

## Relation between Endothelial Protein C Receptor Gene Polymorphism 6936A/G and the Risk of Vascular Access Thrombosis in Patients with End Stage Renal Disease on Hemodialysis

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### ABSTRACT

**Background:** The most significant complication of chronic kidney disease (CKD) is end-stage renal disease (ESRD). Hemodialysis (HD) necessitates a well-functioning vascular access with enough blood flow for clearance and blood dialysis. Thrombosis of the veins can be caused by both acquired and inherited factors.

**Aim of The Work:** To assess EPCR levels in patients with ESRD on hemodialysis & study relationship between the presence of EPCR gene polymorphisms (6936A/G) and incidence of vascular access thrombosis.

**Patients and Methods:** This research was carried out on 50 studied cases admitted to the Theodor Bilharz Research Institute (TBRI), Imbaba, Giza, Hemodialysis Unit, Nephrology Department from November 2018 to May 2019. In addition, 25 age and sex-matched normal people (control cases) were included.

**Results:** The mutant EPCR (6936A/G) genotypes were found to be substantially more prevalent in our investigation and the polymorphic type allele (G allele) in ( $p < 0.05$ ) in ESRD patients on long-term hemodialysis with thrombosis compared to healthy subjects and ESRD patients without thrombosis. the flow cytometric expression of CD 201 was statistically reduced in ESRD patients on long-term hemodialysis with thrombosis ( $p < 0.001$ ) & without thrombosis ( $p < 0.005$ ) when compared to healthy subjects. CD201 Expression levels showed negative correlation with EPCR 6936 A/G heteromutant genotype.

**Conclusion:** The result obtained via the recurrent study provide evidence that EPCR 6936A/G (rs867186) genetic polymorphisms in ESRD patients on long-term hemodialysis could be a potential reason for Venous thrombosis.

**Keywords:** ESRD; CKD; HD; EPCR; CD201.

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### INTRODUCTION

The most significant complication of chronic kidney disease (CKD) is end-stage renal disease (ESRD). In the following years, the global increase in the number of patients with CKD is likely to continue and, as a result, ESRD, necessitating renal replacement therapy (RRT), threatens to approach pandemic proportions. CKD with consequent ESRD is becoming more widely recognised as a public health issue, particularly in Egypt, associated with increased morbidity, mortality and healthcare costs.<sup>1</sup> Hemodialysis (HD) necessitates a well-functioning vascular access with enough blood flow for clearance and blood dialysis. Vascular access problems increase morbidity and account for 20–25 percent of all dialysis complication, with thrombosis accounting for roughly 85 percent of all occurrences.<sup>2</sup>

Thrombophilia is caused by genetic or acquired alterations to the coagulation factors or a deficiency in the fibrinolytic pathway, resulting in abnormal clot formation.<sup>3</sup> Both acquired and inherited variables are risk factors for venous thrombosis. Prolonged bed rest, surgery, pregnancy or puerperium, hormone treatment, varicose veins, long flights, acute inflammatory, rheumatological disorders & malignancies are all acquired risk factors.<sup>4</sup> Mutations in coagulation-fibrinolytic system factors such as antithrombin, protein C and S (PC and PS), factor V Leiden, prothrombin 20210A, and fibrinogen 10034T are among the genetic risk factors.<sup>5</sup>

Endothelial Protein C Receptor (EPCR), a transmembrane protein produced on the endothelium of big arteries, is also implicated in the activation of the protein C (PC) anticoagulant pathway by binding PC and increasing its activation.<sup>6</sup> It is a critical component of the PC pathway because it can boost the activity of PC/APC (activated protein C) by 5- to

20-fold via the thrombin–thrombomodulin complex, resulting in significantly increased anticoagulant action. EPCR mutants with various expression levels and functions have been discovered.<sup>7</sup>

For normal haemostasis to retain the integrity of the blood arteries following vascular injury, a successful balance between the coagulation and fibrinolytic pathways is required.<sup>8</sup>

Plasma levels and gene polymorphisms of particular cytokines have predictive value in various distinct clinical settings, in addition to playing a role in disease pathogenesis. In fact, individual allelic polymorphisms can affect outcomes not just in acute or chronic kidney disease, but also in a variety of glomerulonephritis, ESRD, and renal transplantation.<sup>9</sup>

In the EPCR gene, two polymorphisms have been identified: 6936A/G (rs 867186) and 4678G/C (rs 9574). Within the area of EPCR encoded by exon 4, near the 4600 position, polymorphism 6936A/G (equivalent to 4600A/G) occurs. They're single-nucleotide polymorphisms (SNPs) that correspond to two of the EPCR gene's four haplotypes, haplotype 3 (H3) & haplotype 1 (H1), respectively.<sup>10</sup>

Soluble EPCR (sEPCR) is the soluble version of EPCR seen in normal human plasma and is result of metalloprotease cleavage.<sup>11</sup> Increased sEPCR levels may be linked to increased risk of thrombosis because sEPCR competes for PC with membrane-associated EPCR, which can reduce (APC) activity and PC activation.<sup>10</sup>

The goal of this research was to assess EPCR levels in studied cases with ESRD on hemodialysis, as well as to look into the link between the EPCR gene polymorphisms (6936A/G) as well as the occurrence of vascular access thrombosis in order to evaluate the usage of these parameters as predictive thrombotic markers in those patients.

## PATIENTS AND METHODS

This was case-control research including 50 patients who were admitted to the hemodialysis Unit, Theodor Bilharz Research Institute (TBRI) from November 2018 to May 2019. In addition, a control group of 25 healthy people of similar age and sex was added.

The following criteria were used to select fifty individuals with end-stage renal disease (ESRD) for inclusion in this study:

ESRD cases on long-term hemodialysis (> 6 months).

Exclusion Criteria

Children and non-Egyptians.

Pregnant woman

Oral contraceptive pills or oral anticoagulation therapy.

Malignancy.

Sepsis.

Autoimmune diseases.

All studied cases were subjected to:

Taking a complete history

Personal history: age, residence, occupation.

Family history of malignancy.

Patients were categorized into 2 groups, 25 cases each:

ESRD studied cases on hemodialysis (> 6 months) without vascular access thrombosis.

ESRD studied cases on hemodialysis (> 6 months) with vascular access thrombosis diagnosed by Doppler ultrasonography.

The Research Ethics Committee of Theodor Bilharz Research Institute accepted the current study research protocol (TBRI). The entire procedures were carried out in conformity with the Helsinki Declaration of 1964 & subsequent revisions, and related ethical standards. Prior to enrolment in the trial, all individuals signed informed written consents. Before being included in the study, all subjects gave their informed consent according to the rules of the TBRI Institute's Human Research Ethics Committee.

Statistical analysis

Statistical tool R version 4.0.2 for Windows was used to analyzed the data which is WHO approved. Numerical variables were described using mean, median, standard deviation, minimum and maximum.

For categorical variables frequency and percentage were used. Chi-square Test ( $\chi^2$ ) was for comparing changes in categorical variables. For comparing normally distributed numerical variables ANOVA test (analysis of variance) followed by Tukey HSD were performed.

For comparing non-parametric numerical variables Kruskal-Wallis Test after that Dunn Test were performed. Pearson's Chi-squared Test with Monte Carlo Simulation was used to evaluate the association between genotypes.

Correlation analysis was conducted using Wilcoxon Rank Sum Test and Point-Biserial Correlation. Two-tailed tests were used in all tests. Statistical the p values of <0.05 & <0.01 were taken into consideration be statistically significant and extremely significant.

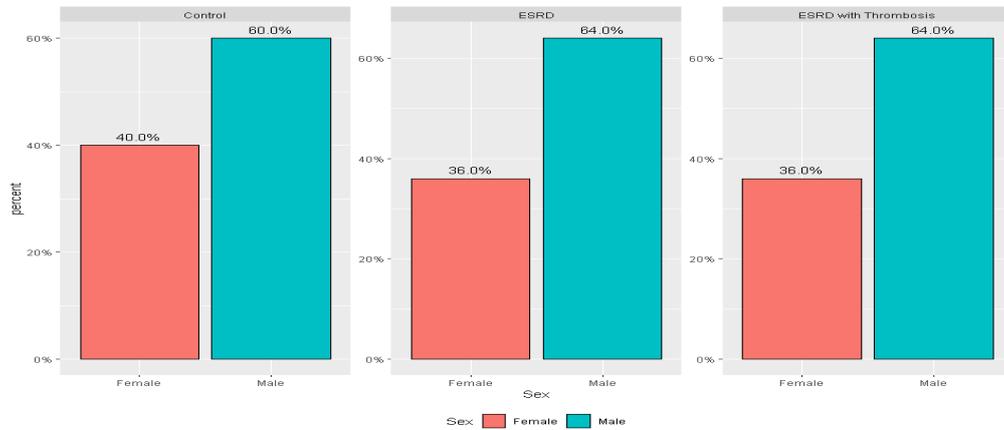
## RESULTS

The research was conducted on 50 studied cases admitted to the Hemodialysis Unit, Theodor Bilharz Research Institute (TBRI), Imbaba, Giza, Nephrology Department from November 2018 to May 2019. In addition, a total of 25 healthy adults of similar ages and sexes took part in the study and acted as a control group, they were made up of Fifteen males (60 percent) and Ten women (40 percent) ranging in aging from 35 to 59 years.

Patients were categorized into 2 groups (25 cases) for each:

ESRD patients on long-term hemodialysis without vascular access thrombosis. There were 16 males (64 percent) & 9 females (36 percent) (Figure 1). Their age range between 37-58 years

ESRD patients on long-term hemodialysis with vascular access thrombosis diagnosed by Doppler ultrasonography. There were 16 males (64 percent) and 9 females (36 percent) (Figure 1). Their ages range from 36 to 60 years old.



**Fig. 1:** Sex distribution of ESRD cases on long-term hemodialysis with and without thrombosis and healthy subjects.

Clinico-demographic of ESRD studied cases on long-term hemodialysis with and without thrombosis and healthy individuals were presented Figure (1).

Data revealed that the flow cytometry expression of CD 201 was statistically reduced in ESRD patients on long-term hemodialysis with thrombosis ( $p < .01$ ) & without thrombosis ( $p < .05$ ) compared to healthy subjects.

In addition, a further reduction ( $p < .01$ ) in CD 201 expression was noticed in ESRD patients on long-term hemodialysis with vascular access thrombosis compared to ESRD patients without.

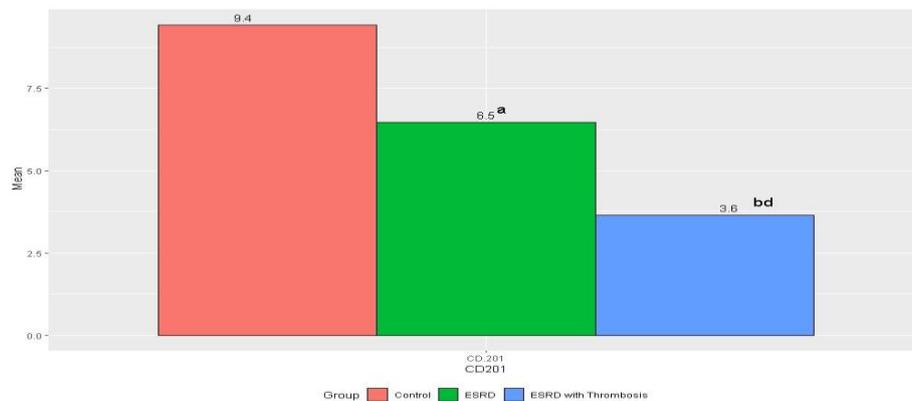
|                               |               | Controls      | ESRD                       | ESRD with Thrombosis        |
|-------------------------------|---------------|---------------|----------------------------|-----------------------------|
|                               |               | (n=25)        | (n=25)                     | (n=25)                      |
| <b>CD 201 expression* (%)</b> | Mean $\pm$ SD | 9.4 $\pm$ 2.8 | 6.5 <sup>a</sup> $\pm$ 2.7 | 3.6 <sup>bd</sup> $\pm$ 2.0 |
|                               | Median        | 10.3          | 5.89                       | 3.4                         |
|                               | Range         | 4.5 - 14.3    | 1.5 - 12.7                 | 0.1 - 10.0                  |

ap < .05, bp < .01: Controls against other groups.

cp < .05, dp < .01: ESRD patients without thrombosis against ESRD with thrombosis.

**Table 1:** CD201 expression levels in ESRD patients on long-term hemodialysis with and without thrombosis and healthy subjects.

Expression levels of CD201 in ESRD cases on long-term hemodialysis with and without thrombosis and healthy subjects were presented in Table (1) and Figures (2).



**Fig. 2:** CD201 expression levels in ESRD cases on long-term hemodialysis with and without thrombosis and healthy subjects.

| SNP & Genotypes<br>n (%)  |           | Controls<br>(n=25) | ESRD<br>(n=25) | ESRD with<br>Thrombosis<br>(n=25) |
|---------------------------|-----------|--------------------|----------------|-----------------------------------|
| EPCR 6936<br>(rs: 867186) | AA        | 24 (96%)           | 24 (96%)       | 19 (76%)                          |
|                           | AG*       | 1 (4%)             | 1 (4%)         | 6 (24%)                           |
|                           | allele A  | 98 %               | 98 %           | 88 %                              |
|                           | allele G* | 2 %                | 2 %            | 12 %                              |

p&lt;0.05.

**Table 2:** Genotyping of EPCR 6936 A/G (rs 867186) SNPs in ESRD patients on long-term hemodialysis with and without thrombosis and healthy subjects.

To assess the connection between EPCR expression & its polymorphism a correlation analysis was performed between CD201 Expression levels and the EPCR 6936 A/G hetero mutant genotype in all studied groups.

Statistical analysis, Table (2) show that EPCR 6936 A/G hetero mutant genotype and the polymorphic type allele (G allele) were significantly elevated ( $p < .05$ ) in ESRD patients on long-term hemodialysis with thrombosis compared to healthy subjects & ESRD cases without.

| (n-75)                                       | CD 201 expression (%)       |
|--|-----------------------------|
| Genotyping of EPCR 6936 A/G (rs 867186) SNPs | $r = -0.34^*$<br>$p < 0.01$ |

**Table 3:** Correlation between CD 201 expression and EPCR 6936A/G (rs 867186) SNPs levels in healthy subjects and ESRD patients on long-term hemodialysis with and without thrombosis.

A strong significant inverse negative correlation ( $r = -0.34.000$ ,  $p < .01$ ) was demonstrated Table (3) between CD201 Expression levels and that EPCR 6936 A/G hetero mutant genotype in healthy subjects and ESRD patients on long-term hemodialysis with and without vascular access thrombosis.

## DISCUSSION

Our data revealed that the flow cytometric expression of EPCR (CD201) was statistically reduced ( $p < 0.05$ ) in ESRD patients on long-term hemodialysis without thrombosis compared to healthy subjects. Moreover, an important reduction ( $p < 0.01$ ) in flow cytometric expression of CD201 were also noticed in ESRD patients on long-term hemodialysis with thrombosis compared to healthy subjects. In addition, a further reduction ( $p < 0.01$ ) in CD 201 expression was noticed in ESRD patients on long-term hemodialysis with vascular access thrombosis compared to ESRD patients without thrombosis. Our results demonstrated that the decrease in EPCR (CD201) membranous expression paralleled the advancement of the disease and occurrence of thrombosis in ESRD patients on long-term hemodialysis.

Our findings are consistent with those of others who discovered a link between raised sEPCR levels and increase risk of thrombosis in patients with deep venous thrombosis.<sup>10, 12</sup> The soluble form of EPCR (sEPCR), which lacks the transmembrane domain & cytoplasmic tail circulates in plasma, is produced by metalloprotease activity induced by thrombin proteolytic cleavage of membrane bound EPCR. It inhibits PC activation on endothelium and has a similar affinity for PC and APC.<sup>13</sup> It also prevents APC from acting as an anticoagulant by inhibiting its capacity to inactivate factors Va and VIIIa and blocking its binding to phospholipids.<sup>16</sup> Elevated sEPCR competes with membrane-associated EPCR for PC, it may be connected the increased risk of thrombosis is a result of this.<sup>10</sup>

Because EPCR is involved in the anticoagulant pathway of hemostasis, it may have a role in thrombosis aetiology.<sup>13</sup>

In vivo, blocking EPCR appears to hasten thrombus formation.<sup>14</sup>

In our work, data revealed that incidences of EPCR 6936 A/G heteromutant genotype and polymorphic type allele (G allele) were encountered in 4% and 2%, respectively, in both groups of controls and ESRD patients on long-term hemodialysis without vascular access thrombosis. On the other hand, these 2 markers were encountered in 24% and 12%, respectively, of ESRD patients on long-term hemodialysis with vascular access thrombosis.

Moreover, data analysis using statistics revealed that mutation of EPCR 6936 A/G heteromutant genotype and the polymorphic type allele (G allele) was significantly increased ( $p < 0.05$ ) in ESRD studied cases on long-term hemodialysis with vascular access thrombosis compared to healthy subjects and ESRD cases without.

In our results, EPCR 6936A/G heteromutant gene were found to have statistically significant higher frequencies in ESRD patients on long-term hemodialysis with vascular access thrombosis (24%) than control group (4%).

These findings approve prior research that found that in human umbilical vein endothelial cell conditioned media, 4600AG is connected to decreased membrane bound EPCR and increased sEPCR, greater levels of a shorter messenger RNA isoform, and a lower rate of PC activation.<sup>10</sup> Furthermore, the EPCR gene serine-219-glycine polymorphism has been linked to an increased risk of venous thromboembolism, and those with the G allele of the EPCR gene at the 6,936th or 4,600th position have been found to have a higher risk of venous thromboembolism.<sup>15</sup>

Our findings are also in accordance with other studies which individuals with the mutant EPCR (6936A/G) genotype had a greater risk of venous thromboembolism and had higher levels of plasma sEPCR, according to the study Gandrille et al.<sup>7</sup>; Yin et al.<sup>16</sup>. They claimed that the 6936A/G polymorphism is a potential risk factor for deep venous thrombosis (DVT), and that it is connected to greater sEPCR plasma levels in DVT patients.<sup>17</sup>

Likewise, EPCR 6936A/G polymorphism, in cases with deep venous thrombosis, it was utilised to detect the danger of vascular thrombosis.<sup>18, 16,19,10,17</sup>. The frequency of mutant EPCR (6936A/G) genotypes (AG, GG) was greater in patients with deep venous thrombosis than in the control group, and patients with polymorphic type allele (G) had a four-fold increased risk of deep venous thrombosis compared to those with wild type (A) Yin et al.<sup>16</sup>; Zoheir et al.<sup>17</sup>.

Furthermore, Li et al.<sup>15</sup> performed a meta-analysis on 13 studies to better understand the Ser219Gly a putative relationship between a variation in the EPCR gene and venous thrombosis. According to the researchers, the mutant EPCR (6936A/G) genotypes (AG) were connected to a statistically significant increased risk of venous thrombosis. In this research, correlation analysis of the data established a strong significant negative ( $p < 0.01$ ) correlation between CD201 expression levels and EPCR 6936 A/G heteromutant genotype in all studied cases denoting that the reduction in the levels of membranous CD201 expression is inversely correlated with elevation in levels of EPCR 6936 A/G heteromutant genotype and was strongly correlated with increased incidence of vascular thrombosis. Our results confirm those of others who Higher levels of sEPCR were associated with mutant genotypes, an investigation of sEPCR levels in relation to EPCR genotypes (AG and GG) revealed.<sup>17</sup>

Our information, therefore, propose that the reduction of membranous EPCR, assessed by the flow cytometric analysis of CD 201 expression levels, may be used as a relevant potential clinical indicator for predicting the development of vascular access thrombosis in ESRD cases on long-term hemodialysis. Similarly, EPCR gene serine-219-glycine polymorphism or EPCR 6936 A/G heteromutant genotype was found to be associated with higher susceptibility for development of thrombosis of vascular accessibility in ESRD studied cases on long-term hemodialysis. The persons with the A/G heteromutant genotype polymorphism may be more prone to develop thrombosis.

### CONCLUSION

We, therefore, may conclude that EPCR 6936A/G polymorphism (rs867186) in ESRD patients on long-term hemodialysis could be possible molecular risk factor for venous thrombosis. Patients having coinheritance of EPCR 6936A/G (rs867186) genetic polymorphisms are at a higher risk for developing vascular thrombosis.

Conflict of interest : none

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