The relation between soluble endothelial protein C receptor and factor VIII levels and FVIII/sEPCR index in healthy infants

Sağlıklı süt çocuklarında çözünür endotelyal protein C reseptörü ve faktor VIII düzeyleri arasında ilişki ve FVIII/sEPCR indeksi

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Abstract

Objective: Both soluble endothelial protein C receptor (sEPCR) and factor VIII (FVIII) seem to be potential mediators in thrombotic and inflammatory states. The aim of the present study was to determine the relation between plasma sEPCR and FVIII levels in a group of healthy Turkish infants.
Materials and Methods: The study population consisted of 50 healthy infants aged 6 months (Group 1, n=23) and 12 months (Group 2, n=27) having no acute or chronic infection and/or disease. sEPCR levels and FVIII levels were measured by ELISA and one stage factor assay method, respectively.
Results: The sEPCR levels of the infants aged 6 months were found higher than those of the infants aged 12 months (p<0.001). There was a correlation between sEPCR and FVIII levels of the infants in Group 1 (6-month-old infants) (r=0.678, p<0.001). FVIII/sEPCR index was 0.73±0.3 and 1.0±0.5 in Group 1 and Group 2, respectively (p=0.027). A correlation between infant age and FVIII/sEPCR index was found (r=0.312, p=0.027).
Conclusion: The FVIII/sEPCR index in healthy infants reflects the physiological condition of this population. The finding showing a positive relationship between sEPCR and FVIII levels suggests a possible interaction between these mediators in healthy infants aged six months. *(Turk J Hematol 2011; 28: 27-32)*
Key words: Soluble endothelial protein C receptor (sEPCR), factor VIII, healthy infants, thrombosis

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Özet

Amaç: Çözünür endotelyal protein C reseptörü (sEPCR) ve faktör VIII (FVIII) trombotik ve inflamatuvar durumlarda potansiyel arabileşenler olarak görülmektedir. Bu çalışmanın amacı; bir grup sağlıklı süt çocuğunda plazma sEPCR ve FVIII düzeyleri arasındaki ilişkiyi tanımlamaktır.
Yöntem ve Gereçler: Çalışma grubunu herhangi bir akut ya da kronik hastalığı ve/veya enfeksiyonu olmayan, sağlıklı 6 aylık (Grup 1, n=23) ve 12 aylık (Grup 2, n=27) çocuklar oluşturmaktadır. sEPCR düzeyleri ve FVIII düzeyleri sırasıyla; ELISA ve one stage factor metodu ile çalışılmıştır.
Bulgular: Altı aylık çocukların sEPCR düzeyleri oniki aylık olanlardan daha yüksek bulunmuştur (p<0.001). Grup 1'i oluşturan 6 aylık çocuklarda sEPCR ve FVIII düzeyleri arasında bir korelasyon vardır (r=0.678,

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p<0.001). FVIII/sEPCR indeksi Grup 1'de 0.73±0.3 ve Grup 2'de 1.0±0.5 olarak bulunmuştur (p=0.027). Çocuğun yaşı ve FVIII/sEPCR indeksi arasında bir korelasyon saptanmıştır (r=0.312, p=0.027).
Sonuç: Çalışmada sağlıklı çocuklarda kullanılan FVIII/sEPCR indeksi bu populasyonun fizyolojik durumunu yansıtmaktadır. Altı aylık çocuklarda sEPCR ile FVIII arasındaki pozitif ilişki bu yaş grubundaki çocuklarda bu mediatörler arasında muhtemel bir etkileşim olduğunu gösterebilir. *(Turk J Hematol 2011; 28: 27-32)*
Anahtar kelimeler: sEPCR, Factor VIII, sağlıklı süt çocukları, tromboz

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Introduction

The protein C anticoagulant pathway is critical to both regulation of the blood coagulation process and control of the innate inflammatory response and some of its associated downstream pathologies [1,2]. The endothelial protein C receptor (EPCR) plays an important role in this pathway [3]. EPCR is preferentially expressed by endothelial cells of large blood vessels and increases the rate of protein C activation by the thrombin/thrombomodulin complex [3,4]. Increased levels of a soluble form of EPCR (sEPCR) in plasma lead to dysfunction of the EPCR-mediated coagulation [5]. sEPCR levels increase in conditions associated with considerable thrombin production such as vasculitis, sepsis and systemic lupus erythematosus [6,7].

Factor VIII (FVIII) is a plasma glycoprotein in the coagulation cascade, and it is the cofactor of factor IXa in the activation of factor X. FVIIIa can be inactivated by activated protein C [1,2]. Previous studies have shown that the coagulant activity of FVIII is increased as an acute phase reaction in thrombosis [8,9]. Elevated plasma levels of FVIII are associated with an increased risk of venous and arterial thrombosis [10,11]. Possible mechanisms thought to be associated with elevated FVIII are the enhancement of thrombin formation or the induction of acquired activated protein C resistance, but the molecular mechanisms that underlie elevated FVIII are still not clear.

Although *in vivo* physiological mechanisms and their importance are still unknown, both EPCR and FVIIII seem to be potential mediators in thrombotic and inflammatory states because of their roles in the protein C anticoagulant pathway. Thus, the determination of the association between sEPCR and FVIII may provide new knowledge about the pathogenesis of thrombotic and inflammatory conditions. The aim of this study, therefore, was to determine the relation between plasma sEPCR and FVIII levels in a group of healthy Turkish infants.

Materials and Methods

This study was conducted at the Department of Pediatrics, Divisions of Pediatric Molecular Genetics and Social Pediatrics. Ethics approval was obtained from the Ethics Committee of the School of Medicine. The study population consisted of healthy infants aged 6 and 12 months who were admitted for well-child visits. They had no acute or chronic infection and/or disease. The written informed consent to participate was obtained from the parents of all subjects.

Peripheral blood samples were collected from the subjects into tubes containing 1 ml 0.109 M trisodium citrate. Plasma was obtained by centrifugation at 2500xg for 10 minutes (min) at room temperature. Plasma specimens obtained were maintained until the date of measurement at 20±5˚C for 4 hours (h), at 2-8˚C for 24 h, and at -20˚C for 1 month. Soluble EPCR levels were determined in plasma by using sEPCR Asserachrom enzyme-linked immunosorbent assay (ELISA) kits from Diagnostica Stago (Asnières, France), according to the manufacturer's instructions. Factor VIII levels were measured concomitantly with one stage factor assay method and FVIII-absent plasma (Sigma Diagnostica Inc, St. Louis, MO).

Statistical analysis

Statistical analysis was performed using the SPSS 11.5. Descriptive analysis summarizing the characteristics and the levels of sEPCR and FVIII is presented. Since the plasma sEPCR and FVIII measurements were not normally distributed, nonparametric tests were conducted to compare these parameters. The Mann-Whitney U test was used to compare sEPCR and FVIII levels between the groups. Nonparametric correlations between sEPCR and FVIII levels were evaluated using the Spearman correlation test. *FVIII/sEPCR index* was calculated in the groups. The Student t test was used to compare *FVIII/sEPCR index* between the groups, and the Pearson correlation test was used to evaluate the correlation between this index and age.

Results

Totally, 50 healthy infants were studied. These infants were divided into two groups according to age. Group 1 consisted of 23 infants aged 6 months (9 boys and 14 girls), and Group 2 consisted of 27 infants aged 12 months (13 boys and 14 girls).

Table 1 shows the sEPCR and FVIII levels of the infants. There was a significant difference between Group 1 and Group 2 with respect to sEPCR levels ($p<0.001$), with the sEPCR levels of infants aged 6 months found to be significantly higher than those of the infants aged 12 months. There was no difference between the groups with respect to the FVIII levels. Concerning all infants and groups, there was no statistical difference between boys and girls in sEPCR or FVIII levels.

In the correlation analysis, there was a correlation between sEPCR and FVIII levels of the infants in Group 1 (aged 6 months) ($r=0.678$, $p<0.001$) (Figure 1). However, no correlation was present between the sEPCR and FVIII levels in Group 2 (aged 12 months) ($r=-0.251$, $p=0.206$).

With respect to all infants, the mean *FVIII/sEPCR index* was found as 0.88 (median: 0.85, SE: 0.06, min: 0.3, max: 3.1). There was a statistically significant difference in the *FVIII/sEPCR index* between the groups ($p=0.027$; 0.73 ± 0.3 and 1.0 ± 0.5 for Group 1 and Group 2, respectively). In the correlation analysis, there was a correlation between the infant age and the *FVIII/sEPCR index* ($r=0.312$, $p=0.027$).

Discussion

This is the first study in the literature to examine the relationship between plasma sEPCR and FVIII levels in healthy infants as an indicator of the physiological condition. This study also highlights the

Table 1. sEPCR and FVIII concentrations according to groups

		All infants (n=50)	Group 1 (n=23)	Group 2 (n=27)
sEPCR (ng/ml)				
	Mean ± SE	145.4±10.7	175.1±17.9	120.0±10.8
	Median	112.0	130.0	102.0
	Min-Max	67.0-346.0	95.0-346.0	67.0-280.0
	95% CI[1]	123.8-166.9	137.9-212.3	97.8-142.3
	P*		***<0.001***	
FVIII (U/dl)	**Mean ± SE**	109.6±6.2	116.1±11.8	104.1±5.4
	Median	100.5	103.9	96.6
	Min-Max	42.0-284.0	42.0-284.0	72.0-209.0
	95% CI[1]	97.2-122.0	91.6-140.6	92.9-115.2
	P*		***>0.05***	

[1]95% CI: Confidence intervals, * Mann-Whitney U test

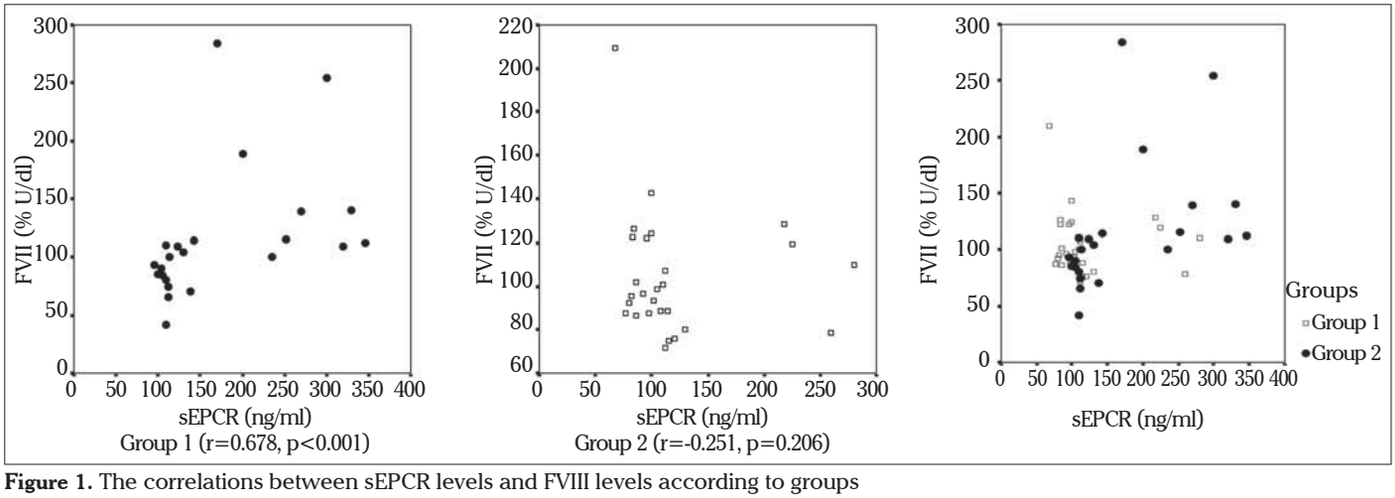

Figure 1. The correlations between sEPCR levels and FVIII levels according to groups

relation between infant age and the *FVIII/sEPCR index*.

Protein C activation is regulated by numerous mediators in the coagulation process, and activated protein C has a variety of anti-inflammatory activities [1]. Increased levels of sEPCR in plasma lead to dysfunction of the EPCR-mediated coagulation [5]. sEPCR levels vary among healthy subjects, and the bimodal distribution has been reported several times [12-14]. A recent study showed a negative relationship between sEPCR levels and an individual's age; that is, sEPCR levels of healthy children were found to be higher than those of healthy adults (14). The levels of sEPCR and FVIII reflect the physiological condition of these mediators in this healthy population; however, the physiological importance and influence of sEPCR levels *in vivo* and the relation to FVIII are unknown.

Increased plasma sEPCR levels were found associated with an increased risk of venous thrombosis and thrombin generation [12,15]. Further, it was reported that increased FVIII levels are an important risk factor for venous and arterial thrombosis [11,16]. Yürürer *et al.* [17] showed a negative relationship between sEPCR and FVIII levels in a group of pediatric stroke patients. They suggested that this association makes each of them regulate the action of the other, and may play a role in the stroke pathophysiology. Under the physiological circumstances, we found that the sEPCR levels of infants aged 6 months were significantly higher than of infants aged 12 months. We also found a positive relationship between sEPCR and FVIII levels in the 6-month-old healthy infants, but not in the 12-month-old infants. These findings, which indicate the physiological state in healthy infants, may reflect the interactions among the mediators in the coagulation system over the first months of life. Thrombin is a multifunctional serine protease generated at the site of vascular injury and has a key role in blood coagulation. Cellular effects of thrombin are mediated by protease-activated receptors (PARs), members of the G protein-coupled receptors. Their expression is low in contractile vascular smooth muscle cells (SMC), but becomes markedly upregulated upon injury [18]. We suggest that the overexpression of PARs or the presence of unknown mechanisms during the early stage of life may play a role in the tendency towards thrombotic conditions. We may speculate that the positive relation between sEPCR and FVIII levels in 6-month-old infants may be associated with the enhancement of thrombin formation and probably with the overexpression of PARs. Further studies are needed to evaluate the associations among the mediators in the coagulation system in a variety of healthy groups, including newborns, infants, children, and adults, under physiological circumstances.

As the first in the literature, we proposed the ratio of FVIII/sEPCR as an index of the physiological status of the coagulation system. We found a correlation between infant age and the *FVIII/sEPCR index* in this healthy group. In our study population, this index reflects the physiological conditions regarding the coagulation pathway process under normal conditions. Although the *in vivo* importance of this index is unknown, determining the difference in this index between physiological and pathological conditions may confirm the potential interactions between the mediators in the coagulation process. We consider the possibility of using this index in further studies in pathologic states in which the pathogenesis involves thrombotic and inflammatory processes.

In this study, we aimed primarily to determine the actual physiological condition in healthy infants. Because of the potential tendency to thrombotic states over the first months of life and the possible risk of complexity in our findings, the younger infants were not included in the study. On the other hand, the interactions among the mediators in the coagulation system over the first month of life need to be determined, in order to clarify the detailed mechanisms under the tendency of thrombosis. Therefore, further studies with the aim of assessing the relations among the mediators, such as EPCR, FVIII, other factors, PARs, or other receptors, may be conducted in healthy infants in the first month of life.

Various genetic and environmental conditions have been discussed as risk factors for thrombotic events in adult and pediatric patients [19]. Functional polymorphisms in the EPCR gene may increase/decrease the risk of thrombosis, especially in carriers of prothrombotic mutations [20]. Familial clustering of high factor VIII levels in patients with venous and arterial thromboembolism and retinal artery occlusion were reported in

previous studies [21-23]. These findings have pointed to genetic influences on these mediator levels. It is conceivable that the genetic polymorphisms of the FVIII and/or EPCR genes are linked with high FVIII and/or sEPCR levels in patients with thrombosis. The evaluation of these polymorphisms may be an important clinical indicator for determination of the thrombosis risk in a healthy population. In a recent study, Ay *et al.* [24] found no evidence of an association between observed single nucleotide polymorphisms in exons of the FVIII gene and high thrombosis levels. The findings in the literature seem to be contradictory about the polymorphisms in the genes of the mediators regarding the protein C system; therefore, further detailed studies are needed to assess these polymorphisms to determine their roles in the pathogenesis of thrombosis.

In conclusion, this is the first study in the literature to examine the relationship between plasma sEPCR and FVIII levels in healthy infants and also to determine the ratio of FVIII/sEPCR as an index. Both sEPCR and FVIIII seem to be potential mediators in thrombotic and inflammatory states. The levels of sEPCR and FVIII in our study group reflect the physiological levels in this healthy population. We found a positive relationship between sEPCR and FVIII levels in 6-month-old healthy infants, but not in 12-month-old infants. This may reflect the interactions among the mediators in the coagulation system over the first months of life under physiological circumstances. We also found a correlation between infant age and the *FVIII/sEPCR index* in this healthy group. Although the *in vivo* importance of this index is unknown, we consider the possibility of using this index in further studies in pathologic states in which the pathogenesis involves thrombotic and inflammatory processes. On the other hand, future studies are needed to evaluate the physiological associations among the mediators, such as sEPCR and FVIII, in the coagulation system in a variety of healthy groups, including newborns, infants, children, and adults.

Conflict of interest statement

None of the authors of this paper has a conflict of interest, including specific financial interests, relationships, and/or affiliations relevant to the subject matter or materials included.

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