. Medical College and Hospital, Jaipur. The studies comprised of total 160 subjects and were divided into groups as follows: Group 1: Comprised of 40 patients with a duration of diabetes of more than five years and less than ten years.

Group 2: Comprised of 40 patients with a diabetic duration between 10 to 15 years Group 3: comprised of 40 patients with a history of diabetes for more than 15 years. Control group – 40 age and sex-matched healthy individuals were taken as control.

Inclusion Criteria

- Diabetics with a history of > 5 yrs duration
- Age > 30 years and <70 years
- HbA1c> 8%

Exclusion Criteria

- Patients with thyroid disorders.
- Patients on glucocorticoid therapy.
- Past history of renal impairment.

Venous Blood was withdrawn for investigations taking all aseptic precautions. Serum was separated and investigated for Cystatin-C, Vascular endothelial growth factor (VEGF) by ELISA method.

Ethical approval and Informed consent

The Protocol was approved by the institutional Ethics committee. Informed written consent was obtained from all study subjects.

Statistical Analysis

The data was analyzed using SPSS version 20 (SPSS Inc., Chicago, Illinois, USA). Descriptive statistics included in the computation of percentages, means and standard deviations were calculated. The statistical tests applied for the analysis were one-way ANOVA with Post-Hoc Bonferroni test. The confidence interval and p-value were set at 95% and ≤ 0.05 respectively.

Results

Table 1 shows serum cystatin-C and VEGF concentration in control and diabetic groups. Both serum cystatin-C and VEGF concentrations were higher in diabetic subjects as compared to controls (Figure 1).

Table 1: Serum Cystatin-C and Vascular Endothelial Growth Factor (VEGF) level in Control v/s Diabetic Groups

Character		Control	Group 1 (5-10 years)	Group 2 (10-15 years)	Group 3 (>15 years)
	Cases	40	40	40	40
Cystatin C (ng/l)	Mean±SD	4.13±2.14	5.17±2.09	11.58±1.59	15.34±2.52
VEGF (pg/ml)	Mean±SD	71.71±23.56	103.33±36.54	316.80±71.86	759.67±158.18

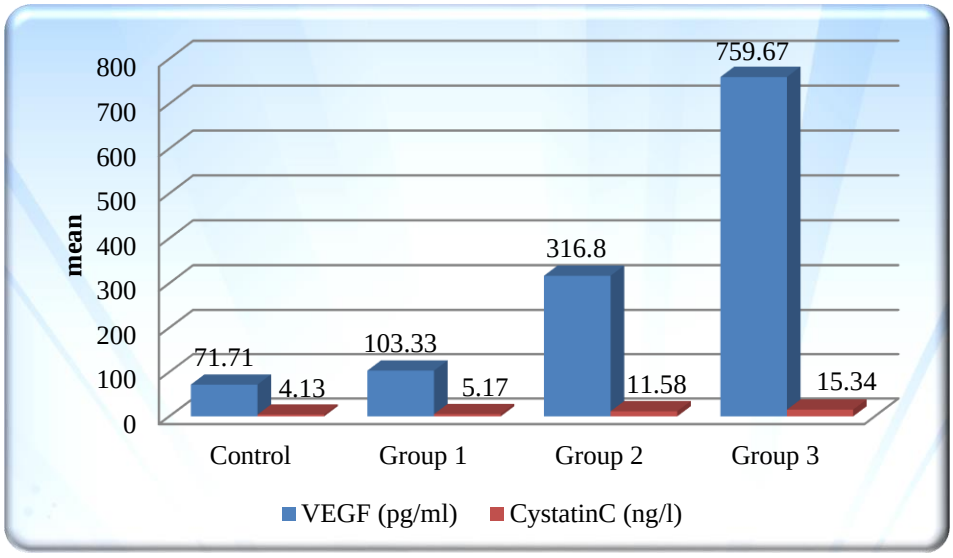

Fig 1: Serum cystatin-C and VEGF in control v/s Diabetic groups

Table 2: Multiple (Intra groups) Comparisons of Serum Cystatin–C by Post hoc Bonferroni test

Groups		Mean Difference	P value
Group 1 (5-10 years)	Group 2	-6.41370*	0.000
	Group 3	-10.17398*	0.000
	Control	1.03672	0.180
Group 2 (10-15 years)	Group 1	6.41370*	0.000
	Group 3	-3.76028*	0.000
	Control	7.45042*	0.000
Group 3 (>15 years)	Group 1	10.17398*	0.000
	Group 2	3.76028*	0.000
	Control	11.21070*	0.000

*=significant

Table 3: Multiple (Intra groups) Comparisons of Serum VEGF by Post hoc Bonferroni test

Groups		Mean Difference	P value
Group 1 (5-10 years)	Group 2	-213.46615*	0.000
	Group 3	-656.33640*	0.000
	Control	31.62210	0.698
Group 2 (10-15 years)	Group 1	213.46615*	0.000
	Group 3	-442.87025*	0.000
	Control	245.08825*	0.000
Group 3 (>15 years)	Group 1	656.33640*	0.000
	Group 2	442.87025*	0.000
	Control	687.95850*	0.000

*=significant

Table 2 and 3 demonstrate multiple (Intra groups) comparisons of serum cystatin–C and VEGF in control and diabetic groups respectively. Both cystatin-C and VEGF concentration is significantly higher in group 2 patients having a diabetic history of more than 10 years and the level increases further with the duration of the disease.

Discussion

Our study result shows that diabetic individuals were found to have higher concentrations of serum cystatin-C as compared to control subjects and the level raised significantly in patients specifically with a history of more than 10 years. Our result was in agreement with another study conducted by Rao *et al*. [8] indicating that levels of cystatin-C are related to subclinical renal damage and can be an earlier measurable marker of renal involvement in type 2 diabetes. Cystatin-C is produced by a housekeeping gene in all nucleated cells and is freely filtered by the glomerulus without stearic restrictions, and does not appear to be secreted by the renal tubules [8]. Subsequently, it is reabsorbed and almost completely catabolized in the proximal renal tubule and is not affected by age, gender, body mass or any inflammatory condition [9]. If kidney function and GFR decline, the blood levels of Cystatin-C rise.

VEGF concentration was also observed higher in group 2 patients with a diabetic history of more than 10 years and further increases with the progression of the disease. A similar result was reported by Shao *et al*. [11] that VEGF in patients with diabetic kidney disease (DKD) was significantly higher than in control subjects. Vascular endothelial growth factors can affect the filtration of large molecular weight proteins through the glomerular filtration barrier by promoting endothelial cell proliferation and increasing vascular permeability. In type 2 diabetes mellitus subjects, both plasma and urinary VEGF level was higher in normal buminuric patients and gradually increased along with the diabetic nephropathy stages, suggesting it be an effective biomarker for early diagnosis of diabetic nephropathy [12]. Tavafi M [13] also observed an increased rate of VEGF in diabetic nephropathy.

Conclusion

Diabetic nephropathy is one of the leading causes of end-stage renal disease and the gap between the onset and clinical diagnosis of diabetic nephropathy causes premature mortality. Hence, early detection is necessary to treat patients for their prognosis and better survival. Our study results suggested that as both serum cystatin-C and VEGF raised significantly in diabetic patients specifically having a history of 10 years or more so they can be used to predict diabetic nephropathy in early stages.

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