Research Article

Endothelial Markers in patients with Renal Impairment.

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Abstract

Background: Chronic kidney disease CKD is an abnormality of kidney structure or function that is present for greater than three months. Endothelial dysfunction is prevalent in patients with CKD. **The aim of this study is to** study changes in endothelial markers in CKD and evaluate the relationship between these changes and presence of prethrormbotic state. **Materials and methods:** The present study included sixty patients with CKD in addition to twenty apparently healthy volunteers who served as a control group of matched age and sex. Patients with CKD were subdivided into three subgroups. Group I: stage 2 CKD, Group II: stage 3 and 4 CKD and Group III: stage 5 CKD. All the study subjects were submitted to Clinical History, Clinical Examination and Laboratory Investigations (Serum Creatinine and urea, Serum total cholesterol and Triglycride, Fasting blood glucose, HbAlc, Urine protein / Creatinine ratio, eGFR, PT, APTT, thrombomodulin, tissue factor, Tissue factor pathway inhibitor, D-dimer and Fibrinogen. **Results:** There were statistically significant higher fibrinogen levels in group II and III as compared with group I, (P. = 0.046 and 0.001) respectively. There were statistically significant higher levels of D-dimer in group II and III as compared with group I, (P. = 0.049 and 0.003) respectively. There were statistically significant higher levels of TFPI in group III as compared with group I and II, (P. = < 0.001 and < 0.001) respectively. There were statistically significant higher levels of TF in group III as compared with group I and II, (P. = < 0.001 and < 0.001) respectively. **Conclusion:** Lower eGFR was associated with higher levels of Fibrinogen, D-dimer, TFPI, TM and TF in subjects with CKD than normal subjects.

Key words: Chronic kidney disease (CKD), Estimated glomerular filtration rate (eGFR), Tissue factor (TF), Thrombomodulin (TM).

Introduction

Chronic kidney disease is an abnormality of kidney structure or function that is present for greater than three months[1]. Endothelial dysfunction is highly prevalent in patients with advanced chronic kidney disease[2] and is linked to the elevated cardiovascular risk of this patient population[3].

Prolonged exposure to risk factors, such as inflammation and oxidative stress chronically present in CKD patients after the normal homeostatic properties of the endothelium and activate endothelial cells [4].

Injured endothelial cells release sTM into the circulation, where it bind thrombin, inhibiting it from forming fibroin and activating platelets sTM has additional antithrombotic activity as a cofactor for the thrombin catalyzed activation of protein C[5]. Endothelial cells also express TF after injury that initiates the extrinsic coagulation cascade[6].

Injured endothelial cells also release TFPI is a major physiological circulating inhibitor. TFPI binds to factor Xa and, in this combination, binds to and inhibits tissue factor/factor VIIa complex and activated FX (FXa) and thus TFPI is currently being included as a natural coagulation inhibitor[7].

Patients with CKD have elevated levels of plasma fibrinogen. Inflammation associated with lower eGFR in those patients causing elevation of markers that are acute phase reactants as these patients have a state of persistent low grade inflammation also elimination of fibrinogen decreased in renal insufficiency and ESRF [8].

Also kidney dysfunction may generate a thrombotic milieu indirectly through electrolyte or aced-base abnormalities which may alter activities of enzymes involved in coagulation. The higher molecular weight of D-dimer is elevated in these patients due to process initiated by the smaller molecular and inflammation associated with lower eGFR [5].

Subjects and methods

The present study included sixty patients with Chronic Kidney Disease in addition to twenty apparently healthy volunteers who served as a control group of matched age and sex.

Patients with Chronic Kidney Disease were further subdivided according to estimated glomerular filtration rate into three subgroups: **Group I:** Included 20 patients with stage 2 Chronic Kidney Disease (eGFR 60-89 ml/min/1.73m^2). **Group II:** Included 20 patients with stage 3 and 4 Chronic Kidney Disease (eGFR 15-59 ml/min/1.73m^2). **Group III:** Included 20 patients with stage 5 Chronic Kidney Disease (eGFR less than 15 ml/min/1.73m^2) and **Group IV (control group):** Consisted of 20 apparently healthy subjects.

All the study subjects were submitted to Clinical History, Clinical Examination, Laboratory Investigations: *Routine investigations***:** Serum creatinine and urea, Serum total cholesterol and triglyceride, Fasting blood glucose, HbAlc, Urine protein/creatinine ratio, eGFR, Prothrombin concentration and Activated partial thromboplastin time**.** *Special investigations:* Soluble thrombomodulin, soluble tissue factor, Tissue factor pathway inhibitor, D-dimer and Fibrinogen.

Results

All results summarized in tables (1-3) and figures (1-4).

Demographic and laboratory data for the studied groups are shown in table (1):

Group II and III had statistically significant higher serum creatinine (P values = 0.002 and < 0.001 respectively) as compared with group IV. Also on comparing group II and III with group I they had statistically significant higher serum creatinine (P values = 0.008 and < 0.001 respectively), higher serum total cholesterol (P values < 0.001 and < 0.001 respectively), higher APTT (P values < 0.001 and < 0.001 respectively), higher P/C ratio (P. values < 0.001 and < 0.001 respectively) and lower eGFR (P. values < 0.001 and < 0.001 respectively). Group I, II and III were compared with group IV they had significantly increased prevalence of hypertension (P values = 0.017, < 0.001 and < 0.001 respectively), higher serum total cholesterol (P values < 0.001, < 0.001 and < 0.001 respectively), higher APTT levels (P values = 0.038, < 0.001 and < 0.001 respectively) and higher P/C ratio(P. values < 0.001, < 0.001 and < 0.001 respectively) but they had statistically significant lower prothrombin concentration (P values = 0.014, 0.03 and < 0.001 respectively) and lower eGFR (P values < 0.001, < 0.001 and < 0.001 respectively). Group III had statistically significant higher serum creatinine, higher P/C ratio and lower eGFR as compared to group II (P values < 0.001, < 0.001 and < 0.001 respectively) while it had statistically significant lower prothrombin concentration as compared with group I and II (P values = < 0.001 and < 0.001 respectively).

Results of hemostatic markers of studied groups are shown in table (2) & figures (1-4):

There were statistically significant higher fibrinogen levels in group I, II and III as compared with group IV (P values < 0.001, < 0.001 and < 0.001 respectively) and higher fibrinogen levels in group II and III as compared with group I (P values = 0.046 and 0.001 respectively). There were statistically significant higher levels of D-dimer in group II and III as compared with group IV (P value = 0.001 and < 0.001 respectively) and higher levels of D-dimer in group II and III as compared with group I (P values = 0.049 and 0.003 respectively). There were statistically significant higher levels of TFPI in group I, II and III as compared with group IV (P values < 0.001, < 0.001 and < 0.001 respectively) and higher levels of TFPI in group III as compared with group I and II (P values < 0.001 and < 0.001 respectively). There were

statistically significant higher levels of TF in group II and III as compared with group IV (P value = 0.007 and < 0.001 respectively) and higher levels of TF in group III as compared with group I and II (P values < 0.001 and < 0.001 respectively).

Correlations of FIB, DD, TF, TM and TFPI with eGFR are shown in table (3): There were significant negative moderate correlation of FIB and eGFR (r = -0.695, p= < 0.001), significant negative fair correlation of DD and eGFR (r = -0.342, p= 0.002), significant negative moderate correlation of TFPI and eGFR (r = -0.675, p= < 0.001), significant negative weak correlation of TM and eGFR (r = -0.015, p= 0.897) and significant negative fair correlation of TF and eGFR (r = -0.421, p= < 0.001). Fibrinogen had moderate correlation (r= 0.590) with TFPI (p= < 0.001)**.**

Table (1): Demographic and clinical characteristics of the studied group

eGFR	Group I (60-89) (n=20)	Group II (15-59) (n=20)	Group III (<15) ESRF (n=20)	Group IV Control (n=20)	P. value					
Age: Years Range. Mean ± SD	(18-71) 50.3 ± 15.4	(28-75) 55.4±13.8	(40-85) 59.7± 12.3	(21-70) 48.4 ± 6.5	0.023*					
					IV vs I	**IV vs II**	**IV vs III**	**I vs II**	**I vs III**	**II vs III**
					0.640	0.082	0.005*	0.199	0.019*	0.237
Male, n (%) Female	12(60%) 8 (40%)	12 (60%) 8 (40%)	11(55%) 9(45%)	12(60%) 8 (40%)	0.985					
					IV vs I	**IV vs II**	**IV vs III**	**I vs II**	**I vs III**	**II vs III**
					1	1	0.749	1	0.749	0.749
BMI, kg/m^2 Range. Mean ± SD	(18.5-31.2) 24.6 ± 3.7	(18.7- 37.1) 27.8 ± 4.9	(23.2-37.1) 30.05 ± 4.6	(20.7-32.4) 24.1 ± 3.2	< 0.001*					
					IV vs I	**IV vs II**	**IV vs III**	**I vs II**	**I vs III**	**II vs III**
					0.729	0.007*	0.017*	<0.001*	<0.001*	0.091
Current Smoker, n(%)	4 (20)	4 (20)	5 (25)	2 (10)	0.853					
					IV vs I	**IV vs II**	**IV vs III**	**I vs II**	**I vs III**	**II vs III**
					0.637	0.667	0.313	0.888	0.801	0.563
Prevalent Diabetes, n(%)	3 (15)	3 (15)	1 (5)	0 (0)	0.238					
					IV vs I	**IV vs II**	**IV vs III**	**I vs II**	**I vs III**	**II vs III**
					0.072	0.072	0.311	1	0.292	0.292
Prevalent Hypertension, n (%)	5 (25)	10 (50)	16 (80)	0 (0)	< 0.001*					
					IV vs I	**IV vs II**	**IV vs III**	**I vs II**	**I vs III**	**II vs III**
					0.017*	<0.001*	<0.001*	0.102	<0.001*	0.047*

=significant** *= highly significant**

Table (2): Results of hemostatic markers of studied groups:

eGFR	Group IV Control (n=20)	Group I (60-89) (n=20)	Group II (15-59) (n=20)	Group III (<15) ESRF (n=20)	P. value					
FIB: (g/dl) Range. Mean±SD	(1.8-3) 2.2 ± 0.3	(2.4-4.7) 3.78 ± 0.7	(2.1-5.5) 4.2 ± 1.03	(2.4-6.4) 4.6 ± 1.2	< 0.001*					
					IV vs I	**IV vs II**	**IV vs III**	**I vs II**	**I vs III**	**II vs III**
					<0.001*	<0.001*	<0.001*	0.046*	0.001*	0.129
DD (ng/ml) Range. Mean ±SD	(100-200) 145 ± 35.9	(100-1500) 390.3 ± 443.7	(100-1500) 740 ± 559.04	(100-2500) 980 ± 834.9	< 0.001*					
					IV vs I	**IV vs II**	**IV vs III**	**I vs II**	**I vs III**	**II vs III**
					0.129	0.001*	<0.001*	0.049*	0.003*	0.4
TFPI (pg/ml) Range. Mean ±SD	(11000-15000) 13375 ± 1098.7	(10000-36000) 23800 ± 6143.9	(10000-54000) 25055.6 ± 11201.2	(25000-60000) 39950 ± 9207.9	< 0.001*					
					IV vs I	**IV vs II**	**IV vs III**	**I vs II**	**I vs III**	**II vs III**
					<0.001*	<0.001*	<0.001*	0.621	<0.001*	<0.001*
TM (pg/ml) Range. Mean ±SD	(1000-2350) 1551±350.5	(620-4400) 1677±1091.2	(620-3400) 1730.5 ± 1029.6	(620-3200) 675.5 ± 822.9	0.850					
					IV vs I	**IV vs II**	**IV vs III**	**I vs II**	**I vs III**	**II vs III**
					0.266	0.533	0.797	0.903	0.606	0.839
TF (pg/ml) Range. Mean ±SD	(30-92) 51.9 ± 20.9	(30-124) 59.1 ± 24.2	(46-190) 84.1 ± 37.4	(64-250) 136.7± 55.04	< 0.001*					
					IV vs I	**IV vs II**	**IV vs III**	**I vs II**	**I vs III**	**II vs III**
					0.542	0.007*	<0.001*	0.035	<0.001*	<0.001*

=significant** *= highly significant**

Table (3): correlations of FIB, DD, TF, TM and TFPI with eGFR:

eGFR verus:-	r (correlation coefficient)	P. value	Degree
FIB	-0.695	<0.001*	Moderate
DD	-0.342	0.002*	Fair
TEPI	-0.675	<0.001*	Moderate
TM	-0.015	0.897	Weak
TF	-0.421	<0.001*	Fair

Correlation degrees:
Weak: (r= 0 - 0.24)
Fair: (r = 0.25 - 0.49)
Moderate: (r = 0.5 - 0.74)
Strong: (r = 0.75 - 1)
***: Statistically Significant.**

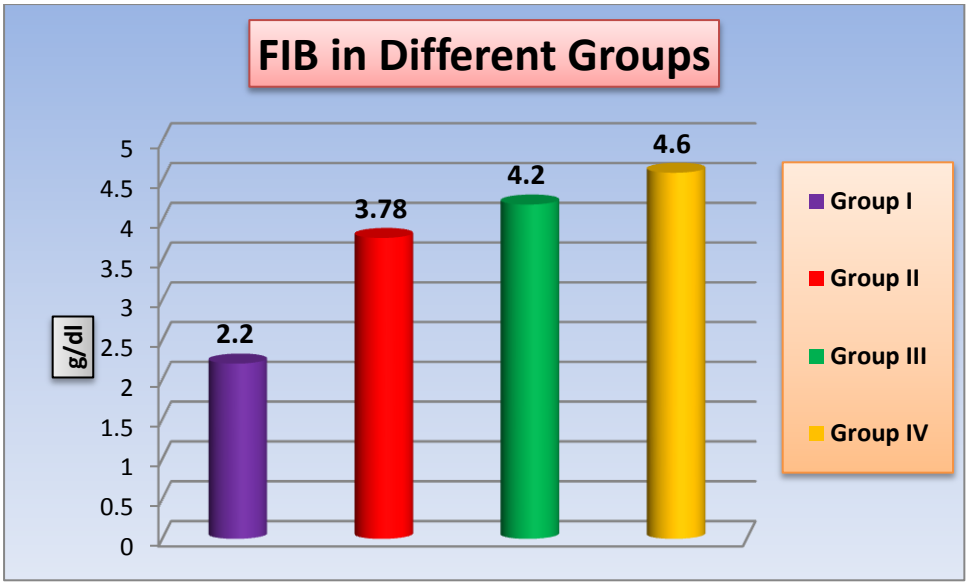

Fig. (1): Mean Values of Fibrinogen in different studied groups

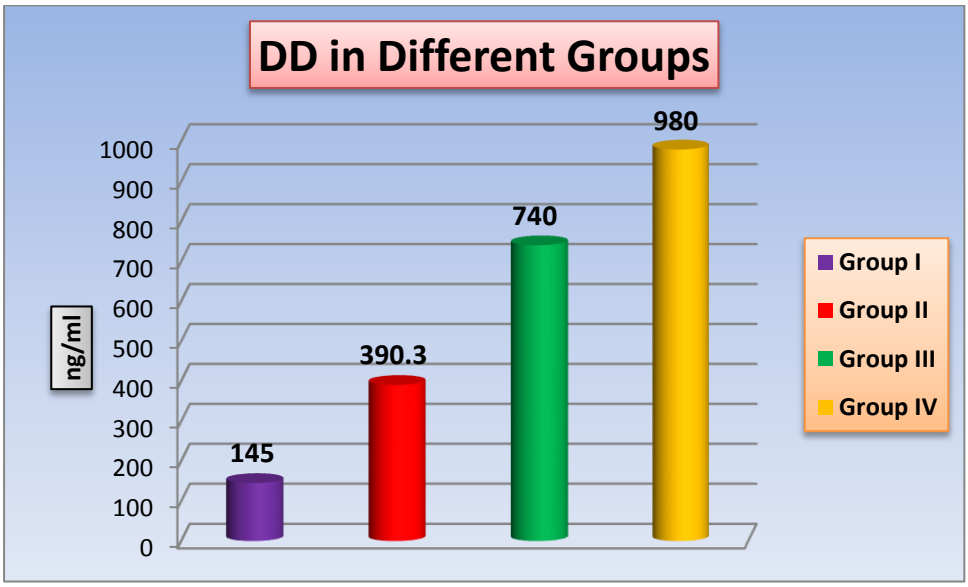

Fig. (2) : Mean values of D- dimer in different studied groups

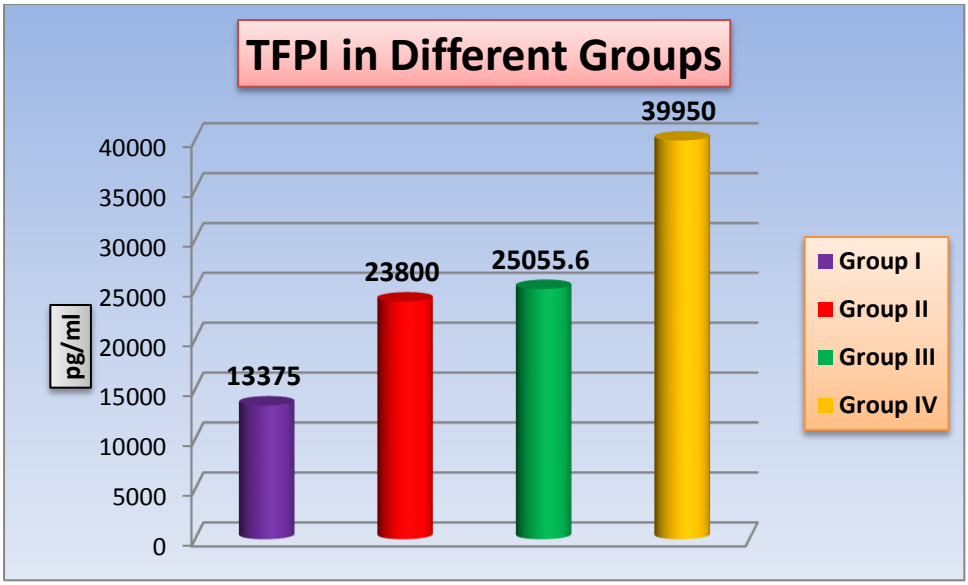

Fig. (3) : Mean values of TFPI in different studied groups

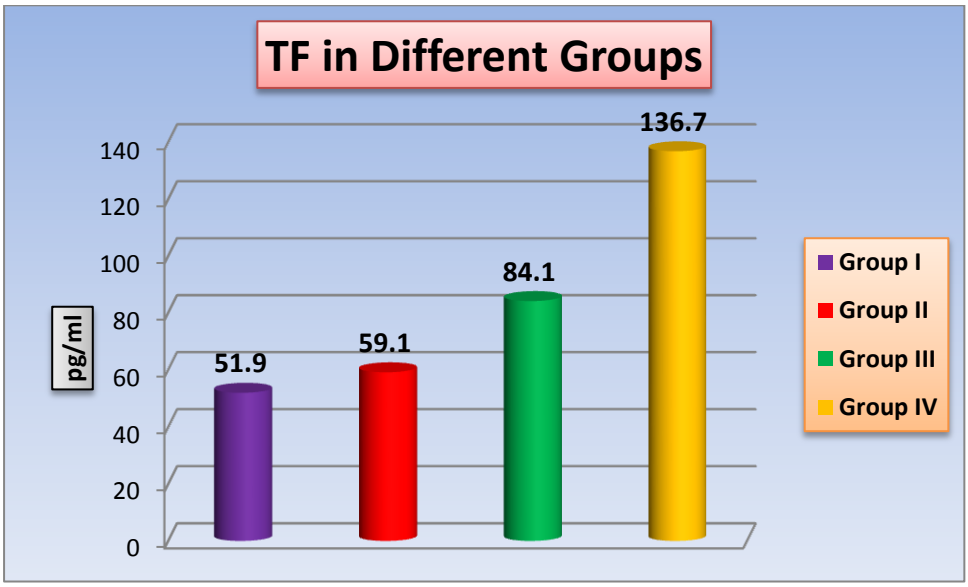

Fig. (4) : Mean values of TF in different studied groups

Discussion

CKD is a serious health problem, often associated with other common chronic diseases such as diabetes, hypertension, and cardiovascular disease (CVD) 9. CKD and associated conditions (e.g., CVD, diabetes and hypertension) place persons at risk of end stage renal disease (ESRD) requiring RRT (e.g., dialysis or transplantation)10.
Several hemostatic abnormalities have been described in patients with even mild chronic Kidney disease. Endothelial cell injury in these patients is due to uremic toxins retention and hypertension11.

Injured endothelial cells release soluble thrombomodulin, tissue factor pathway inhibitor and express tissue factor. Patients with CKD have increased levels of plasma fibrinogen and D-dimer that directly contributable to hypercoagulable state12.

The D-dimer antigen is a unique marker of fibrin degradation that is formed by the

sequential action of 3 enzymes: thrombin, factor XIIIa, and plasmin. D-dimer levels correlated with the grade of renal function impairment and inversely with eGFR13.

Fibrinogen (Fg) is a high molecular weight plasma adhesion glycoprotein. Plasma fibrinogen is an important component of the coagulation cascade and a major determinant of blood viscosity and blood flow. Elevated plasma fibrinogen concentrations are associated with an increased risk of cardiovascular events, including ischemic heart disease, stroke. There were positive associations between plasma fibrinogen and cardiovascular mortality among patients undergoing dialysis treatment, there were elevated levels of Plasma fibrinogen in patients with stage 3–stage 4 CKD that predicted future cardiovascular events. Plasma fibrinogen associates with mortality among subjects with mild to moderate kidney impairment8.

Tissue factor pathway inhibitor (TFPI) is a major physiological circulating inhibitor. TFPI binds to factor Xa and, in this combination, binds to and inhibits tissue factor/factor VIIa complex and activated FX (FXa) and thus TFPI is currently being included as a natural coagulation inhibitor. TFPI is synthesized primarily by the vascular endothelium. Lower eGFR was significantly associated with higher levels of TFPI in subjects with CKD 5. As the initiator of blood coagulation, TF has a central role in maintaining hemostasis 14. It is exposed on the surface of fibroblasts as a result of vessel wall injury and joins activated factor VII (FVIIa) normally present in the circulating blood and the TF-FVIIa complex converts FX to FXa.5. As the initiator of blood coagulation, TF has a central role in maintaining hemostasis

Increased levels of plasma TF have been observed in patients with renal failure and in patients with CKD15.
TM is a cell surface-expressed transmembrane glycoprotein which is originally identified on vascular endothelium, so it is an important molecule in human natural anticoagulation system. Endothelial injury has been reported to be the unique stimulation for sTM to be released from the surfaces of endothelial cells16.

Increased levels of plasma sTM can therefore serve as a marker of endothelial injury. Furthermore, increased plasma levels of sTM have been reported to be associated with kidney injury induced by sepsis17 and diabetes18.

Soluble TM concentrations significantly increase in the CKD patients and are associated with the severity of the disease. Soluble TM may play critical roles in the development of CKD, as a biomarker of endothelial cells damage, anticoagulation and anti-inflammation19.

The current study found that subjects with lower eGFR were older, obese and have high prevalence of hypertension and diabetes 52% and 7% respectively than subjects without CKD. This in agreement with Ruth et al 2011 5 who found that subjects with CKD were older and had high prevalence hypertension and diabetes 45% and 14% respectively.

The present study found that the levels of urine protein/ creatinine ratio increase in subjects with CKD than subjects without CKD, $p = < 0.001$. This is in agreement with Johannes and George, 2014 20 and Ruth et al 2011 5 who found that subjects with CKD had higher urine/protein/ratio than subjects without CKD, $p = < 0.001$. This can be explained by glomerular damage as proteinuria typically occur with progressive, CKD even in primary tubule interstitial diseases such as chronic pyelonephritis due to reflux nephropathy. Interstitial fibrosis and primary glomerular disease or secondary to intra glomerular hypertension and glomeruli hypertrophy can course glomeruli damage.

Our study found that the level of cholesterol and triglycerides were elevated in patients with CKD than the subjects without CKD. This is in agreement with Ruth et al 2011 5 and Robert 2014 21.

Our study found that the level of prothrombin time and APTT are elevated in patients with CKD than subjects without

chronic kidney disease, p= < 0.001 and p= < 0.001 respectively. This is in agreement with Ramaprobhaetal 2014 22, Subhard and Shahide et al, 2013 23 who found that the level of prothrombin time and APTT are elevated in patients with CKD than subjects without chronic kidney disease. This could be explained by diseases associated with renal disease as gastroenteritis, septicemia, pregnancy induced renal failure and DLC.

The present study found that the levels of serum FIB, D-dimer, TFPI, TM and TF were elevated in patients with CKD than subjects without CKD p= < 0.001, p= < 0.001, p= < 0.001, p= < 0.850 and p= < 0.001. This is in agreement with Ruth et al 2011 5, who found that the levels of serum FIB, D-dimer, TFPI, TM and TF were elevated in patients with CKD than subjects without CKD p= < 0.001, p= < 0.001, p= < 0.001, p= < 0.001 and p= < 0.001. There are several possible mechanisms to explain the association of lower eGFR and higher levels of hemostatic factors. A direct effect of decreased renal clearance may explain an increase in levels of smaller molecular weight hemostatic markers such as sTF and sTM, as these may be filtered at the glomerulus.

The higher molecular weight of D-Dimer is elevated due to processes initiated by smaller molecules. Inflammation associated with lower eGFR may cause elevation in markers that are acute phase reactants.

The present study found that lower eGFR was significantly associated with higher levels of fibrinogen, p=< 0.001. This is in agreement with Stack et al., 2014 8 who found that subjects categorized as having eGFR < 60 ml / min had highest concentration of plasma fibrinogen. Similar results were reported by Evangelia et al., 2012 24 who found that subjects with CKD had high concentration of plasma fibrinogen than without CKD p = < 0.001. Also Ruth et al., 2011 5 found that lower eGFR was significantly associated with higher levels of fibrinogen p= < 0.001. There were elevated levels of serum fibrinogen in patients with chronic kidney disease than the normal control. This could be explained by inflammation associated with lower eGFR cause elevation of markers that are acute phase reactants.

A clinically important system may be involved in the hypercoagulable state of patients with CKD is the renin-angiotensin-aldosterone system as its activation has been associated with increased levels of plasma fibrinogen and D-dimer. Also inhibition of release of platelet derived growth factors and endothelial growth factors increase the circulation plasma fibrinogen level.

The present study found that lower eGFR is associated with higher levels of D-dimer p= < 0.001. This is in agreement with Lindner et al., 201425 who found that D-dimer levels were elevated in patients with an eGFR < 60 ml / min p= < 0.001. Also Subhan and Shahida 2013 23 found that patients with CKD had significantly elevated level of D-dimer, p= 0.001. This came in accordance with previous study of Ruth et al., 2011 5 who found that lower eGFR is associated with higher levels of D-dimer p= < 0.001. This could be explained by renal insufficiency was associated with increased levels of inflammatory and procoagulant biomarkers. This association caused by either their increased production, decreased clearance, or a combination of both mechanisms. Patients with CKD have increase fibrin clot formation and breakdown also related to process initiated by smaller molecules that cause decreased renal clearance. Activation of renin-angiotensin-aldosterone system cause increased in D-dimer level.

The present study found that there was increased level of TFPI in patients with chronic kidney disease than the subjects without p= < 0.001. This is in agreement with Ruth et al, 2011 5 who found that increased level of TFPI in patients with chronic kidney disease than the subjects without p= < 0.001. This also in agreement with Amal et al., 2015 26 who found that TFPI was significantly higher in patients with CKD than control group p= < 0.05. This could be explained by endothelial injury or dysfunction in patients with chronic kidney disease as it caused by several factors as oxidative stress, inflammation, hemostatic derangements, uremia, protein energy wasting, vascular

calcification, anemia, molecular alterations and proteinuria. Also high level of TFPI in these patients caused by reduced kidney catabolism and increase expression tissue factor from endothelial cells often injury.

This study found that there was elevated level of sTM in patients with chronic kidney disease stage II, III and IV than normal control. This is in agreement with Bao et al, 2014 16. Who found that there were higher levels of TM in patients with CKD stage II, III and IV than the control group. Also Ruth et al., 2011 5 found that patients with CKD stage II, III and IV had higher levels of TM than control group. High levels of sTM cleaved from endothelial cells indicate disorders associated with vascular damage including a variety of infection, sepsis, inflammation and uremia accompanying patients with chronic kidney disease. Injured endothelial cells release sTM into circulation. Association between lower eGFR and elevated sTM in patients with chronic kidney disease occurs as a direct effect of decreased renal clearance as they filtered at the glomerulus. Also sTM is increased in patients with CKD due to the anti-inflammatory and the anti-coagulant effect of this factor.

Our study found that the level of TM in patients with ESRF were decreased than control group and this could be explained by in patients with ESRD, endothelial cell damage can lead to coagulation disorders together with thrombophilia. Hemocysteine can play a role as a mediator between renal dysfunction and endothelial cell damage. It can inhibit the TM – dependent activated protein C system lead to permanent activation of thrombin with subsequent form of fibrin. Also hemocysteine reduce the expression of TM on the surface of endothelial cells. Hemocysteine decrease the amount of intact TM in endothelial cells and both reduce and unreduced forms of TM.

This study found that there were elevated levels of sTF in patients with chronic kidney disease than normal control. P= < 0.001. This is in agreement with Ruth et al, 2011 5 and Amal et al., 2015 26 who found that elevated levels of sTF in patients with chronic kidney disease than normal control. P= < 0.001and p= < 0.001.Elevated levels of sTF in patients with chronic kidney disease is due to endothelial damage or dysfunction as TF is expressed on the surface of the injured endothelial cells. Also decrease renal clearance of sTF which is filtered at the glomerular results in elevation of sTF with lower eGFR.

In constant to present study bleeding has been reported in 40 – 50% of patients with chronic kidney disease in the study of Pavorad and Myers 2011 27. Also Jens et al., 2014 12 reported that previous study found that 24% of patients with CKD had bleeding event. Increased risk of bleeding in patients with CKD is the result of changes in platelets related to the composition of α-granules, a deregulation of arachidonic acid and prostaglandin metabolism, circulating fibrinogen fragments, changed calcium content and calcium mobilization, as well as oxidative stress.

Furthermore, the changed platelet–vessel wall interaction with a reduced amount of GP Ib receptor for adhesion molecules on endothelial cells, a reduced binding of vWF and fibrinogen with decreased function of the GP IIb/IIIa complex and an increased concentration of vasoactive substances (i.e.NO) also contribute to the risk of bleeding. In addition, anemia contributes to the increased risk of bleeding in CKD through a reduced platelet–vessel wall interaction together with a reduced ADP release/inactivation of PGI2, and a reduced scavenging of NO by hemoglobin. Moreover, bleeding episodes can be the result of an accumulation of anticoagulants in patients with CKD.

Conclusions

Lower eGFR was associated with higher levels of Fibrinogen, D-dimer, TFPI, TM and TF in subjects with CKD than normal subjects. Increased levels of smaller molecular weight molecules such as sTM and sTF may be a direct result of decreased renal clearance. Elevations of larger molecules may represent increased fibri-nolytic activity and higher clot burden of a

thrombotic milieu induced by declining kidney function.

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