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VEGF-460T/C polymorphism and changes in serum VEGF levels after coronary artery bypass surgery

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Vascular endothelial growth factor (VEGF) is a highly specific growth factor for vascular endothelial cells by both stimulating endothelial cell growth and enhancing vascular permeability. We investigated the effects of VEGF-460 T/C polymorphism on serum levels of VEGF in coronary artery disease. 26 first time elective coronary artery bypass grafting (CABG) patients with cardiopulmonary bypass (CPB) were enrolled in the study. VEGF -460T/C polymorphism analysis was performed by a PCR-RFLP method. VEGF levels were determined by enzyme immunoassay method. There was no significant relationship between VEGF levels before and after bypass surgery in T allele group. But there was a significant decrement of VEGF levels in C allele carriers after surgery.

Key words: VEGF, coronary artery disease, polymorphism, PCR-RFLP

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Introduction

Endothelial dysfunction plays an important role in the pathogenesis of systemic inflammatory response syndrome. Vascular Endothelial growth factor (VEGF) is a highly specific growth factor for vascular endothelial cells by both stimulating endothelial cell growth and enhancing vascular permeability.[1-3] The effect of cardiopulmonary bypass on circulating VEGF levels and whether different VEGF polymorphisms have any influence on VEGF levels are largely unknown. VEGF has a 7-exon gene located on chromosome 6 and non-lethal hypoxia has been found to be the strongest inducer of VEGF.[4-7] Because of alternative splicing of VEGF gene, there are four major isoforms expressed: $VEGF_{121}$, $VEGF_{165}$, $VEGF_{189}$, $VEGF_{206}$.[8] VEGF levels and polymorphisms were evaluated for several diseases

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before, and it was demonstrated that different polymorphisms on promoter regions of VEGF genes were effecting the expression and activity levels of VEGF, possibly by disrupting transcription binding sites.[3,5,8-10] In o ur study we investigated the possible reationship between serum VEGF levels of coronary artery disease patients preoperatively and after bypass surgery and we evaluated the effects of VEGF-460 T/C polymorphism on serum levels of VEGF.

Materials and Methods

After institutional ethical comittee approval, 26 first time elective coronary artery bypass grafting (CABG) patients with cardiopulmonary bypass (CPB) were enrolled in the study. All operations were performed in a standardized approach by a Jostra HL-20 roller pump (Jostra AG, Hirrlingen, Germany), membrane oxygenator (Jostra Quadrox, Hirrlingen, Germany), and a 40 μm arterial blood filter (Jostra AG, Hirrlingen, Germany). Mild to moderate (28-32°C) hypothermia and pulsatile flow of 2.2 to 2.4 L/m^2 were used. Myocardial protection was achieved with cold antegrade blood cardioplegia. Perfusion pressure was kept over 70 mmHg in all times. Induction and maintenance of general anesthesia with endotracheal intubation were standardized an all the patients (phentanyl, midazolam, pancuronium and isoflurane in oxygen with air). Venous blood samples for VEGF levels and genetic analysis were taken during induction and 4 hours after CPB. Venous blood samples were collected in tubes containing ethylenediaminetetra-acetic acid (EDTA) and centrifuged immediately at 3000xg for 10 min. Plasma were stored at -20°C. Measurement was performed using a commercially available enzyme immunoassay kit(human VEGF Biosource; CA;USA). The assay recognized hVEGF165. VEGF -460T/C polymorphism analysis was performed by a previously described PCR-RFLP method.[11] After the amplification, restriction endonuclease BsrUI was used and all products were evaluated by using 2% agarose gel electrophoresis. The homozygous individuals for C allele (CC genotype) were identified by the presence of 155 and 20 bp. The homozygous individuals for the T allele (TT genotype) were identified by the presence of a single 175bp product. On obtaining cell frequencies less than 5, frequency analyses were performed with Fisher's exact test. Frequency labels and statistical analyses were performed with SPSS for Windows standard version 7.5 software (SPSS Inc, Chicago, Illinois).

Results

There was no statistically significant relationship between any VEGF genotypes in this patient group. The frequencies of VEGF alleles among the study group were 73% for T allele and 27% for C allele. The VEGF genotype and allele frequencies are

Table 1
The genotype and allele distributions of the serum VEGF levels before and after cardiopulmonary bypass (CPB) surgery

	n (%)	Preoperative VEGF levels (mean ± std. deviation)	Postoperative VEGF levels (mean ± std. deviation)
Genotype			
CC	3 (11.5%)	99.00 ± 71.46	49.16 ± 17.41
TC	8 (30.7%)	69.27 ± 42.68	41.80 ± 13.16
TT	15 (57.6%)	64.58 ± 47.51	72.71 ± 39.93
Alleles			
T	38 (73%)	66.21 ± 44.95	61.96 ± 36.01
C	14 (27%)	77.38 ± 49.89	43.80 ± 13.92

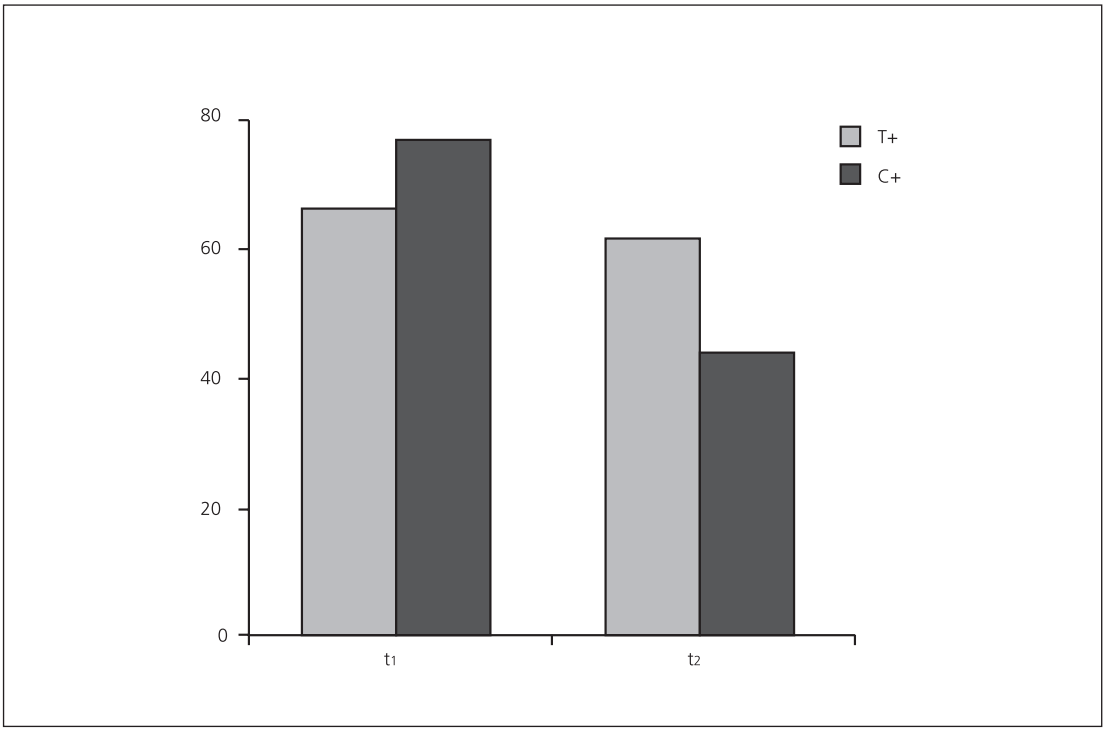

Figure 1
The serum VEGF levels before (t_1) and 4 hours after (t_2) CBP distribution according to alleles.

given in Table 1. We determined the changes in serum VEGF levels before and after bypass surgery for both alleles separately. T allele did not cause any difference between expression levels of VEGF in this patient group. There was a statistically significant decrement in VEGF levels after surgery in patients carrying C allele (p=0.031) (Figure 1).

Discussion

Hypoxia has been known as a strongest inducer of VEGF, which serves as a major angiogenic factor in humans. Previously it was found that repetetive cycles of coronary artery occlusions followed by short durations of reperfusion are known to trigger myocardial angiogenesis.[5] Hearts obtained from rats after hypoxia followed by 24-hour period of reoxygenation were shown to have increased stainings of VEGF around the capillary areas with increased durations of hypoxia.[5,8] It has been shown that VEGF enhances the survival of endothelial cells in toxic and oxygen deficient enviroment. Another study has shown that VEGF upregulated the expression of intracellular adhesion molecule-1 (ICAM-1) by phosphorylation of P13K/AKT pathway which is a general mediator of cytokine induced survival and anti-apoptotic signals. Therefore, VEGF is also a key element in determining whether an endothelial cell is going to survive or will undergo programmed cell death. Endothelium maintains a non-thrombogenic surface at the blood tissue interface and plays an important role in the metabolism of circulating substances, regulation of smooth muscle proliferation and control of vasomotor tone. Cardiopulmonary bypass results in endothelial activation and dysfunction.

We found that there were no statistically significant difference between serum VEGF levels before and 4 hours after surgery in T-allele carrying people. In a recent article by Arab et al.[12] it was found that

gene expressions are inreased early at one hour after cardioplegic arrest and reduced at reperfusion period.[12] In another article by Kusumanto et al, it was shown that levels were increased up to 6 days after cardiopulmonary bypass.[13] So our results might also mean that serum VEGF levels return to the preoperative levels in 4 hours time. There was a significant decrement in VEGF levels of C allele carrying people; this might mean that VEGF -460T/C polymorphism caused a decrement in VEGF expression and this might be a disadvantage for those patients. Although the numbers are relatively small and may be open to interpretation, there may be a correlation between these alleles and changes in VEGF levels before and after cardiopulmonary bypass surgery.

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