

# Serum Vitronectin and Related Molecules in Chronic Kidney Disease

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## Abstract

One in eight people are reported to have chronic kidney disease (CKD). The renal function slowly deteriorates when nephrons become impaired by inflammatory and fibrotic processes. In this study, the relationships between vitronectin (VTN), plasminogen activator inhibitor-1 (PAI-1) and growth factor translation  $\beta$ 1 (TGF- $\beta$ 1) are studied in CKD to assess the role as predict about progression stages of diseases. 105 patients with early stages (1 to 3) of CKD and 35-69 age matched healthy controls were included in the study. The VTN, PAI-1, TGF- $\beta$  levels of all participants were examined by Enzyme linked immune sorbent assay, creatinine, and urea by enzymatic method. Early morning urine sample was collected to be used for determination albumin creatinine ratio in patient with early stages of CKD. The renal function tests were significantly elevated in CKD group compared with healthy controls. The serum concentration of VTN increase in early stages(1-2) of CKD and decrease significantly with progression of disease (stage 3). Serum PAI-1 antigen level increased significantly. With the development of CKD, the effective role of TGF- $\beta$  became more severe, the correlation among VTN, TGF- $\beta$  and PAI-1 was positive.

**Conclusions:** These results indicate that VTN is important indicator for predict of CKD progression and both VTN, PAI-1 are connected to TGF- $\beta$ 's active form and can be used as a prediction for progression of CKD.

**Keywords:** CKD, Vitronectin, PAI-1, TGF- $\beta$ .

## Introduction

The role of VTN in the pathogenesis of chronic kidney injury is of particular interest due to its high binding affinity to the potent fibrosis-promoting molecule (PAI-1) <sup>[1]</sup>. VTN is known to be a cofactor in proteolytic inhibitory activities of PAI-1 <sup>[2]</sup>. Once PAI-1 is attached to VTN, it stabilizes its effective verification and raises its half-life nearly fourfold <sup>[3]</sup>. when serine proteases become active, the VTN/PAI-1 complex effectively inhibits plasmin production and proteolytic responses induced by plasmin. Nevertheless, during kidney damage, plasmin has pleiotropic and even opposite consequences. Recent surveys promote the belief that plasmin is beneficial in acute glomerular disease but in chronic tubulointerstitial disease it induces fibrosis <sup>[4]</sup>. PAI-1 elevated expression is observed in mesangial cells endothelial cells glomeruli podocyte cells, interstitial narrow arteries, proximal tubular epithelial cells, and

fibroblast cells. However, some primary renal fibrosis modulators induced PAI-1. The most prominent molecular characteristic of progression kidney diseases is over-expression of TGF- $\beta$ , a 28-kDa dimeric protein composed of two 14-kDa subunits produced by different cell types, including T cells and monocytes <sup>[5]</sup>. TGF- $\beta$  significantly increases the development of PAI-1 by cultivated glomeruli, mesangial cells and tubular cells and it is associated with increased expression of PAI-1 in disease <sup>[6]</sup>. All main components of the renin-angiotensin-aldosterone system; renin, ang II and aldosterone quickly and significantly enhance the production of PAI-1 via pathways that are independent of and dependent on TGF- $\beta$  <sup>[7]</sup>.

## Material and Method

A total of 105 Patients most of them were early staged included in this study (54 female, 65 male), age

range ((35-69) years), they were seen from Nov. 2018 till April 2019 at Baghdad Teaching Hospital/Medical City, A group of 30 subjects matched for age and sex, served as healthy control were, 14 (60%) female and 16(40%) male age range (35-67) years. None of them was CKD, according to laboratory findings of renal function tests that were considered as control. Blood, and urine were collected at the same visit from each subject. Venous blood samples were aspirated following a 12-hour fasting into plain tubes and centrifuged to obtain serum for the measurement of serum VTN, PAI-1 and TGF- $\beta$  levels in the fasting state, which were determined using ELISA. Blood urea and serum creatinine to assess renal function were estimation by Jaffe method. Early morning (1st am urination) sample was collected by patient for microalbuminuria test and be examined in early morning. Microalbumin in urine was estimated by particle enhanced turbid metric inhibition immunoassay (PETINIA).

## Result and Discussion

Function examination of kidney includes numerous parameters that should be estimated to determine disorder in kidney biological function. These parameters represent each of serum S.Cr and urea, albumin creatinine ratio (ACR) in urine and glomerular filtration rate (eGFR) were estimated on the basis of the Modification of Diet in Renal Disease (MDRD) formula which was measured for CKD and control groups also for subgroups of patients as recorded in Table 1. The mean  $\pm$  SD of S.Cr values of groups including each of patients group and control group are (1.803 $\pm$ 0.967) and (0.792 $\pm$ 0.279) respectively. Whereas mean  $\pm$  SD values of urea for the mentioned groups are including (10.544 $\pm$ 6.926) and (3.948 $\pm$ 1.084) respectively. The results showed a high significant increase ( $p < 0.001$ ) in level of both S.Cr and urea in the CKD group comparison with control group.

**Table 1: Mean  $\pm$  SD and range of renal function parameter for the studied groups**

	CKD	Healthy Controls	P value
S.Cr (mg/dl)	1.803 $\pm$ 0.967 (0.501-4.2)	0.792 $\pm$ 0.279 (0.501-1.567)	0.0001*
Blood Urea (mg/dl)	10.544 $\pm$ 6.926 (2.1-25.5)	3.948 $\pm$ 1.084 (2.1-6.97)	0.0001*
GFR (ml/min/1.73m <sup>2</sup> )	61.462 $\pm$ 21.444 (30.22-110.1)	127.897 $\pm$ 12.108 (110.1-150.56)	0.0001*
ACR	268.681 $\pm$ 232.525 (9.23-599.0)	12.037 $\pm$ 2.311 (7.28-16.05)	0.0001*

\*Significant difference between two independent means using Student-t-test at 0.05 level.

In earlier research, an increased incidence of kidney failure was associated with high levels of S.Cr and renal impairment depending on stages of eGFR. Creatinine is easily processed at a constant rate by the glomerulus, but is excreted by the tubules (10-40%). Creatinine tubular secretion rises with CKD resulting in unpredictable GFR overestimation<sup>[8]</sup>. Seki et al (2019) proposed that complete blood urea might have a predictive ability for development of kidney disease. These findings can indicate that in earlier stages of CKD, blood urea rates has a strong effect of on the development of renal disease<sup>[9]</sup>.

In table 1 the mean  $\pm$  SD values of eGFR for the mentioned groups were (61.462 $\pm$ 21.444) and

(127.897 $\pm$ 12.108) respectively. The results showed high significant decreases in CKD group ( $p < 0.001$ ) compared to control groups. The mean  $\pm$  SD level of ACR showed that there are a high significant differences ( $p < 0.001$ ) in the value of ACR among the studied groups that included CKD and control groups (268.681 $\pm$ 232.525) and (12.037 $\pm$ 2.311) respectively. The ACR between 30 mg/g is used as a kidney harm indicator and is used to describe CKD with low eGFR. Albuminuria is an indicator of diabetic and nondiabetic kidney disease progression and development<sup>[10]</sup>. Such results are compatible with latest studies, Eknoyan et al (2013) found that patients with regular or mild decline in GFR and Albuminuria may occur during the early stages; later it develops, lead to end stages of renal disease<sup>[11]</sup>.

Coresh et al (2014) reported that decline in eGFR in CKD and non-CKD patients must be tested for threat of accident and mortality because the eGFR gradient in CKD with eGFR < 60 ml/min/1,73 m<sup>2</sup> is more important and critical than eGFR > 60 ml/min/1,73 m<sup>2</sup>. [12]. Emily et al (2014) showed in patients with CKD, increased

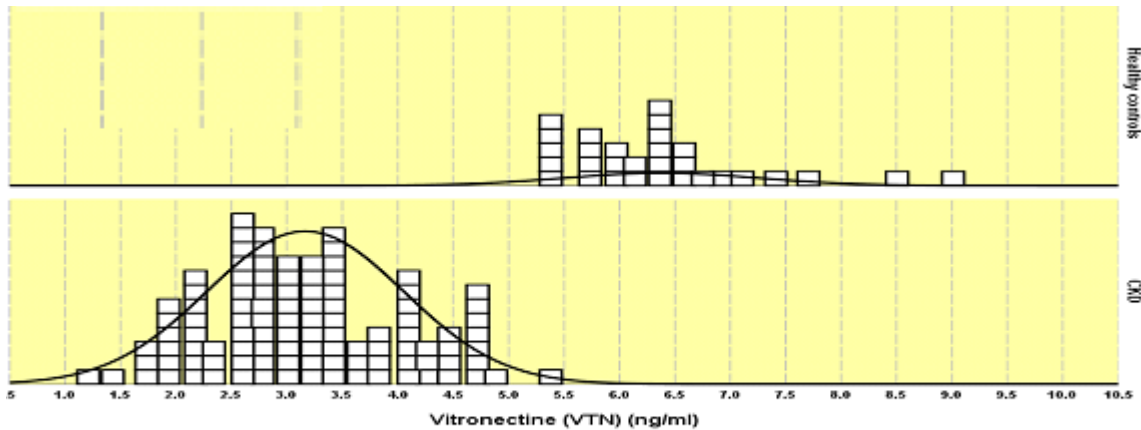
proteinuria (or albuminuria) is a major predictor of CKD progressions<sup>[13]</sup>.

Table (2) shows mean ± SD of fibrosis markers for CKD and control groups.

**Table 2: Assessment mean ± SD of fibrosis markers for CKD and healthy groups**

Fibrosis Markers	CKD	Healthy Controls	P value
VTN (ng/ml)	3.166±0.888 (1.21-5.42)	6.379±0.881 (5.32-9.01)	0.0001*
PAI-1 (ng/ml)	32.544±13.481 (10.353-58.02)	9.452±2.290 (4.661-14.426)	0.0001*
TGF-β1 (pg/ml)	22.702±10.163 (10.33-46.4)	1.713±1.303 (0.37-6.38)	0.0001*
-Data were presented as Mean±SD (Range)			
*Significant difference between two independent means using Student-t-test at 0.05 level.			

The results revealed a significant decrease level VTN in CKD patients as compared to control subjects (3.166±0.888), (6.379±0.881) respectively While serum VTN level ranged between (5.42-1.21) and (5.32-9.01) in CKD, and healthy control groups respectively, as shown in figure (1).



**Figure 1: Distribution of VTN values in the CKD and control groups**

The present result revealed the following: a) the serum concentration of VTN increase in early stages of CKD, and decrease with disease progression: b) increased serum VTN might be released from activated platelet c) progression renal injury may also contribute to the decreased serum VTN concretion because of VTN precipitated in kidney. VTN, one of the αvβ3 ligands of integrin, is a multifunctional glycoprotein found in the blood and ECM [14]. This protein, which is an adhesive glycoprotein in structure, is initially defined as serum spreading factor in blood. VTN plays an important role

in fibrinolysis, the immune defense, and hemostasis by providing cell adhesion and migration through interaction with collagen<sup>[15]</sup>. In the present study, demonstrates for the first time that VTN is instrumental in CKD. The one reason of the decreased VTN levels may be that it functions as a cofactor for PAI-1 proteolytic inhibitory activities<sup>[16]</sup>.

The mean ± SD values of PAI-1 for the CKD and controls groups include (32.544±13.481), and (9.452±2.290) respectively while serum PAI-1 level

ranged between (10.353-58.020), and (4.661-14.426) in CKD and control cases respectively control as shown in the table and figure. Statistically higher significant differences were found between mean serum PAI-1 level of CKD and control groups.

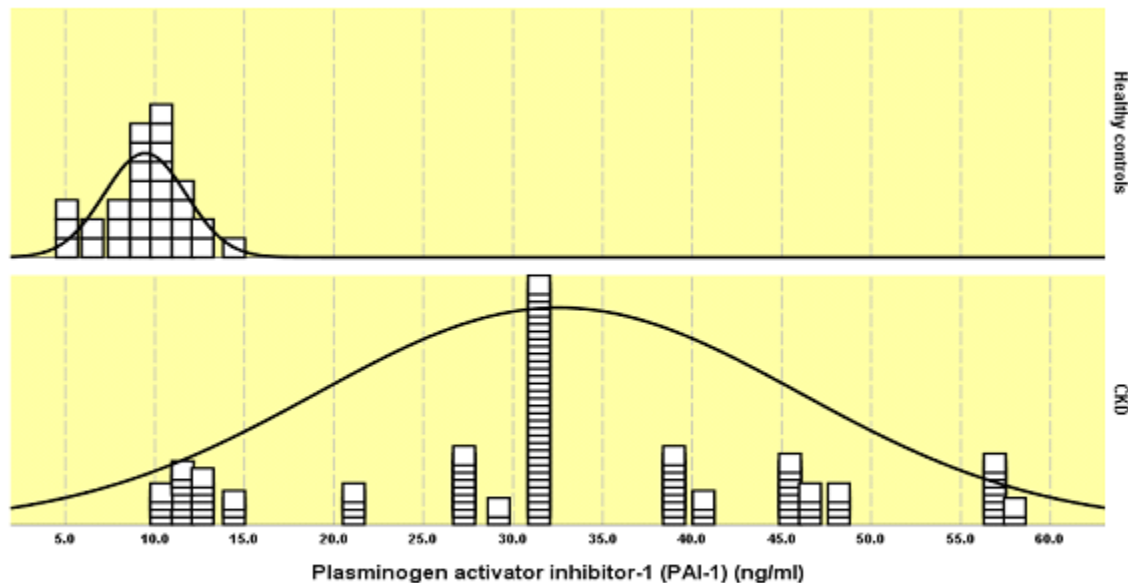


Figure 2: Distribution of PAI-1 values in the CKD and control groups.

In several other pathophysiological disorders, PAI-1 plays an important role, include metabolic syndrome, wound healing, diabetes, cancer and heart disease<sup>[17]</sup>. PAI-1 has appeared recently as an important fibrogenic mediator in renal diseases, which include glomerulonephritis and diabetic nephropathy<sup>[18]</sup>. By contrast PAI-1 dysfunction mitigates diabetic nephropathy and PAI-1 function disturbance dramatically lead thrombosis and fibrosis in mice<sup>[19]</sup>. Consequently, repression of PAI-1 genetic expression could have important renoprotective effects and the development of different antagonists of PAI-1 could produce new therapeutic strategies<sup>[20]</sup>. In humans, only trace amounts of PAI-1 are produced by healthy kidneys, whereas it is synthesized in higher levels in acutely or chronically injured kidneys<sup>[21]</sup>

Latest observational studies have linked CKD to a threat of venous thrombosis closely associated with increased PAI-1 levels<sup>[22]</sup>. The correlation between low eGFR and higher levels of hemostatic factors is clarified by several possible mechanisms. Reduced renal clearance can lead to higher levels of smaller hemostatic molecular weight markers. Results of a Dubin et al study showed that PAI-1 was 6.5 percent higher in patients with eGFR < 60 ml/min/1.73m<sup>2</sup> comparison with subjects

with eGFR > 90. The study suggested that hemostasis deregulation might play a significant pathology role in CKD<sup>[23]</sup>.

Ma and Fogo (2016) reported that fibrosis and glomerulosclerosis were strongly associated with increased regional PAI-1. In contrast PAI-1 inhibition blocks CKD development and can even promote glomerulosclerosis relapse, probably due to proteolysis effects. PAI-1 interactions with vitronectin, on the other hand, appear key in the renal interstitium and promote cell migration. There are numerous dynamic associations with angiotensin, aldosterone, TGF- $\beta$  and kidney. More analysis of PAI-1's regional activity and its interactions with other fibrosis modulators may lead different active strategies to the treatment of progressing renal disease<sup>[24]</sup>. The results of the present study correspond to the above results.

The present study showed a significant increase level of TGF- $\beta$ 1 in CKD patients as compared to control subjects (22.70 $\pm$ 10.16 vs. 1.71 $\pm$ 1.30pg/ml), while serum TGF- $\beta$ 1 level ranged between (10.33-46.40), and (0.37-6.38) in CKD, and healthy control groups respectively. As shown in table (2) and figure (3). TGF- $\beta$ 1 as dependent outcome variable demonstrated a

strong association with the mild to moderate CKD patients. This observation fits well with the results of Wong et al. (2013) who indicates that elevated levels of TGF- $\beta$  in this patient population indicate progressing renal disease [25].

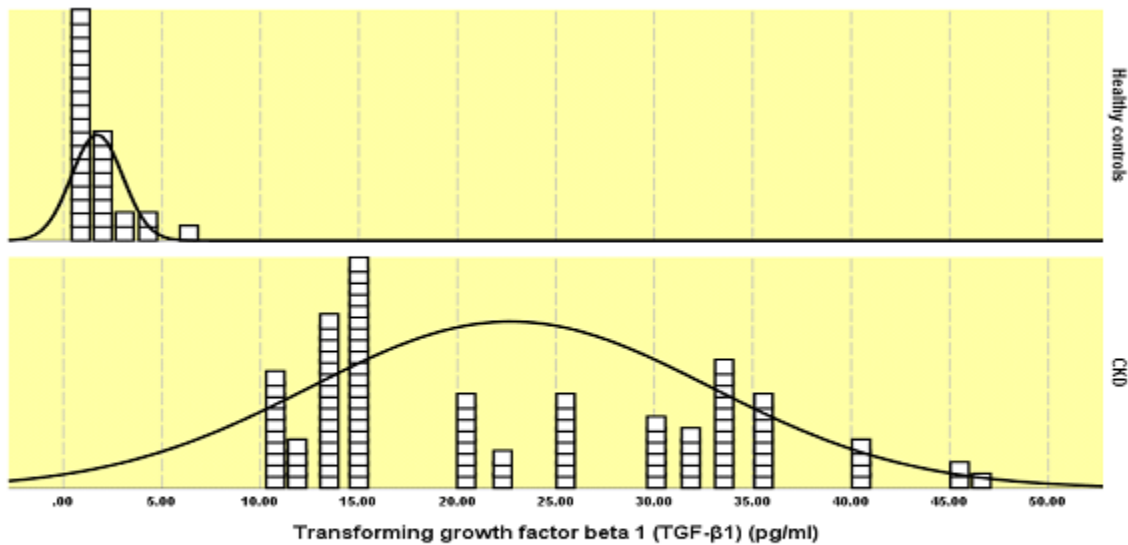
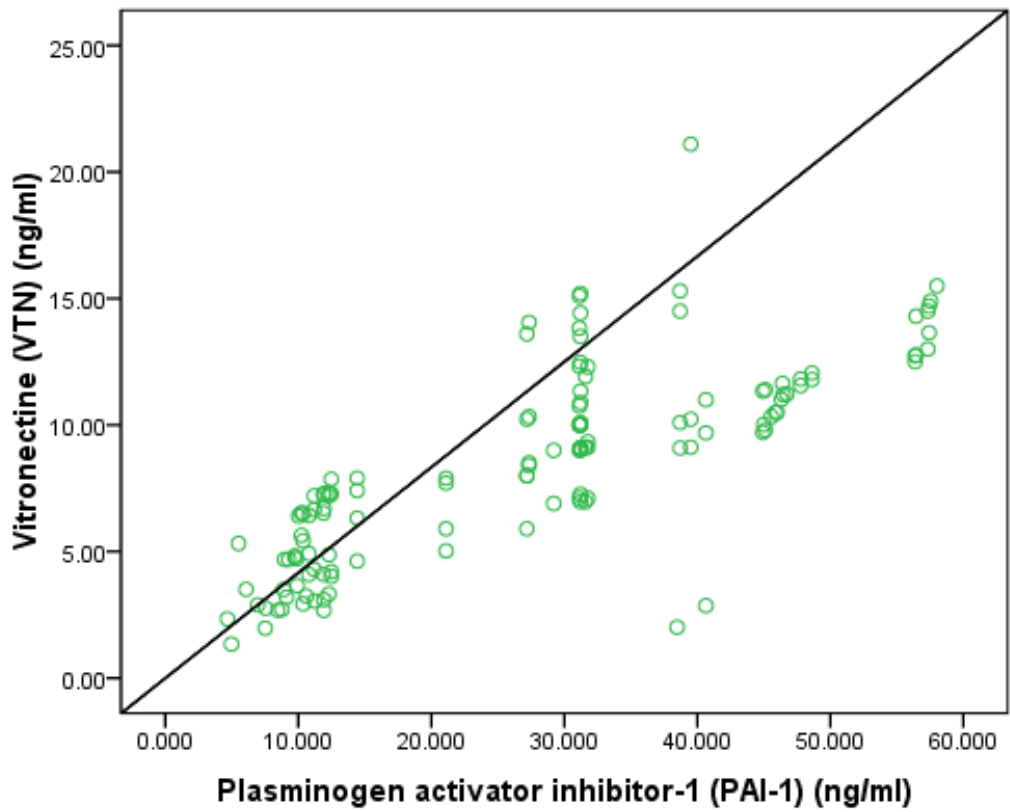


Figure 3 : Distribution of TGF- $\beta$  values in the CKD and control groups

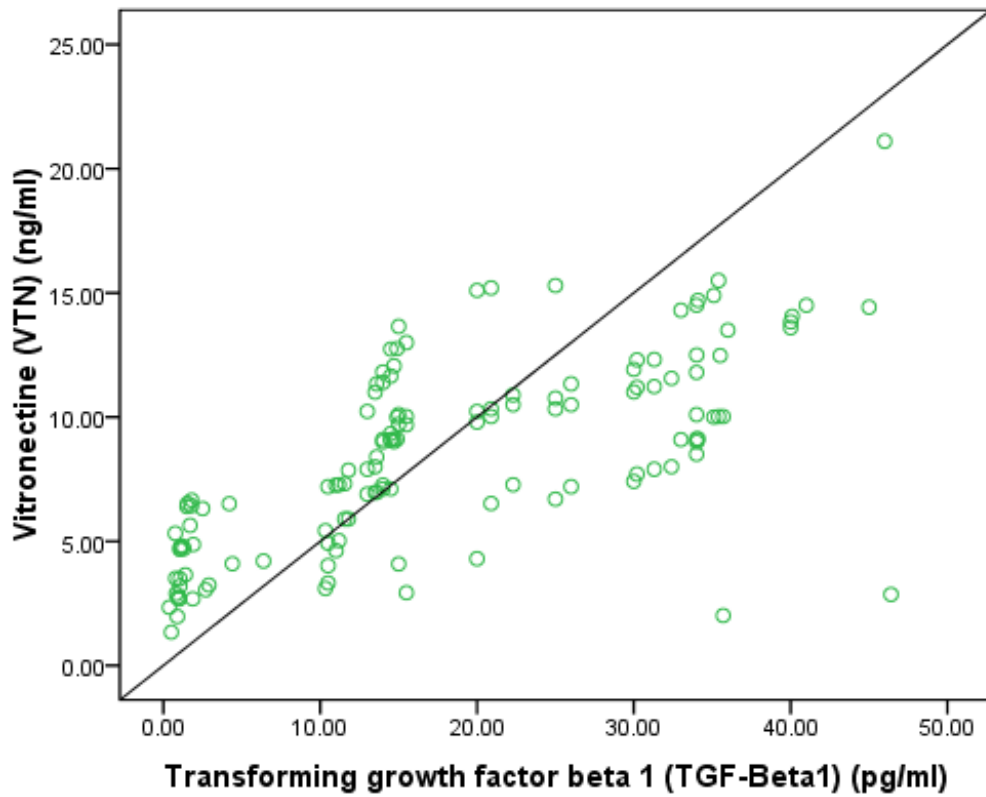
These findings indicate that TGF- $\beta$  plays a significant role in renal disease glomerular and tubulointerstitial pathobiology by having contributed to pathological modifications which cause modifications in the glomerular filtration membrane, glomerulosclerosis and fibrosis, and tubular degeneration leading to irreversible renal dysfunction [26]. Mehta et al (2017) propose that elevated TGF- $\beta$  can be a vascular disease marker which leads to decline in GFR, age mortality and CV events [27]. Unlike these results, an examination of TGF- $\beta$  levels in the Chronic Renal Insufficiency prospective study in 3791 participants showed no cross-sectional correlation between TGF- $\beta$  levels and CKD measurements [28].

The results showed a presence of strong positive correlation between Vtn and PAI-1, and TGF- $\beta$  ( $r=0.72$

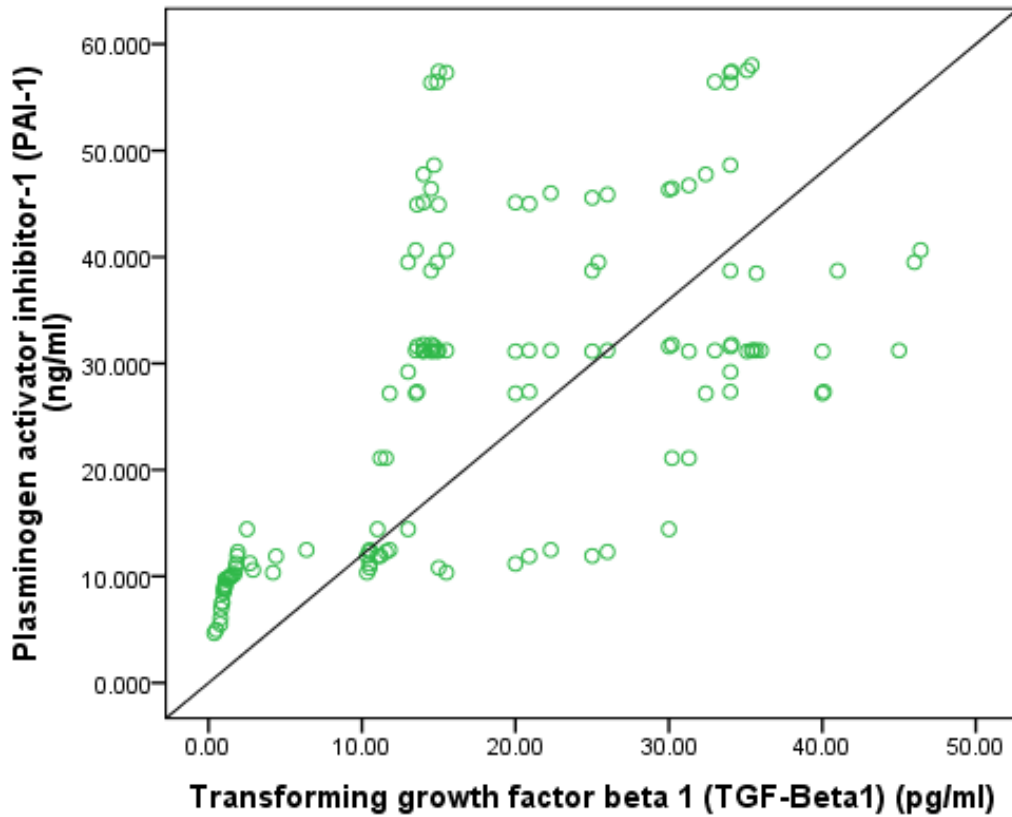
$1, p=0.0001$ ), ( $r=0.698, p=0.0001$ ) and ( $r=0.648, p=0.001$ ) in patients group as shown in fig 4(A,B and C) These result in agreement with [29]. VTN attaches with a high affinity to PAI-1 through its NH<sub>2</sub>-terminal somatomedin B (SMB) domain, a connection favored to VTN receptor cooperation, resulting in the active conformation of PAI-1 stability. PAI-1 is a powerful renal fibrosis-promoting molecule, at least partially mediated by its ability to improve interstitial myofibroblast recruitment [30]. While less thoroughly investigated, VYN has been documented to associate a number of other molecules involved in chronic renal disease, include hepatocyte growth factor TGF- $\beta$  1, epidermal growth factor, connective tissue growth factor, fibroblast growth factor, vascular endothelial growth factor, anti-thrombin complexes, insulin-like growth factor II and proteoglycans [29].



(A)



(B)



(C)

**Figure 4: The correlation between VTN & PAI-1 (A) VTN & TGF-Beta 1 (B) PAI-1 & TGF-Beta 1(C) in CKD**

**Conclusion**

These results indicate that VN and PAI-1 are connected to TGF-β’s active form and can be used as a prediction for CKD’s progression

**Conflict of Interest:** Null

**Source of Funding:** Self

**Ethical Clearance:** This study was conducted with the consent of the volunteers and without mentioning the names with the complete privacy of volunteers.

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