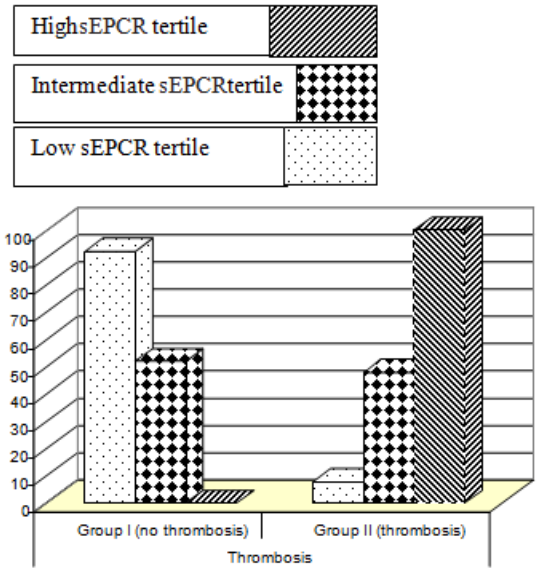
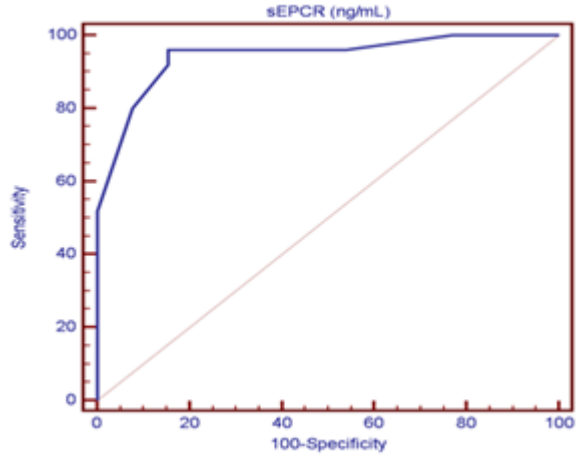


Figure 2: Comparison between three sEPCR tertiles and percentages of thrombosis occurrence. The percentage of thromboses development was significantly higher in the highest sEPCR quartile [all the 12 patients had history of thrombosis (100.0%)] compared with the other two groups [7.69% in the lowest quartile (1\13 patients had history of thrombosis) and 48.0% in the intermediate quartile (12/25 patients had history of thrombosis); p =0.000]



Cut off point	AUC	Sensitivity	Specificity	+PV	- PV	Accuracy
>200 ng/ml	0.943	96.00	84.62	85.7	95.7	90.31

AUC: Area under the curve, +PV: Positive predictive value, -PV: Negative predictive value

Figure 3.ROC curve analysis showing the prognostic performance of sEPCR to differentiate between HD patients as regards thrombotic manifestations. The best cut-off for sEPCR was 200 ng/ml.

Table 1: Laboratory data of the two patients' groups showing that Groups 1 and 2 were similar with regard to possible risk factors for thrombosis except for the PTH which was statistically significant higher in group 2 (HD patients with thrombosis history)

Laboratory data		Group1		Group 2		Test of significance	
		Mean	SD	Mean	SD	t	p-value
CBC parameters							
Hb (g/dL)		10.49	1.73	10.48	2.75	0.019	0.985
RBC (10^6/mL)		3.89	0.78	3.74	1.10	0.575	0.568
WBC's (10^3/mL)		6.21	2.18	6.76	2.52	0.832	0.410
Plat (10^3/mL)		207.27	75.65	199.4	63.11	0.403	0.689
Hct (%)		32.88	5.75	31.31	9.18	0.733	0.467
MCV (fL)		82.03	13.67	85.36	10.51	0.971	0.336
MCH (pg)		31.30	11.28	28.11	4.35	1.322	0.192
MCHC (g/dL)		32.65	1.36	32.34	2.54	0.533	0.597
Kidney parameters							
Urea (mg/dL)		166.96	41.30	175.96	40.23	0.788	0.435
Creatinine (mg/dL)		7.03	2.18	7.54	2.30	0.819	0.416
Uric acid (mg/dL)		4.79	1.06	4.47	1.02	1.084	0.284
Others							
Ca (mg/dL)		7.76	1.42	10.48	12.42	1.109	0.273
Ph (mg/dL)		4.98	1.28	5.14	1.49	0.430	0.669
PTH (pg/mL)		279.68	182.1	432.65	328.1	2.069	0.044*
Demographic data		**Group1**		**Group 2**		**Chi-square test**	
		Mean	**SD**	**Mean**	**SD**	**X^2**	**p-value**
Age (yrs)		38.96	12.86	37.76	11.65	0.346	0.731
BMI (kg/m^2)		21.35	3.62	22.5	2.99	0.320	0.751
Dialysis duration(yrs)		6.77	4.17	8.16	3.66	1.264	0.212
Sex	**Female N (%)**	17(68.0%)		10(40.0%)		2.899	0.088
	Male N (%)	8 (32%)		15(60%)			

BMI: Body Mass Index N: Number, SD: Standard deviation CBC: Complete blood count, Hb: Hemoglobin, RBCs: Red blood cells, WBCs: White blood cells, Plat: Platelets, Hct: Hematocrite, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, Ca: Calcium, Ph: Phosphorus, PTH: Parathyroid hormone. * Significant p< 0.05; ** highly significant p< 0.01

5. Discussion

Vascular complications represent 20-25% of all hospitalized patients on hemodialysis. The spectrum of thromboembolic events in ESRD patients is wide, the most frequent thrombotic event being arteriovenous (AV) access thrombosis [12].Changes in the haemostatic system may play a major role in the pathogenesis of cardiovascular complications and vascular thrombosis; during haemodialysis, platelets, coagulation and fibrinolytic systems could be importantly affected due to several known factors (e.g. alterations in vessel wall integrity and platelet function, reduced blood flow in the native arteriovenous fistula, velocity of procedure, type of membrane, artificial vascular access, circuit composition, and the type of anticoagulation)[13], [14].

Thus, it is essential to investigate haemostatic alterations in patients on haemodialysis so that adequate regimes for anticoagulant therapy could be implemented. The aim of the present study was to explore the level of sEPCR in haemodialysis patients, to correlate its level with other parameters that are possible risk factors for thrombotic events (i.e., demographic features, dialysis duration, body mass index, and various laboratory parameters such as whole blood count and serum levels of albumin, calcium, phosphorus, uric acid, creatinine, urea, parathyroid hormone (PTH) and correlate its level with the thrombotic events.

The study was carried on 50 HD patients, divided into 2 groups; group 1 with no history of thrombosis and group 2 with history of thrombosis within the previous 6 months. Patients with increased tendency toward thrombotic events (diabetic patients, malignancy patients, smokers, autoimmune disorders patients and patients with known clotting disorders) and patients on anti coagulant therapy were excluded.

Our results showed statistically higher significant serum level of sEPCR in both groups of HD patients compared to the healthy control group in addition to a statistically higher significant serum level of sEPCR among group 2 (with thrombosis) patients compared to group1(without thrombosis)patients. Indeed ***Bilgic et al., (2007)*** [15] demonstrated a relationship between plasma sEPCR levels and development of AVF thrombosis in HD patients.

Likewise, numerous studies investigated the correlation of sEPCR to thrombosis in various disorders. ***Ulu et al., (2007)*** [16] reported that sEPCR level was higher in a group of a stroke patients compared to a control group and those patients with high sEPCR level were found to have the A3 haplotype of the EPCR gene and thus concluded that sEPCR level is higher than normal in those with the A3 haplotype and that this might be associated with a tendency to develop thrombosis. Also, many studies reported the association of A3 haplotype with increased plasma level of sEPCR and proposed it as a candidate risk factor for thrombosis [17], [18], [19], [20]. Also, ***Gumus et al., (2006)*** [21] observed that the sEPCR level was statistically significantly higher in patients with central venous occlusion compared to control group. Similarly ***Ducros et al., (2012)*** [22] correlated the leukemia-associated hypercoagulability to high sEPCR plasma level and recommended its measurement in routine work up for these patients. Also, ***Elgammal et al., (2012***) [23] found a higher level of sEPCR in β thalassemia patients in relation to controls and suggested that sEPCR could be implicated in the haemostatic derangements and endothelial dysfunction in β thalassemia patients. In addition ***Stearns-Kurosawa et al., (2002)*** [24] previous study demonstrated a relationship between plasma sEPCR levels and thrombin generation in healthy subjects and in patients undergoing anticoagulant therapy and thus suggested that sEPCR levels may be a marker for a hypercoagulable state.

Conversely, in a prospective study published by ***Kallel et al., (2012***) [6] who investigated the relationship between sEPCR and the risk of cardiovascular events (CVE), although they found sEPCR level was elevated in association with classical cardiovascular risk factors but there was no significant relation to long term incidence of CVE. However, they attributed these discrepancies to differences in study design (retrospective vs prospective) and differences in patients' ages. ***Yamagishi et al., (2009)*** [11] documented that there was no overall association between sEPCR and venous thromboembolism risk (VTE) in the Longitudinal Investigation of Thromboembolism Etiology (LITE) pooled data from the Cardiovascular Health Study (CHS) and also suggested that sEPCR levels might reflect damage to venous endothelium at or after a VTE event and this might explain why sEPCR was not associated with VTE overall in their study compared to other studies. Also ***Atalay et al., (2013)*** [25] found no statistically significant difference in sEPCR level between essential thrombocytosis (ET) and polycythemia vera (PV) patients with and without thrombosis. Likewise ***Javanmard et al. (2013)*** [26] found the plasma sEPCR level wasn`t different between cerebral venous and sinus thrombosis (CVST) patients and the control group and documented no significant quantitative association between the risk of CVST and level of sEPCR. These discrepancies confirm and highlight the need for further larger study group on different patient conditions, ages and longer follow up time to be able to measure sEPCR before and after a thrombotic event.

Other possible risk factors for thrombosis were studied and compared between the 2 patients groups, there was no statistically significant difference between the 2 patients groups as regards hematological and biochemical profile. However, a statistically significant difference was found as regards PTH level.

Regarding PTH, there were several studies suggesting the involvement of PTH directly or through the calcium/phosphorus levels, in the development of atherosclerotic lesions in patients with end-stage renal disease (ESRD) and failure of AVF. In our study, we found, a statistically significant increase in serum PTH among group 2 (with thrombosis) compared to group 1(without thrombosis). This was previously reported by ***Grandaliano et al. (2003)*** [12] who found that patients with at least one episode of AVF dysfunction had mean

PTH plasma concentration significantly higher than the ones with no AVF dysfunction and they suggested that there was a potential association between PTH plasma levels and the incidence of AVF failure. In contrast, ***Bilgic et al. (2007)*** [15] found no relationship between PTH level and the development of AVF thrombosis.

In our study, a cut-off point of 200 ng/ml for sEPCR was able to differentiate between HD patients with and without thrombosis with prognostic accuracy of 90.3%. Likewise, ***Ducros et al. (2012)*** [22] proposed the same cut off and documented that the thrombotic risk in patients increased when level of sEPCR was higher than 200 ng/ml, suggesting that sEPCR released from endothelial cells of HD patients could serve as a 'trap' for PC preventing its binding to EPCR on the surface of endothelial cells and favoring a hypercoagulable state.

We classified the 50 HD patients into 3 tertiles according to sEPCR level. Low tertile included patients with sEPCR less than 25th quartile, median tertile included patients with sEPCR between 25th and 75th quartile and high tertile included patients with sEPCR above 75th quartile. We found that the rate of thrombosis development was significantly higher in the high sEPCR tertile group compared to the other two tertiles. So, the incidence of thrombosis increased with increasing sEPCR levels. These results are in agreement with those of ***Bilgic et al. (2007)*** [15] ***and Ducros et al. (2012)*** [22] who revealed that detection of plasma sEPCR levels provides a powerful insight into thrombotic risk assessment.

In conclusion, our findings demonstrate that high sEPCR level in HD patients is a marker for a hypercoagulable state. Quantification of plasma sEPCR level would allow clinicians to discriminate between patients at imminent risk of thromboembolism and those who are not and thereby adopt the required care. Also, the determination of plasma sEPCR provides us not only a powerful tool for the assessment of thrombotic risk factor in patients but also arms us for taking preventive measures in time. Testing in larger populations, normal individuals and serial measurements are warranted for confirmation of such an association which would be a first step toward identification and subsequent validation of a novel biomarker of vasculopathy and hypercoagulability.

6. Acknowledgment

AbdelgaffarAA, Ismail MM, Farweez BA and Elmasry RF contributed to the design of this study. Farweez BA and Elmasry RF contributed to data acquisition. Farweez BA and Elmasry RF participated to the statistical analysis of data under the supervision of AbdelgaffarAA, Ismail MM. Farweez BA wrote the manuscript. All authors read and approved final manuscript.

7. Disclosure

The authors state that they have no conflict of interest.

References

[1] G. Ocak, W. Lijfering, M. Verduijn, et al,"Risk of venous thrombosis in patients with chronic kidney disease: identification of high-risk groups," J Thromb Haemost, 11, pp. 627–33, 2013.
[2] L. Casserly, and L. Dember,"Thrombosis in end-stage renal disease," Semin Dial, 16,pp. 245–256, 2003.
[3] D. Stearns-Kurosawa, S. Kurosawa, J. Mollica, et al,"The endothelial cell protein C receptor augments protein C activation by the thrombin-thrombomodulin complex,"Proc Natl Acad Sci USA, 93, pp. 10212–10216, 1996.
[4] F. Taylor, G. Peer, M. Lockhart, et al,"Endothelial cell protein C receptor plays an important role in protein C activation in vivo," Blood, 97, pp. 1685–1688, 2001.
[5] C. Wu, D. Dwivedi, L. Pepler, et al,"Targeted Gene Sequencing Identifies Variants in the Protein C and Endothelial Protein C Receptor Genes in Patients With Unprovoked Venous Thromboembolism," Arteriosclerosis, Thrombosis, and Vascular Biology, 33, pp. 2674-2681, 2013.
[6] C. Kallel, W. Cohen, N. Saut, et al, "Association of soluble endothelial protein C receptor plasma levels and PROCR rs867186 with cardiovascular risk factors and cardiovascular events in coronary artery disease patients: the Athero Gene study,"BMC Med Genet, 8 (13), pp. 103-11, 2012.
[7] J. Gu, Y. Katsuura, G. Ferrell,et al,"Endotoxin and thrombin elevate rodent endothelial cell protein C receptor mRNA levels and increase receptor shedding in vivo," Blood, 95(5),pp. 1687–1693, 2000.
[8] C. Sesin, X. Yin, C. Esmon, et al,"Shedding of endothelial protein C receptor contributes to vasculopathy and renal injury in lupus: in vivo and in vitro evidence," Kidney Int,68,pp. 110–20, 2005.
[9] M. Van de Wouwer, D. Collen, and E. Conway,"Thrombomodulin-protein C-EPCR system: integrated to regulate coagulation and inflammation," Arteriosclerosis, Thrombosis, and Vascular Biology, 24, pp. 1374–1383, 2004.
[10] C. Esmon,"The endothelial protein C receptor," Current Opinion in Hematology, 13, pp. 382–385, 2006.
[11] K. Yamagishi, M. Cushman, S. Heckbert, et al,"Lack of association of soluble endothelial protein C receptor and PROCR 6936A/G polymorphism with the risk of venous thromboembolism in a prospective study,"British Journal of Haematology, 145, pp. 221–226, 2009.
[12] G. Grandaliano, A. Teutonico, A. Allegretti A, et al,"The role of hyperparathyroidism, erythropoietin therapy, and CMV infection in the failure of arteriovenous fistula in hemodialysis," Kidney Int, 64,pp. 715–719, 2003
[13] B. Naumnik, J. Borawski, and M. Mysliwiec,"Different effects of enoxaparin and unfractionated heparin on extrinsic blood coagulation during haemodialysis: a prospective study,"Nephrol Dial Transplant, 18, pp. 1376–1382, 2003.
[14] A. Aggarwal, DA. Whitaker, JM. Rimmer, et al, "Attenuation of platelet reactivity by enoxaparin compared with unfractionated heparin in patients

undergoing haemodialysis,"Nephrol Dial Transplant, 19, pp. 1559–1563, 2004.
[15] A. Bilgic, O. Nurhan, B. Nilufer, et al,"Soluble Endothelial Protein C Receptor: Influence on Arteriovenous Fistula Thrombosis Development in Hemodialysis Patients," Am J Nephrol., 27, pp. 366–372, 2007.
[16] A. Ulu, D. Gunal, S. Tiras, et al, "EPCR gene A3 haplotype and elevated soluble endothelial protein C receptor (sEPCR) levels in Turkish pediatric stroke patients," Thromb Res.,120, pp. 47-52, 2007.
[17] B. Saposnik, J. Reny, P. Gaussem, et al,"A haplotype of the EPCR gene is associated with increased plasma levels of SEPCR and is a candidate risk factor for thrombosis," Blood, 103,pp. 1311–1318,2004.
[18] D. Qu, Y. Wang, Y. Song, et al."The Ser219->Gly dimorphism of the endothelial protein C receptor contributes to the higher soluble protein levels observed in individuals with the A3 haplotype," J Thromb Haemost., 4, pp. 229–35,2006.
[19] S. Navarro, P. Medina, Y. Mira, et al,"Haplotypes of the EPCR gene, prothrombin levels, and the risk of venous thrombosis in carriers of the prothrombin G20210A mutation," Haematologica, 93, pp. 885–891,2008.
[20] J. Dennis, C. Johnson, A. Adediran, et al,"The endothelial protein C receptor (PROCR) Ser219Gly variant and risk of common thrombotic disorders: a HuGE review and meta-analysis of evidence from observational studies," Blood, 119, pp. 2392–2400,2012.
[21] K. Gumus,S. Kadayifcilar, B. Eldem, et al,"Is elevated level of soluble endothelial protein C receptor a new risk factor for retinal veinocclusion?," Clin Experiment Ophthalmol., 34, pp. 305-311,2006.
[22] E. Ducros, S. Mirshahi, D. Azzazene, et al,"Endothelial protein C receptor expressed by ovarian cancer cells can be a biomarker of cancer expansion," Int. J. Oncol., 41, pp. 433–440,2012.
[23] M. Elgammal, Z. Mourad, N. Sadek, et al, "Plasma levels of soluble endothelial protein C-receptor in ppatients with β thalassemia,"Alexandria journal of medicine, 48 (4), pp.283-288, 2012.
[24] D. Stearns-Kurosawa, K. Swindle, A. D'Angelo A, et al, "Plasma levels of endothelial protein C receptor respond to anticoagulant treatment. Blood, 99, pp. 526–530, 2002.
[25] F. Atalay, S. Toprak, E. Koca, et al, "sEPCR Levels in Chronic Myeloproliferative Diseases and Their Association with Thromboembolic Events: A Case-Control Study,"Turk J Hematol., 31, pp. 121-127, 2014.
[26] S. Javanmard, T. Shahsavarzadeh, and M. Saadatnia,"Soluble thrombomodulin and endothelial cell protein C receptor levels in patients with cerebral venous and sinus thrombosis,"Eur Neurol.,70 (3-4), pp. 156-158.2013.