

Research Article



Serum Levels of Endothelin-1 and Vascular Endothelial Growth Factor in Chronic Renal Failure Patients Receiving Haemodialysis with Different Etiology

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ABSTRACT

The objective of present study was to verify the changes of serum levels of ET-1 and VEGF in Chronic Renal Failure (CRF) Patients undergoing haemodialysis. Sixty five of haemodialysis patients were enrolled in this study and compared with forty healthy subjects. Thirty from these patients were followed up to six months for serial assessment of these biomarkers as well. The results indicated significant elevations of VEGF and ET-1 levels when compared with the control group ($P < 0.0001$). Also the statistical analysis showed a significant difference in serum levels of VEGF ($P < 0.003$) according to different causes of chronic renal failure while there was no significant difference in serum levels of ET-1 within different etiology of haemodialysis patients. Moreover there was a significant differences in 3rd and 4th reading of ET-1 and VEGF and a significant differences in 2nd reading of ET-1 level ($P < 0.05$) when compared to first reading of VEGF and ET-1 through the period of serial assessment. Further, a significant negative correlation were observed for ET-1 and VEGF levels with the GFR of patients ($P < 0.05$) and a significant positive correlation were demonstrated for the level of ET-1 ($P < 0.05$) while not with VEGF when they have correlated with the duration of dialysis of patients. The results pointed out that ET-1 and VEGF levels are involved in the pathophysiology of the disease and they were useful in the prediction of the disease earlier to the clinical manifestations of atherosclerotic and cardiovascular disease.

Keywords: Chronic Renal Failure, Haemodialysis, ET-1, VEGF.

INTRODUCTION

Chronic renal failure (CRF) is defined as a glomerular filtration rate less than 60 ml/min/1.72 m² for at least 3 months duration, it is usually the end result of conditions such as diabetes mellitus, hypertension, primary glomerular nephritis, autoimmune disease, obstructive uropathy, polycystic kidney disease, renal artery stenosis, infection, tubular dysfunction and the use of nephrotoxic drugs. The most important biochemical evidents in CRF are increase in serum urea and creatinine concentration, Other findings include: metabolic acidosis, and fixed urine osmolality¹. End Stage Renal Disease (ESRD) includes patients treated by dialysis or transplantation, irrespective of the level of GFR². With the improvement in conservative management and dialysis, the life span of patients with chronic renal failure (CRF) has been increased. As the patient's survival has approached the 10 years, there is an increasing indication that accelerated atherosclerosis may remain a major unresolved problem threatening the longevity of CRF patients³. Infections and cardiovascular diseases remain the leading causes of complications and death in end stage renal disease patients⁴. The clinical course of patients with chronic renal failure (CRF) is conditioned by a series of immunological and inflammatory alterations which, in turn, lead to cardiovascular diseases⁵. A number of angiogenic growth factors are involved in the development of the kidney and in the maintenance of glomerular structures and the glomerular filtration barrier function in adults. Vascular endothelial growth factor

(VEGF), a potent pro-angiogenic factor, is involved in the development of the kidney, and plays an important role in maintaining the glomerular capillary structure and in the repair process following injuries of glomerular endothelial cells and peritubular capillaries⁶. Loss of these capillaries is strongly associated with the progression of chronic kidney disease (CKD) to end-stage renal disease⁷. Physiological levels of VEGF are also required for maintenance of the glomerular filtration barrier. In the early stages of chronic kidney disease increases in the number of glomerular capillaries and in the glomerular levels of VEGF⁸. Endothelin may play an important role in renal regulation in cardiorenal states of endothelin activation⁹. Investigation into the role of endothelin-1 (ET-1) in renal function has revealed dual major direct actions leading to the control of extracellular volume and blood pressure. These are the regulation of renal hemodynamics and glomerular filtration rate and the modulation of sodium and water excretion¹⁰.

In the kidney of chronic renal failure, ET-1 production is increased in blood vessels and renal tissues. These changes are related to an increase in prepro ET-1 expression and correlate with the rise in blood pressure, the development of cardiovascular hypertrophy, and the degree of renal insufficiency and injury¹¹.

Similarly, plasma ET-1 concentrations are markedly increased in patients with end-stage renal disease undergoing dialysis, and this correlates with blood pressure, suggesting that ET-1 may contribute to



hypertension in these patients¹². The increase in ET-1 production can be associated with other local mediators, including angiotensin II, transforming growth factor-beta1 and nitric oxide, the local production of which is also altered in chronic renal failure¹³.

Previous studies showed that patients with chronic renal failure have markedly elevated levels of biomarkers (ET-1 and VEGF) in particular those with haemodialysis therapy, but the pathogenetic mechanisms of this phenomenon are still not completely understood.

PATIENTS AND METHODS

Sixty five patients of CKD (29 females, 36 males) were enrolled in this study. Their ages range from 20-70 years with mean of age (38.43 ± 17.65 year).

Thirty, 12 females, 18 males from these patients were followed up to six months for four readings (zero reading at 1st month and the others at 3rd month, at 5th month and 7th month) for serial assessment for estimation of serum levels of ET-1 and VEGF in haemodialysis patients.

Patients with Acute renal failure, HBs Ag positive and Nephrotic syndrome were excluded from the current study while the control group were consisted of forty subjects who were free from signs and symptoms of renal diseases, lipid metabolism disorders, diabetes mellitus, and hypertension.

18 of them were females and 22 were males. Their ages range from 25 to 65 years with mean of age (33.23 ± 14.75 year).

Five milliliters of venous blood samples were collected from each patient after an overnight fasting before the haemodialysis session was started. a slow aspiration of the venous blood sample via the syringe was carried out, the samples were dropped into clean disposable tubes, left at room temperature for 30 minutes for clot formation and then centrifuged for 20 minutes at 3000 xg.

The sera were separated in a disposable tube and stored at -80 °C for estimation of inflammatory markers later.

Similarly blood samples were taken from the control group by vein puncture and sera subjected to processing exactly as that for patients.

Serum levels of these markers were measured by enzyme linked immuno-sorbent assay (ELISA).

Statistical Analysis

Statistical analyses were performed using SPSS 16.0 for windows.

Results are expressed as mean ± standard deviation, t – test used to estimate differences in each parameter between groups , linear regression analysis also used to study the relations between different parameters and Anova to compare differences in parameter among different groups, accepted significant was P < 0.05.

RESULTS

The evaluation of the data indicated that the enrolled patients were distributed according to different trends. They were distributed according to the age, sex, BMI, cause of disease, GFR and duration of dialysis. The characteristics of the enrolled CRF patients are mentioned in table 1 by mean and the standard deviation (Mean ± SD).

Table 1: Characteristics of patients included in the study

Variable	Mean ± SD
Age, years	38.43 ± 17.65
Duration of dialysis, months	25.39 ± 16.45
GFR, ml/min	13.11 ± 5.6
Body mass index, kg/m ²	22.37 ± 4.17
Blood urea, mmol/L	28.6 ± 5.8
Serum creatinine, µmol/L	701.8 ± 136.3
Hb, g/L	97.7 ± 26
Numbers of session / week	2±1.2

Most causes of CKD patients was found to be of glomerulonephritis (GN) etiology (18, 28%), the second most common one was hypertension (HT) (12, 18%), followed by diabetes mellitus (DM) (10, 15%) , renal stones (6, 10%), polycystic kidney (5, 7%) and chronic pyelonephritis (CPN) (4, 6%) , amyloidosis (2, 3%) , while the rest (8, 13%) of unknown causes. A significant difference was observed in serum levels of ICAM-1 and VCAM-1 while there was no a significant difference in serum levels of CRP within different etiology of haemodialysis patients (Table 2).

Table 2: Serum ET-1 and VEGF levels in chronic renal failure receiving haemodialysis with different etiology.

	NO.	ET-1 (ng/ml)	VEGF pg/ml)(
GN	18	139.5±45.6	393±234 @ ‡
HT	12	158.8±44 † ‡	398±285 @ ‡
DM	10	141.4±30.3	1040±615 * ∞ †
Stones	6	99.7±42 ∞	295±276 @ ‡
Unknown	8	111±62 ∞	915±613 * ∞ †
P- value		N.S.	< 0.003

* means p < 0.05 significantly difference with respect to GN group.

∞ means p < 0.05 significantly difference with respect to HT group.

@ means p < 0.05 significantly difference with respect to DM group.

† means p < 0.05 significantly difference with respect to Stones group.

‡ means p < 0.05 significantly difference with respect to Unknown group.



Table 3: Serum ET-1 and VEGF levels in Chronic Renal Failure patients receiving haemodialysis and the control group.

Parameter	Subject	NO.	Mean ± SD	Range	P-value
ET-1 pg/ml)	Patients	65	122.7 ± 47.9	18- 200	< 0.0001
	Control	40	24.7 ± 19.2	3.3 – 77	
VEGF pg/ml)	Patients	65	590.3 ± 345.2	70.3 – 1900.7	< 0.0001
	Control	40	132.6 ± 179.6	8.9 – 289	

Table 4: Serial assessment of serum levels of ET-1 and VEGF of thirty chronic renal failure patients receiving haemodialysis during six month.

	First reading	Second reading	Third reading	Fourth reading
ET-1	129.84 ± 42.77	137.35 ± 40.04*	145.44 ± 38.22*	145.78 ± 37.67*
VEGF	751.13 ± 650.55	806.63 ± 640.67	850.94 ± 639.43*	888.18 ± 628.89*

* means p < 0.05 significantly difference with respect to the first reading

Thirty patients were followed up to six months for four readings each 2 months for serial assessment of serum levels estimation of ET-1 and VEGF in haemodialysis patients, they were 10 from each of glomerulonephritis, hypertension and diabetes mellitus group patients (Table 4).

In addition to that a significant negative correlation were observed for ET-1 ($r = 0.32, p < 0.01$), VEGF ($r = 0.35, p < 0.004$) levels with the GFR of patients. Further, a significant positive correlation were demonstrated for the level of ET-1 ($r = 0.33, p < 0.006$) while not with VEGF when they have correlated with the duration of dialysis of patients. (Figure 1, 2, 3 and 4 respectively).

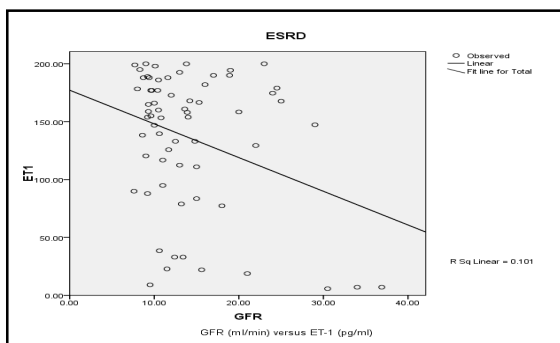


Figure 1: The correlation of GFR with ET-1 in chronic renal failure patients receiving haemodialysis.

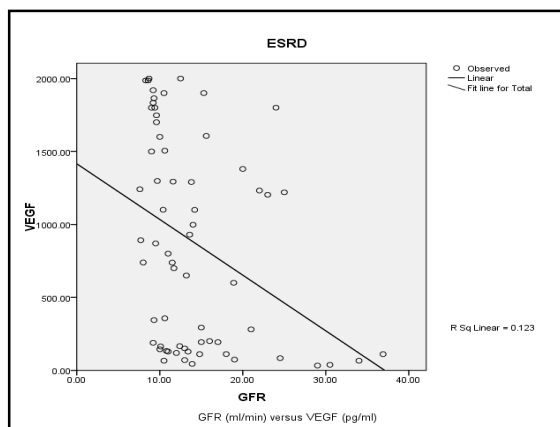


Figure 2: The correlation of GFR with VEGF in chronic renal failure patients receiving haemodialysis.

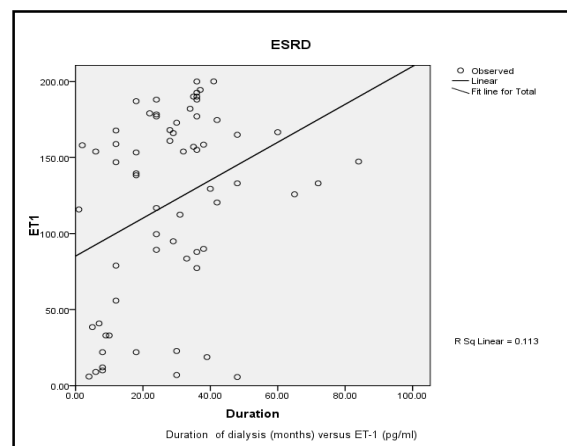


Figure 3: The correlation of duration of dialysis with ET-1 in chronic renal failure patients receiving haemodialysis.

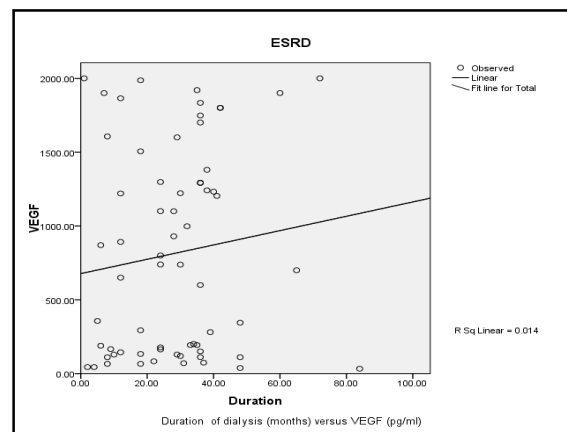


Figure 4: The correlation of duration of dialysis with VEGF in chronic renal failure patients receiving haemodialysis.

DISCUSSION

The current study was evaluated serum levels of ET-1 and VEGF in patients with end-stage renal disease (ESRD) on maintenance hemodialysis. It showed a significant increase in serum level of these biomarkers in haemodialysis patients when compared with the control those reported previously. VEGF is constitutively expressed in the human kidney, primarily in the glomerular visceral epithelial cells (podocytes) and in tubular epithelial cells in the outer medulla and medullary

rays, more commonly observed in distal tubules and collecting ducts than in proximal tubules resulted in proteinuria and glomerular endothelial injury¹⁴. In addition to its role in the maintenance of glomerular endothelial cells, glomerular VEGF is increased in response to hypertension and activation of the renin-angiotensin system in the experimental setting, thus showing a protective role for VEGF signaling in stressful vascular conditions¹⁵. Hypoxia is the main stimulus for VEGF expression and / or production. Several cytokines such as interleukin-1 (IL-1), and IL-6 also have the potential to up-regulate VEGF expression. VEGF may be induced by other factors as well (i.e hyperglycemia, advanced glycation end products (AGEs) and reactive oxygen species (ROS))¹⁶. Most the reasons for higher VEGF levels in uremia are unknown, but excess production, tissue hypoxia, or reduced clearance of VEGF has been suggested¹⁷. Although a large number of studies have been designed to examine this hypothesis, Positive correlations between urinary VEGF levels and proteinuria may relate to urinary podocyte loss rather than to a causative link between renal up-regulation of VEGF and development of proteinuria¹⁸. The paramount of VEGF in diabetic nephropathy where it contributes to glomerular hypertrophy and hyperfiltration, but may be an essential repair mechanism in glomerulonephritis. Similarly, tubular cells may respond to hypoxia or injury with the production of VEGF that stimulates proliferation of peritubular capillaries in order to overcome the tubular damage¹⁹. As the VEGF system is affected in a wide variety of kidney diseases, interventions to manipulate VEGF may be promising therapeutic tools.²⁰ The current study was in agreement with those of (Yuan⁷, Maeshima¹⁴ and Hakrout²¹) whose found a significance changes in VEGF In haemodialysis patient. Endothelin-1 (ET-1) plays a central role in the pathogenesis of proteinuria and glomerulosclerosis via activation of its ETA subtype receptor involving podocyte injury²². The increased formation of pro-inflammatory and fibrogenic peptides such as angiotensin II appears to play an essential role in this process²³. Renal endothelin also activates pathways that have been independently associated with the physiopathology of hypertensive renal disease, such as formation of reactive oxygen species production and inflammation²⁴. Endothelin itself stimulates the formation of angiotensin II by increasing the activity of angiotensin-converting enzyme, it is well possible that angiotensin-mediated renal and podocyte injury is directly mediated via endothelin. Angiotensin II, the main effector peptide of the renin-angiotensin system, activates renal endothelin production by partly pressure-independent mechanisms and causes renal glomerular inflammation²⁵. Other important factors that aggravate glomerular injury via endothelin are hyperglycemia, salt (sodium)-sensitive hypertension, or high levels of aldosterone²⁶. Indeed, the increased endothelin levels in ESRD patients do not imperatively suggest an increased endothelin activity, since the responsiveness of target tissues to hormones and other active substances may be altered in uremia and

since continuously increased plasma levels of endothelin may cause endothelin receptor down regulation or tachyphylaxis²⁴. The increased plasma levels of ET-1 in ESRD are not only due to impaired plasma clearance, but also to a stimulated synthesis since many known stimulators of ET-1 synthesis such as hypoxia, oxidative stress, and cytokine production are active in uremia²⁷, so that plasma levels of ET-1 was more elevated in HD patients with ischemic heart disease than in HD patients without this disorder also ET-1 preserves its vasoactive potency in the presence of ESRD, in addition the increased plasma concentrations of ET-1 in dialysis patients are at least partially due to a decreased plasma clearance²⁸. Several researches were in agreement with the current study that found an increase in plasma concentrations of ET-1 compared with healthy control subjects in ESRD, while inconsistency with the observation of (Ortmann)²⁹ who stated that there was an unclear results whether dialysis reduces or increases plasma concentrations of ET-1.

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