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THE EFFICACY OF TISSUE FACTOR −603A/G AND +5466A>G POLIMORPHISMS AT THE DEVELOPMENT OF VENOUS THROMBOEMBOLISM IN CANCER PATIENTS

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***Background and Aim*: Venous thromboembolism (VTE) is one of the most common complications in cancer patients. Although factor V Leiden (FVL) is the most common genetic defect causing thrombosis, the impact of gene abnormalities on thrombotic tendency in cancer patients remains poorly explored. Tissue factor (TF) is a major physiologic initiator of blood coagulation. This is the first study regarding the association of *TF* gene −603A/G and +5466A>G polymorphisms with VTE in malignancy. *Materials and Methods*: The study consists of two groups: cancer patients with VTE were included as Group 1 (n = 46); Group 2 comprises 196 cancer patients without VTE. Restriction fragment length polymorphism method was used for the detection of polymorphisms of *TF* −603A/G in the 5′upstream region and *TF* 5466A/G in intron 2. *FVL*, *PT* G20210A and *MTHFR* C677T polymorphisms were determined by using commercially available Light Cycler kits. The genotype and allele frequencies between the groups were compared using χ^2 or Fisher exact test, if appropriate. *Results*: No differences were observed in the distribution of *TF* gene −603A/G genotype frequencies between the groups. Although a slightly increased incidence of +5466GA genotype was in Group 1 (17.4% *vs* 11.2%), it did not achieve statistical significance. The prevalence of *FVL* was significantly greater in Group 1 compared with Group 2 (41.3% *vs* 4.1%, $p < 0.05$). Difference in frequency of 677TT+CT (*MTHFR*) + 5466GG (*TF*) genotypes combination was found in women of two investigated Groups ($p < 0.05$). No differences were also in genotypes and allele frequencies of *MTHFR* C677T and *PT* G20210A between two Groups ($p > 0.05$). *Conclusions*: The present study did not show significant association of *TF* gene −603A/G and +5466A>G polymorphisms with VTE in malignancy, however, further larger studies including different ethnic population are needed to confirm our findings.**

***Key Words*: cancer, thrombosis, tissue factor, polymorphism.**

Venous thromboembolism (VTE) is one of the most common complications in cancer patients. VTE can be seen in 15% of cancer patients during the progression of the disease [1]. Thrombosis is multifactorial; decreased native anti-coagulants, increased prothrombotic factors and decreased fibrinolysis can cause thrombosis progress [2]. Hereditary and acquired factors act a part in the progression of idiopathic VTE [3]. Factor V Leiden (FVL) is an important hereditary factor. Recent studies have shown that FVL mutation increased the risk of VTE in cancer patients as compared to cancer patients without VTE. Prothrombin (*PT*) gene G20210A mutation plays also a significant role in VTE related events in cancer patients [4–6].

Tissue factor (TF) or coagulation factor III is a 47 kDa single strand transmembrane glycoprotein. It contains 263 aminoacides and is expressed on the surface of various cells except from the vascular system [7]. TF activates coagulation factor VII and initiates the coagulation system by activating factor VII. Bacterial lipopolysaccharides, various inflamatory cytokines, vascular lesions, cancer and sepsis caused by gram negative bacteria can induce TF expression. This causes procoagulant activity and thrombus development by the activation of TF pathway [8].

As a result of endothelial damage, subendotelial matrix disclosure or direct stimulation of the endothelium, are the most important stimuli for the activation of factors involved in hemostasis. TF expression is the most important event that develops after tissue damage or endothelial activation. TF initiates thrombus formation by turning inactive coagulation proteins into active factors. TF is also associated with microparticles. Microparticles carrying TF normally circulate in blood. After vessel wall injury, they accumulate in the developing thrombus by a P-selectin and PSGL-1 dependent mechanism. Microparticles play a major role in the generation of fibrin, TF and progression of thrombus [7, 9].

Any change that will increase the expression of TF will result in more fibrin formation and severe thrombus development. As the role of TF in thrombus pathophysiology becomes increasingly more important and TF level is shown to increase in thrombosis related clinical conditions, *TF* gene polymorphisms are brought on [7]. In this study, *TF* polymorphisms have been investigated in cancer patients with and without VTE, and this is the first study on this subject.

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Abbreviasion used: FVL – factor V Leiden; PT – prothrombin; TF – tissue factor; VTE – venous thromboembolism.

MATERIALS AND METHODS

In this study, two groups of patients were enrolled. Group 1 was composed of cancer patients with VTE (n = 46). Group 2 was composed of cancer patients without VTE (VTE didn't develop during cancer therapy and the follow-up time) (n = 196). Informed consents of the patients were taken. The ethical committee of Yildirim Beyazit University Medical School has approved the study. The study was supported by a grant from Scientific Research Project of Yildirim Beyazit University (project number: 526).

DNA Isolation. Venous blood samples from the patients and controls were analyzed using QIAamp® Blood Mini Kit (Qiagen). DNA was purified on the QIAcube Instrument (Qiagen, Hilden, Germany). The concentration of extracted DNAs was measured by NanoDrop ND-1000 (PeqLab, Saveen Werner, Sweden) and DNA was kept at −80 °C until analyzed.

Genotyping for TF 5466A>G polymorphism. Genotype of TF 5466A>G (rs3917643) in intron 2 polymorphism was determined by the polymerase chain reaction — restriction length fragment polymorphism (PCR — RFLP) method. This *TF* gene region surrounding +5466A>G polymorphism was amplified by using 5′ATG CAG TCA CTG TGC TGA GGA 3′ and 5′GGC AAA TTA CAG AGC CAT CC 3′ primer pair. PCR was run under standard conditions at annealing temperature of 58 °C. The PCR products were digested by HinfI (Fermentas UAB, Vilnius, Lithuania) restriction endonuclease. Digested fragments were visualized after ethidium bromide staining under UV light. Restriction fragments were separated by 2.5% agarose gel electrophoresis. Minor allele (+5466G) was expected to be found mostly in a heterozygous form. Therefore, allele-specific HinfI restriction product lengths were either 170/39 (+5466A allele) or 149/39/21 bps (+5466A/G allele).

Genotyping for TF −603A/G polymorphism. Genotype of TF −603A/G (rs 1361600) in the 5′upstream region was also determined by the polymerase chain reaction–restriction length fragment polymorphism (PCR — RFLP) method. Primer pair for amplifying this region was 5′-CAT GAG AGA CAT CGC CTC TG-3′ and 5′-GAC CTA ACA TGT TCT AGC CAG AAG-3′. PCR was run under standard conditions at annealing temperature of 56 °C. This procedure was followed by Mva I restriction endonuclease digestion for 16 h at 37 °C and resolution by electrophoresis on 2.5% agarose gel. Digested fragments were visualized after ethidium bromide staining under UV light. The resulting 370 bp PCR product was cleaved into two smaller fragments of 252 and 118 bp in the presence of a A nucleotide, 3 smaller fragments of 101; 118 and 148 bp in the presence of a G nucleotide, finally, four fragments of 104; 118; 148; 252 bp in the presence of A/G nucleotide.

To determine the genetic risk factors for thrombophilia, the subjects were genotyped for FVL, factor II G20210A and C677T of *MTHFR* mutations by PCR with Rotor Gene 6000 LightCycler (Corbett Life Science Science, Concorde, NSW) according to the guidelines of the manufacturer.

Statistical analysis. The results were analyzed using SPSS programme. The genotype and allele frequencies between the groups were compared by χ^2 or Fisher's exact test, if appropriate.

RESULTS

TF polymorphisms were investigated in 242 patients (49 males, 193 females) for. The general data on the patients are shown in Table 1, and the frequencies of the tumor types are listed in Table 2. In both groups breast cancer is the most common type (with and without thrombosis), while the testicular cancer is rare as well as thymoma.

Table 1. General patients' data

	Group 1 (n = 46)	Group 2 (n=196)
Age (m ± SD)	57.0 ± 1.8	51.3 ± 0.9
Gender		
Female	28 (60.9%)	165 (84.2%)
Male	18 (39.1%)	31 (15.8%)

Table 2. The frequencies of tumor types in study groups

		Frequency	%
Group 1	Lung	4	8.7
	Brain	2	4.3
	Liver	3	6.5
	Colon	9	19.6
	Breast	16	34.8
	Stomach	5	10.9
	Ovary	3	6.5
	Sarcoma	3	6.5
	Testis	1	2.2
	Total	46	100.0
Group 2	Lung	19	9.7
	Brain	4	2.0
	Dermis	3	1.5
	Tongue	2	1.0
	Liver	1	0.5
	Skeletal system	1	0.5
	Colon	1	0.5
	Larynx	1	0.5
	Breast	152	77.6
	Bladder	1	0.5
	Nasopharynx	3	1.5
	Lymphoma	2	1.0
	Sarcoma	2	1.0
	Testis	1	0.5
	Tymoma	1	0.5
	Uterus	2	1.0
	Total	196	100.0

The prevalance of *PT* G201210A and *MTHFR* C677T polymorphisms were similar in two groups ($p > 0.05$). The allelic frequencies between two groups also also differed insignificantly. No difference was observed in the distribution of *TF* gene −603A/G genotype frequencies between the groups. Although a slightly increased incidence of +5466 GA genotype in Group 1 was observed (17.4% *vs* 11.2%), it didn't achieve statistical significance. The prevalance of FVL was significantly higher in Group 1 compared with Group 2 (41.3% *vs* 4.1%, $p < 0.05$) (Table 3). There was significant difference in Group 2 between the patients with *TF* 5466 polymorphism (homozygote/heterozygote) and the patients with *MTHFR* C677T mutation (homozygote/heterozygote) ($p < 0.05$). Except from this, there was no significant difference between the *TF* polymorphisms and thrombophylia factors.

There were significant differences in the number of women and men in Group 1 and Group 2. So, we also analysed separately the data for the groups by gender. There were 28 and 165 women in Groups 1 and 2, respectively. The prevalance of FVL was significantly higher in subgroup 1 compared with subgroup 2 (39.3% *vs* 4.8%, $p < 0.05$). In subgroup 2, there was significant difference between *TF* 5466 polymorphism (homozygote/heterozygote) and *MTHFR* C677T mutation (homozygote/heterozygote) ($p < 0.05$). Except from this, there was no significant difference between the *TF* polymorphisms in female patients and thrombophilia factors. There were no differences in the prevalence of all genotypes combinations in patients of studied groups except significantly higher prevalence of 677TT+CT (*MTHFR*) +5466GG (*TF*) genotypes combinations in women with VTE (subgroup 1 (66.7%)) compared to women without VTE (subroup 2 (27.8%)) ($p < 0.05$).

Table 3. The frequencies of *FVL*, *PT* G201210A, *MTHFR* C677T and *TF* gene −603A/G and +5466 A>G polymorphisms in Group 1 and Group 2 (*p < 0,05)

	Group 1 (n = 46)	Group 2 (n = 196)	p value
FVL			
AA or GA	19 (41.3%)	8 (4.1%)	
GG	27 (58.7%)	188 (95.9%)	0.05*
PT G20210A			
AA or GA	3 (6.5%)	8 (4.1%)	
GG	43 (93.5%)	188 (95.9%)	0.442
MTHFR C677T			
TT	2 (4.3%)	16 (8.2%)	
CT	21 (45.7%)	80 (40.8%)	0.623
CC	39 (50.6%)	100 (51.0%)	
TF −603A/G			
GG or AG	31 (67.4%)	131 (66.8%)	
AA (wild type)	15 (32.6%)	65 (33.2%)	0.943
TF +5466A>G			
GG or AG	8 (17.4%)	23 (11.7%)	
AA (wild type)	38 (82.6%)	173 (88.3%)	0.302

DISCUSSION

Recently, there have been some studies regarding the association between *TF* 603A/G and +5466A/G polymorphisms and thrombosis in patients with heart attack [10], but there has been no published study related to the polymorphisms in cancer patients with thrombosis. The present study is the first one that determines the relationship between the *TF* polymorphisms, cancer and VTE.

Various types of hemostatic disorders along with different complications are present in cancer patients, in particular, coagulation abnormalities [11]. VTE is one the most common causes of death in cancer patients [12, 13]. The VTE develops at nearly 15% of all cancer cases during the progression of the disease [14, 15].

Previous studies indicated that the FVL mutation is mostly observed in cancer patients with VTE compared to patients without VTE in Turkish population [16, 17]. In our recent study, there was also a higher frequency of FVL mutations in Group 1 compared to Group 2. But, there was no statistical difference between the groups for *MTHFR* C677T and *PT* G20210A polymorphisms.

In recent studies, there is no association between *MTHFR* C677T polymorphisms and some malignant tumors including breast cancer and colorectal carcinoma [18, 19]. In our study, we investigated the relationship between *MTHFR* C677T polymorphisms and VTE development and demonstrated no association between VTE risk and this polymorphism. However, Ozkan et al. [20] reported a significant difference between cancer patients with and without VTE for *MTHFR* C677T polymorphisms. We also found a significant difference between *TF* 5466 polmorphism and *MTHFR* C677T mutation in Group 2 (cancer patients without thrombosis). So, we suggest that the association of *MTHFR* C677T mutation and *TF* 5466 polymorphism is important and this must be taken into consideration for cancer patients. Difference in frequency of 677TT+CT (*MTHFR*) + 5466GG (*TF*) genotypes combination was found in women between two investigated groups ($p < 0.05$). Although there was a significant difference in the number of women and men in Group 1 and Group 2 ($p < 0.05$), there was no effect of these gender differences on the frequencies of polymophisms between the groups.

It is well known that *PT* G20210A mutation is related to higher levels of PT causing increased thrombin formation. In some studies, it has been reported that *PT* G20210A is an important factor in the development of VTE in cancer patients [4, 6, 21]. On the contrary, some researchers reported no association between *PT* G201210A and VTE risk in cancer patients [5, 17, 20, 22–24]. Our recent findings also support the subject.

Development of cancer-related thrombosis may be associated with *TF* [25], expression of which can differ between tumor types. The VTE risk can be related to high level of *TF* expression in cancer patients [26].

The development of TF is related closely with the transcription level of cellular specific promotor genes. The first study on this subject was published by Arnaud et al. [27]. The promoter region of *TF* gene was investigated and six different polymorphisms (−1812C/T, −1442G/C, −1322C/T, −1208D/I, −603A/G, −21C/T) were identified. Conflicting results were published on the relationship between arterial or venous thrombosis and TF promoter region polymorphisms [27]. There are a few studies on thrombosis and *TF* −603A/G polymorphism. For the first time, Oti et al. [10] have reported that TF plasma levels were higher in patients with *TF* −603GG genotype. The authors also showed that the patients with this genotype had an increased heart attack risk.

In our study, we determined no significant association between the development of thrombosis and 603A/G and +5466A>G polymorphisms of *TF* in cancer patients. Although the frequency of +5466 GA genotype was higher in Group 1 according to Group 2 (17.4–11.2%), there was no statistically significant difference ($p > 0.05$).

In conclusion, we have determined that FVL mutation is an important risk factor for VTE development in cancer patients. However we did not find significant association of *TF* gene −603A/G and +5466A>G polymorphisms with VTE in malignancy. It can be suggested that further larger studies including different ethnic

populations and specific cancer types are needed to confirm our findings.

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