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ORIGINAL RESEARCH ARTICLE

A STUDY OF SERUM ENDOSTATIN LEVELS AND ITS CORRELATION WITH eGFR IN CHRONIC KIDNEY DISEASE PATIENTS IN A TERTIARY CARE HOSPITAL

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Abstract

Background: Chronic kidney disease is one of the most leading public health problems worldwide. Increased endostatin accelerates the development and progression of CKD. Present study was aimed to estimate serum Endostatin levels and to correlate the serum endostatin levels with eGFR in chronic kidney disease patients in a tertiary care hospital. **Material and Methods:** Present study was an observational case control study, conducted in patients of age group 45-65 years, both genders. Patients were divided as Group A (Known case of chronic kidney disease with eGFR <60ml/min/1.73m^2), Group B (Known case of chronic kidney disease with eGFR 60 – 90 ml/min/1.73m^2) & Group C (Age and sex matched healthy control of both genders). **Results:** There was a significant mean difference in endostatin between Group 1 and Group 2; Group 2 and Group 3; Group 1 and Group 3 (p = 0.000; 0.000; 0.000). There was a significant positive strong correlation of endostatin with systolic BP, diastolic BP, FBS, serum urea, creatinine, uric acid, PCR, total cholesterol, TGL, LDL and VLDL with correlation coefficient of r = 0.872; 0.715; 0.899; 0.992;0.811;0.918; 0.947; 0.882; 0.864; 0.923 and 0.864 respectively. There was a significant negative strong correlation between endostatin and serum protein, albumin and HDL with a correlation coefficient r = -0.899; -0.920 and -0.780 respectively. The sensitivity (true positives) was found to be 86.7% and specificity (true negative) was found to be 81.7%. Area under the curve was 96.2. The significant cut off value is 397.65. Endostatin above 397.65 ng/mL was considered to be chronic kidney disease. **Conclusion:** Serum Endostatin levels increases as eGFR decreases. Serum Endostatin levels strongly correlate with eGFR and hence with the severity of the chronic kidney disease. Serum Endostatin negatively correlated with HDL and positively correlated with LDL and triglyceride levels which indicates the risk for the development of atheromatous complications.

Keywords: Serum Endostatin, chronic kidney disease, renal function tests, atheromatous complications, biomarker

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 09, 2024

Introduction

Chronic kidney disease is one of the most leading public health problems worldwide. The global prevalence of chronic kidney disease is estimated to be 13.4%.[1]It is the 12th leading cause of mortality globally and 8th leading cause of mortality in India. Chronic kidney disease refers to decreased kidney function that develops over a period of time. It is defined as kidney damage or glomerular filtration rate of less than 60mL/min /1.73 m^2 or both for at least 3 months.[2]

The persistent and progressive deterioration of renal function tests leads to various complications like end stage renal disease (ESRD), bone disorders and cardio vascular diseases.[3] When there is elevation in the levels of antiangiogenic factors, the development of CKD is hastened. This results in tubulointerstitial fibrosis and glomerulosclerosis which is implicated as one of the causes for the development and progression of CKD.

Endostatin is a carboxy terminal fragment of collagen XVIII. It is a potent antiangiogenic factor. It inhibits angiogenesis of the endothelial cells. This leads to impaired renal repair mechanism and causes apoptosis of endothelial cells.[4] Increased endostatin accelerates the development and progression of CKD. Present study was aimed to estimate serum Endostatin levels and to correlate the serum endostatin levels with eGFR in chronic kidney disease patients in a tertiary care hospital.

Material and methods

Present study was an observational case control study, conducted at Institute of Biochemistry & Institute of Nephrology, Madras Medical College & Rajiv Gandhi Government General Hospital, Chennai-3, India. Study duration was of 1 year (December 2019– December 2020). Study was approved by institutional ethical committee.

Inclusion criteria:

Age group 45-65 years, both genders, Patient willing to give written informed consent for participation. Later they were divided as

- Group A: Known case of chronic kidney disease with eGFR <60ml/min/1.73m^2
- Group B: Known case of chronic kidney disease with eGFR 60 – 90 ml/min/1.73m^2
- Group C: Age and sex matched healthy control of both genders.

Exclusion criteria:

- History of dialysis
- Kidney Transplants
- Immunotherapy (past 6 months)
- Chemotherapy within 2 years
- Known case of Malignancy
- Known autoimmune diseases
- Patient not willing to give written consent

Blood sample was collected after 8-12 hours of overnight fasting. After getting informed consent from the patient, about 5mL of venous blood was collected from antecubital vein under strict aseptic precautions in clot activator tubes. Sample collected in the serum tube was allowed to clot and the serum was separated after centrifugation at 3000 RPM for 15 minutes and used for the estimation of fasting blood glucose, urea, creatinine, total protein, albumin and lipid profile. About 1mL of serum was stored at -20^0C for the analysis of endostatin. Spot urine sample was collected in a sterile container for protein creatinine ratio (PCR) estimation.

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 09, 2024

INVESTIGATIONS:

ANALYTES	METHOD
Serum Endostatin	ELISA
Renal function test	Spectrophotometry
Lipid profile	Spectrophotometry
Serum Total protein and albumin	Spectrophotometry
Estimated GFR	Calculated value (CKD-EPI)
Urine Protein Creatinine Ratio	Calculated value

The data obtained between the cases and controls were analysed using IBM SPSS (Statistical Package for Social Science) software version 21. The significance was determined when the p value was <0.05. Gender was analysed by Chi square test. The difference between mean values of the three groups were analysed using ANOVA. Pair wise comparison between the groups were done using Tukey HSD. Pearson correlation analysis was done to assess the correlation of parameters between the groups. ROC curve was plotted to determine the cut-off level of Endostatin for predicting chronic kidney disease.

Results

The study was conducted among 60 patients with chronic kidney disease and 30 age and sex matched healthy controls. Serum Endostatin levels were estimated among different groups and compared and correlated with renal function tests, lipid profile.

Comparison of mean age of Populations in three groups showed no significant difference (p = 0.215). There was no significant difference in the frequency distribution of gender among the Populations in the three groups (p = 0.673). There was significant difference in the mean distribution of systolic and diastolic blood pressure among the populations in the three groups (p = 0.000). There was a significant difference in the mean distribution of fasting blood glucose among the Populations in the three groups (p = 0.000).

TABLE 1: General characteristics

Variable	**Group I (eGFR < 60mL/min/1.73 m^2)**	**Group II (eGFR 60 – 90 mL/min/1.73m^2)**	**Group III (Controls)**	**F value**	**p value**
	Mean ± SD	**Mean ± SD**	**Mean ± SD**		
Age	56.53 ± 6.257	54.97 ± 5.314	54.13 ± 5.270	1.414	0.215*
Gender					
Male	15 (50)	15 (50)	17 (56.7)	0.178	0.673*
Female	15 (50)	15 (50)	13 (43.3)		
Blood pressure					
Systole	140.67 ± 12.02	130.33 ± 11.62	111.33 ± 7.761	58.777	0.000*
Diastole	90.67 ± 13.37	81.00 ± 10.29	72.00 ± 8.867	21.587	0.000*
Fasting Glucose	151.03 ± 24.541	128.10 ± 17.991	95.10 ± 7.703	72.217	0.000*

*One-Way ANOVA

There was a significant mean difference in systolic blood pressure and diastolic blood pressure between Group 1 and Group 2; Group 2 and Group 3; Group 1 and Group 3.

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 09, 2024

TABLE 2: Pair-wise comparison of blood pressure among the groups

Blood pressure	Pairs	Mean difference	p value	95% Confidence interval
Systole	Group 1 vs Group 2	10.333	0.001*	3.79 to 16.88
	Group 2 vs Group 3	19.000	0.000*	12.46 to 25.54
	Group 1 vs Group 3	29.333	0.000*	22.79 to 35.88
Diastole	Group 1 vs Group 2	9.667	0.003*	2.89 to 16.44
	Group 2 vs Group 3	9.000	0.006*	2.22 to 15.78
	Group 1 vs Group 3	18.667	0.000*	11.89 to 25.44

*Tukey HSD

There was a significant mean difference in fasting blood glucose between Group 1 and Group 2; Group 2 and Group 3; Group 1 and Group 3 (p = 0.000; 0.000; 0.000).

TABLE 3: Pair-wise comparison of fasting blood glucose among the groups

Blood glucose	Pairs	Mean difference	p value	95% Confidence interval
Fasting Glucose	Group 1 vs Group 2	22.933	0.000*	11.78 to 34.09
	Group 2 vs Group 3	33.000	0.000*	21.84 to 44.16
	Group 1 vs Group 3	55.933	0.000*	44.78 to 67.09

*Tukey HSD

There was significant difference in the mean distribution of serum urea, creatinine and uric acid among the populations in the three groups (p = 0.000). There was a significant difference in the mean distribution of PCR among the Populations in the three groups (p = 0.000). There was significant difference in the mean distribution of serum protein and albumin among the Populations in the three groups (p = 0.000).

There was a significant difference in the mean distribution of total cholesterol, TGL, HDL, LDL and VLDL among the Populations in the three groups (p = 0.000). There was a significant difference in the mean distribution of endostatin among the Populations in the three groups (p = 0.000).

TABLE 4: Mean distribution of laboratory parameters among the groups

Serum	Mean ± SD			F value	p value
	Group 1	Group 2	Group 3		
Urea	81.57 ± 9.954	61.63 ± 10.08	20.17 ± 2.984	421.14	0.000*
Creatinine	3.375 ± 0.519	1.084 ± 0.078	0.647 ± 0.078	688.08	0.000*
Uric acid	7.960 ± 1.152	6.147 ± 0.834	4.207 ± 0.680	127.50	0.000*
PCR	1.692 ± 0.287	0.9307 ± 0.163	0.3017 ± 0.056	390.12	0.000*
Protein	5.453 ± 0.537	6.053 ± 0.518	7.083 ± 0.441	81.403	0.000*
Albumin	2.673 ± 0.605	3.007 ± 0.417	4.427 ± 0.476	101.76	0.000*
Total cholesterol	224.57 ± 32.37	202.93 ± 14.98	154.90 ± 15.06	76.322	0.000*
TGL	203.53 ± 28.52	143.60 ± 16.11	114.27 ± 13.64	148.03	0.000*
HDL	26.20 ± 4.11	32.97 ± 4.33	44.20 ± 3.199	162.40	0.000*
LDL	157.70 ± 23.10	141.13 ± 7.87	93.10 ± 8.999	149.77	0.000*
VLDL	40.70 ± 5.70	28.72 ± 3.22	22.85 ± 2.73	148.03	0.000*
Endostatin	480.99 ± 52.52	370.21 ± 46.98	89.809 ± 11.42	717.96	0.000*

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 09, 2024

* One-Way ANOVA

There was a significant mean difference in endostatin between Group 1 and Group 2; Group 2 and Group 3; Group 1 and Group 3 (p = 0.000; 0.000; 0.000).

TABLE 5. Pair-wise comparison of endostatin among the groups

Serum	Pairs	Mean difference	p value	95% Confidence interval
Endostatin	Group 1 vs Group 2	110.77	0.000*	85.40 to 136.15
	Group 2 vs Group 3	280.41	0.000*	255.02 to 305.77
	Group 1 vs Group 3	391.18	0.000*	365.80 to 416.55

*Tukey HSD

There was a significant positive strong correlation of Endostatin with systolic BP, diastolic BP, FBS, serum urea, creatinine, uric acid, PCR, total cholesterol, TGL, LDL and VLDL with correlation coefficient of r = 0.872; 0.715; 0.899; 0.992; 0.811; 0.918; 0.947; 0.882; 0.864; 0.923 and 0.864 respectively. There was a significant negative strong correlation between endostatin and serum protein, albumin and HDL with a correlation coefficient r = -0.899; -0.920 and -0.780 respectively.

TABLE 6. Correlation between endostatin and all serum parameters

Independent variable	Dependent variable	Correlation Coefficient (r)	p value
Endostatin	Systolic BP	0.872	0.000
	Diastolic BP	0.715	0.000
	Fasting blood glucose	0.899	0.000
	Serum urea	0.992	0.000
	Serum Creatinine	0.811	0.000
	Serum uric acid	0.918	0.000
	PCR	0.947	0.000
	Serum protein	-0.899	0.000
	Serum albumin	-0.920	0.000
	Total cholesterol	0.882	0.000
	TGL	0.864	0.000
	HDL	-0.780	0.000
	LDL	0.923	0.000
	VLDL	0.864	0.000

The sensitivity (true positives) was found to be 86.7% and specificity (true negative) was found to be 81.7%. Area under the curve was 96.2. The significant cut off value is 397.65. Endostatin above 397.65 ng/mL was considered to be chronic kidney disease.

TABLE 7: Sensitivity, specificity and area under the curve for endostatin and GFR

Variables	Sensitivity	Specificity	AUC	p value	95% Confidence Interval	Cut-off value
Endostatin * GFR	86.7%	81.7%	0.962	0.000	0.928 – 0.996	397.65

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 09, 2024

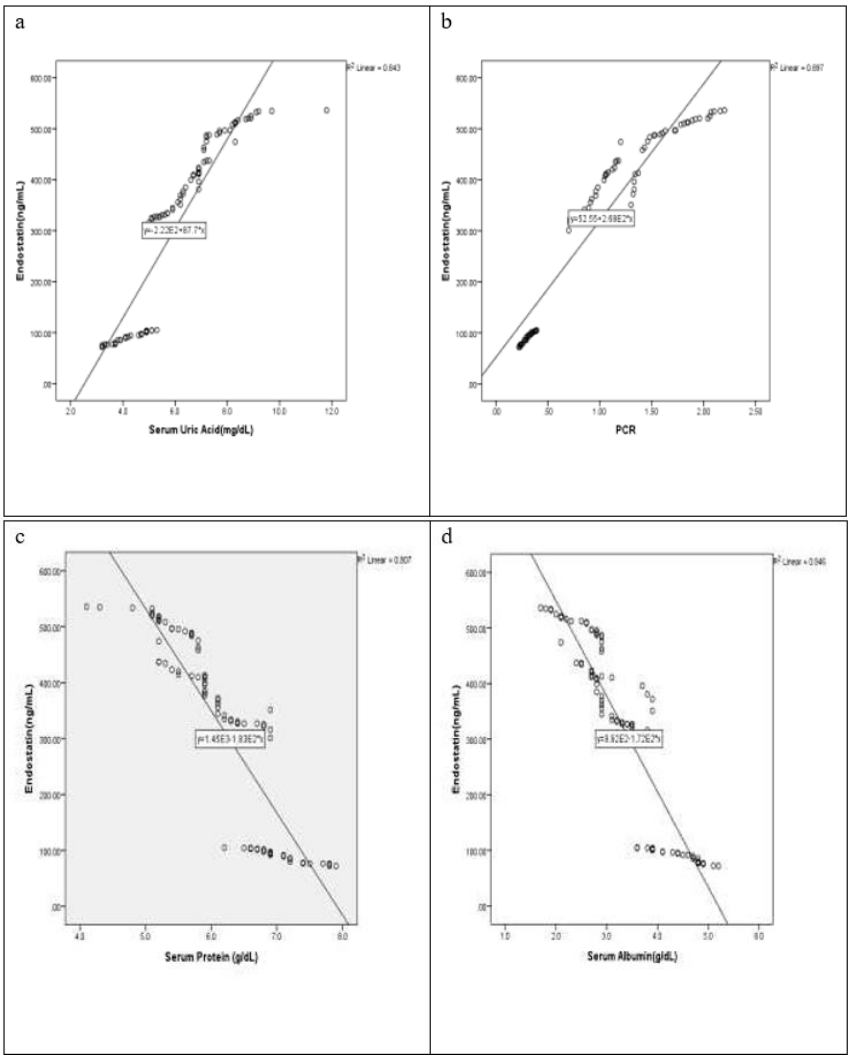

FIGURE 1: Scatter plot showing correlation and regression equation between serum endostatin and uric acid, PCR, serum protein, serum albumin

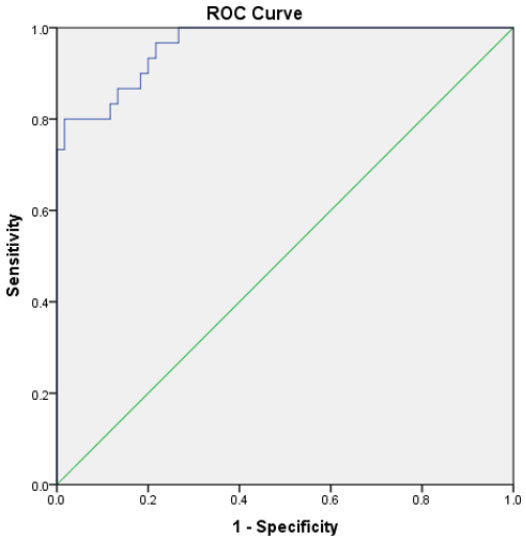

Figure 2: ROC for endostatin and GFR

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 09, 2024

Discussion

Chronic kidney disease is marked by decline in kidney function as it progresses. Earlier intervention by diagnosis and hence treatment is necessary to prevent the complications of CKD. The diagnosis of chronic kidney disease till date depends mainly on measuring serum creatinine. However, measurement of creatinine detects changes in renal function only when the glomerular filtration rate is reduced to 50%. Other markers like Cystatin C are elevated only after the complication sets in.Serum Endostatin, the analyte which was measured in this study begins to raise even before the onset of complications predicting the same. So, this can be used as a biomarker.

Serum Endostatin levels were found to be significantly increased in patients with chronic kidney disease. The mean Endostatin was 480.99 ± 52.52 in CKD patients with eGFR < 60mL/min/1.73 m^2 and 370.21 ± 46.98 in CKD patients with eGFR 60 -90 mL/min/1.73 m^2 compared to the control group (mean 89.809 ± 11.42). A highly significant p value 0.000 was obtained. This shows that the Endostatin level increases as the eGFR decreases. This finding was consistent with Jing Chen *et al.*,[5] who observed increased levels of Endostatin in chronic kidney disease patients.

Endostatin is an antiangiogenic factor. Elevated Endostatin levels inhibits the expression of VEGF. This leads to impaired renal repair which ultimately results in chronic kidney disease.[6] An independent and dose response association was found between serum Endostatin levels and the severity of CKD measured by eGFR and Protein creatinine ratio.

The rate of progression of kidney disease is independent of age and sex. The mean systolic and diastolic blood pressure increases as eGFR decreases. The systolic and diastolic blood pressure positively correlated with endostatin (r value: 0.872 and 0.715 respectively). This shows that hypertension is prevalent in chronic kidney disease patients.

Dan Pugh *et al.*,[7] in his study observed that blood pressure levels were elevated in patients with CKD. Upregulation of Renin Angiotensin Aldosterone Mechanism and endothelial dysfunction in CKD were attributed as the probable mechanism for elevated blood pressure in chronic kidney disease.

There was a significant increase (p value 0.000) in fasting glucose levels as the eGFR decreases. The fasting glucose value shows positive correlation with Endostatin (r value :0.899). Hyperglycemia exerts toxic effects by activation of various biochemical signaling pathways like aldose reductase and protein kinase C. This results in glomerular hyperfiltration and the production of advanced glycation end products which causes renal damage. Glycemic status of these patients must be evaluated further as serum Endostatin levels are increased in patients with diabetic nephropathy. Carlsson AC *et al.* estimated serum Endostatin levels in diabetic nephropathy patients and observed that serum Endostatin levels were elevated in these patients compared to controls.[8]

A strong positive correlation was observed between Endostatin and serum urea, creatinine and uric acid. (r value: 0.992, 0.811 and 0.918 respectively). There was a strong positive correlation between PCR and Endostatin (r value 0.947). Herrick Fisher *et al.*,[9] in his study found that damaged vascular endothelium and impaired tubular reabsorption in CKD causes proteinuria which is consistent with the present study. High PCR values in chronic kidney disease patients indicates the severity of glomerular dysfunction.

There was a strong negative correlation between Endostatin and Total Protein and Albumin (r value: -0.899 and -0.920 respectively). These findings were similar to the work done by Haller C *et al.*,[10] Hypoalbuminemia in chronic kidney disease is due to decreased synthesis as well as due to increased degradation. In addition to this, malnutrition and protein restricted diet also causes hypoalbuminemia.

There was a significant increase(p=0000) in mean LDL values as the eGFR decreases.

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 09, 2024

A strong positive correlation was found between LDL and Endostatin (r value 0.923). HDL levels were found to be significantly decreasing(p=0000) as the kidney function declines. A negative correlation was exhibited between Endostatin and HDL (r value: -0.780).

Tannock L *et al.*,[11] observed elevated LDL and decreased HDL in patients with CKD. Downregulation of LDL receptor and LDL receptor related protein in chronic kidney disease causes elevated LDL levels. Oxidative stress and endothelial dysfunction in chronic kidney disease cause oxidation of the LDL. Decreased Lecithin cholesterol acyl transferase (LCAT) and increase in Cholesteryl ester transfer protein (CETP) contributes to the decreased HDL levels in chronic kidney disease. The ability of the HDL cholesterol to decrease the formed oxidized LDL cholesterol is also impaired and this leads to uncontrolled increase in oxidized LDL level.

A strong positive correlation was found between Endostatin and Triglyceride, VLDL. (r value 0.864 and 0.865 respectively). The elevated triglycerides in chronic kidney disease are primarily due to the downregulation of lipoprotein lipase This downregulation leads to impaired catabolism of triglyceride rich lipoproteins and thereby causing accumulation of Apo-B containing lipoproteins.

The mean total cholesterol value was 187.5 ± 32.37 in CKD patients with eGFR < 60mL/min/1.73m^2 and 183.4± 14.98 in CKD patients with eGFR 60 – 90 mL/min/1.73m^2. Total cholesterol levels also showed significant increase (p-0000), Also a positive correlation was found between Endostatin and total cholesterol. (r value - 0.882). This is in concurrence with the study conducted by Ivana Mikolasevic *et al.*,[12] In his study he found that LDL, VLDL, Triglyceride and Total Cholesterol levels were increased in CKD.

ROC was plotted for Endostatin to determine the optimal cut-off to detect chronic kidney disease. The area under the ROC curve was 0.962 at 95% confident interval and the cut off level is 397.65 ng/mL with a sensitivity of 86.7% and specificity of 81.7%.

Limitations of the study were small sample size; diabetic patients were not excluded hence a separate study may be conducted for diabetic patients. Similarly, patients on haemodialysis and who were on renal transplant were not included in the study.

Conclusion

Serum Endostatin level begins to raise even before the complication sets in. Serum Endostatin levels strongly correlate with the severity of the chronic kidney disease. Serum Endostatin levels positively correlated with Renal function tests. Unlike creatinine, it is unaltered by muscle mass which makes it more reliable. Serum Endostatin levels negatively correlate with nutritional parameters like serum Total protein and serum albumin.

Serum Endostatin negatively correlated with HDL and positively correlated with LDL and triglyceride levels which indicates the risk for the development of atheromatous complications. Hence Endostatin can be used as an independent biomarker for the diagnosis of chronic kidney disease.

Conflict of Interest: None to declare
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ISSN: 0975-3583,0976-2833 VOL15, ISSUE 09, 2024

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