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There is no correlation between the functional polymorphism −460C>T of vascular endothelial growth factor (VEGF) gene promoter and uncomplicated recurrent urinary tract infection among young women

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ABSTRACT

Introduction: The −460C>T polymorphism in vascular endothelial growth factor (VEGF) gene promoter (rs833061) has robust association with various diseases including renal parenchymal scarring following urinary tract infection (UTI). However, the association of this polymorphism with uncomplicated recurrent UTI (RUTI) is still unclear.

Aim: The objective of this study was to assess the correlation between VEGF −460C>T polymorphism and uncomplicated RUTI among young women.

Material and methods: Polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP) with *Bst*U1 restriction enzyme – and DNA sequencing were used to determine the VEGF −460C>T polymorphism among 34 young adult women with uncomplicated RUTI and 34 healthy controls. Differences in genotype and allele frequencies between case and control groups were analyzed with χ^2 test or Fisher's exact test.

Results and discussion: This study found that there was no significant difference in genotype frequencies between cases (CC 23.5%; CT 26.5%; TT 0%) and controls (CC 85.3%; CT 14.7%; AA 0%). Dominant model analysis found that there was no significant difference between uncomplicated RUTI and normal groups ($P = 0.368$). Similarly, allele analysis also found that there was no association between VEGF −460C>T polymorphism and uncomplicated RUTI ($P = 0.398$).

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Conclusions: This study found that there was no correlation between VEGF −460C>T polymorphism and uncomplicated RUTI among young women.

1. Introduction

Recurrent urinary tract infection (RUTI) is an episode of urinary tract infection (UTI) at least two times in six months or three or more re-infections with clinical symptoms in a year. RUTI is one of the major causes of renal scars; it also increases the incidence of renal insufficiency and hypertension, contributes to high morbidity and increases health care costs. Some studies found that RUTI was related to behavioral risk factors (sexual activities, diaphragm and spermicidal use, antibiotics use or estrogen use),[1–3] host factors for instance a short anatomical distance between the urethra and anus,[1,2] and genetic factors such as polymorphisms in several genes that encode cytokines and inflammatory mediators.[4,5]

Vascular endothelial growth factor (VEGF, referred to VEGF-A in this study) is a key mediator of normal and abnormal angiogenesis (proliferation, sprouting, migration and tube formation of endothelial cells) and an important regulator of vascular permeability.[6,7] In addition, several studies found that VEGF is an important molecule in several diseases.[7–11] VEGF belongs to VEGF family that includes VEGF-B, VEGF-C, VEGF-D and placenta growth factor (PlGF).[6] Because VEGF has pivotal roles in endothelial cells – an important medium of transporting immune system components between blood circulation and epithelial compartment – therefore, it could has important roles in infection including UTI.

VEGF gene, located on chromosome 6 at 6p21.3, is organized in 8 exons separated by 7 introns and the coding region encompasses approximately 14 kb.[12] VEGF gene is highly polymorphic and numerous single nucleotide polymorphisms (SNPs) can be found in the promoter and 5′ untranslated region (5′-UTR).[13,14] Common SNPs in VEGF gene have been studied and one of the most consistent SNP with several diseases is VEGF −460C>T. Study found that the person with VEGF −460C allele had higher VEGF protein expression[15] and VEGF −460C>T polymorphism had strong correlation with susceptibility, development, progressivity and prognosis of several diseases.[16–22]

Focusing on urinary tract diseases, a previous study revealed that VEGF −460C>T polymorphism is related to renal parenchymal scarring in childhood UTI.[23] Another study found that UTI cases (with or without vesicoureteral reflux (VUR) complication) had association with VEGF −460C>T polymorphism.[24] However, there was no association of this polymorphism with uncomplicated-UTI.[24] Another study also found similar findings.[25] They found that VEGF −460 CC genotype was more frequent in UTI with VUR complication cases compared to healthy controls or UTI without VUR complication cases. However, the role of this polymorphism in uncomplicated RUTI is still unknown.

2. Aim

The objective of this present study was to determine the correlation between VEGF −460C>T polymorphism and uncomplicated RUTI among young women.

3. Material and methods

3.1. *Subjects*

This case–control study was conducted with 34 culture-confirmed uncomplicated RUTI cases in young women (age range was 15–50 years old) and 34 normal women. Uncomplicated RUTI criteria that were applied in this study have been published previously.[26] Uncomplicated RUTI criteria in this study were: (a) UTI that occurred three times or more in 12 months or two times or more in 6 months; (b) no structural and functional abnormality of the urinary tract (based on standard blood urea nitrogen and creatinine analysis and sonography); (c) mid-stream sample of urine $<10^3$ cfu/mL of uropathogen; and (d) infection was confirmed with mid-stream urine culture. All complicated RUTI cases were excluded from this study. In addition, several exclusion criteria such as post-menopause, post-manipulated bladder, diabetes mellitus, liver cirrhosis, immunosuppressive diseases, use of immunosuppressive drug, and kidney transplant were applied to potential subjects. As control, a group of young women (doctors, nurses and medical students) who were free of the clinical symptom of UTI in the last 3 years and had no sign and symptom of infection was recruited. The subject recruitment and sample collection were conducted only after obtaining written informed consent of the participants. The work was carried out in accordance with The Code of the World Medical Association (Declaration of Helsinki) for experiments involving humans.

3.2. *Deoxyribonucleic acid (DNA) extraction*

For DNA extraction purpose, 2.5 mL peripheral venous blood was collected from 68 subjects (34 cases and 34 controls) and DNA was extracted from whole blood using the salting-out method as described previously.[27]

3.3. *SNP genotyping*

Genotyping was carried out as described in a previous report.[24] Briefly, to amplify VEGF gene, forward primer:

5′TGTGCGTGGGGTTGAGCG3′ and the reverse primer: 5′TACGTGCGGAGGGCCTGA3′ were used. Amplification was performed with 35 cycles (denaturation at 94°C for 1 min, annealing at 57.5°C for 30 s, extension at 72°C for 1 min) in a total reaction volume of 20 μL of genomic DNA, 5 μL of each primer, 250 μM/L deoxynucleotide triphosphate, 1.5 mM/L magnesium chloride, 10 mM Tris–HCl, and 1 unit of Taq DNA polymerase. Restriction fragment length polymorphism (RFLP) procedures were performed by digesting 10 μL of PCR products (175 bp fragments) with 10 μL of restriction enzyme *Bst*U1. Digested products were electrophoresed through ethidium bromide-stained 3% agarose gels. The amplified products were confirmed by DNA sequencing using an ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit and a 3730 DNA analyzer (Applied Biosystems) in 1st BASE Pte Ltd. Laboratory, Singapore.

3.4. *Statistical analysis*

The correlations between risk factors and uncomplicated RUTI were analyzed with χ^2 test and Fisher's exact test or *t*-test as appropriate with data type. The χ^2 test was used to assess the associations between VEGF −460C>T polymorphism (genotype and allele frequencies) and RUTI. Deviation of the genotype frequencies from the Hardy–Weinberg equilibrium (HWE) was assessed by Fisher's exact test. Two sided testing was used for all significant comparisons to evaluate statistically a *P*-value of less than 0.05 as significant.

4. Results

4.1. *Subject characteristics and risk factors*

In this study, 34 young women with uncomplicated RUTI (mean age was 32 ± 6.59 years old) and 34 controls (mean age was 26.1 ± 5.2 years old) were enrolled. Among the cases, 11 had three times UTI in a year and 23 cases suffered from UTI three times in six months. There were 27 with lower UTI (cystitis or bladder infection) and 7 with upper UTI (ureters infection); 80% of infection were caused by *Escherichia coli*.

The list of RUTI risk factors is presented in Table 1. Briefly, age, marital status, the frequency of sexual intercourse, and intrauterine device use were correlated significantly with RUTI. There was significant difference in the marital status between the cases (married 85.3%) and controls (married 29.4%) ($P < 0.001$). In terms of contraception, about a fifth of the cases were using intrauterine device, and this number was significantly different compared to control group (2.9%) ($P = 0.027$). In addition, the average sexual intercourse frequency of the case group was 1.9 times per week and it was significantly higher compared to control group ($P < 0.001$).

4.2. *Distribution of VEGF −460C>T genotype and allele*

The location of −460C>T polymorphism in VEGF gene promoter is shown in Fig. 1. In genotyping procedure, the PCR products were digested with *Bst*U1 and VEGF −460C>T polymorphism was characterized as TT homozygote (indigestible, DNA fragment size 175 bp), CC homozygote (digestible, DNA fragment size 155 bp and 20 bp), and CT heterozygote (DNA fragment size 175 bp, 155 bp and 20 bp). This PCR-RFLP-based genotyping has genotyping success rates of 95%–100% and repeatability rates of 98%–100%. Furthermore, direct DNA sequencing with automated DNA sequencing platform was used to confirm the VEGF −460C>T genotypes from both groups (10% samples each group) and the concordance rate between RFLP and DNA sequencing was 100%.

NCBI data reveal that the minor allele frequency of VEGF −460C>T polymorphism is 0.3802. Based on HWE calculation, this study found that the genotype distributions among cases and overall samples were consistent within HWE, with $P = 0.374$ and $P = 0.344$, respectively. It means that the genotype distribution among the subjects of this present study had no HWE deviation.

In this study, 73.5% of cases and 85.3% of controls were homozygous CC and 26.5% of cases and 14.7% of controls were heterozygous CT. There was no subject either from case or control group who had TT genotype (Table 2). These genotype distributions were not significantly different between cases and controls ($P = 0.486$). It was also true for dominant and recessive models. Similarly, allele frequency analysis revealed that there was no association between VEGF −460C>T alleles and uncomplicated RUTI ($P = 0.398$).

5. Discussion

There are several risk factors of RUTI such as frequent sexual intercourse, use of contraception (intrauterine device, diaphragm and spermicidal), use of antibiotics, estrogen levels, and anal and urethra anatomy.[1,2,28,29] This study results

Table 1 – Risk factors of RUTI between uncomplicated RUTI group (*n* = 34) and control group (*n* = 34).

Risk factors	Group		OR (CI 95%)	P
	RUTI	Control		
Married, *n* (%)[a]	29 (85.3)	10 (29.4)	13.9 (4.2–46.3)	0.000
Intrauterine device use, *n* (%)[a]	7 (20.6)	1 (2.9)	8.6 (0.9–73.9)	0.027
Age, years[b]	32 ± 6.59	26.1 ± 5.20		0.000
Sexual intercourse frequency (per week)[b]	1.9 ± 1.31	0.62 ± 2.11		0.000

Abbreviations: CI – confidence interval; OR – odd ratio; RUTI – recurrent urinary tract infection.
[a] Analyzed with χ^2 and Fisher's exact test.
[b] Analyzed with *t*-test.

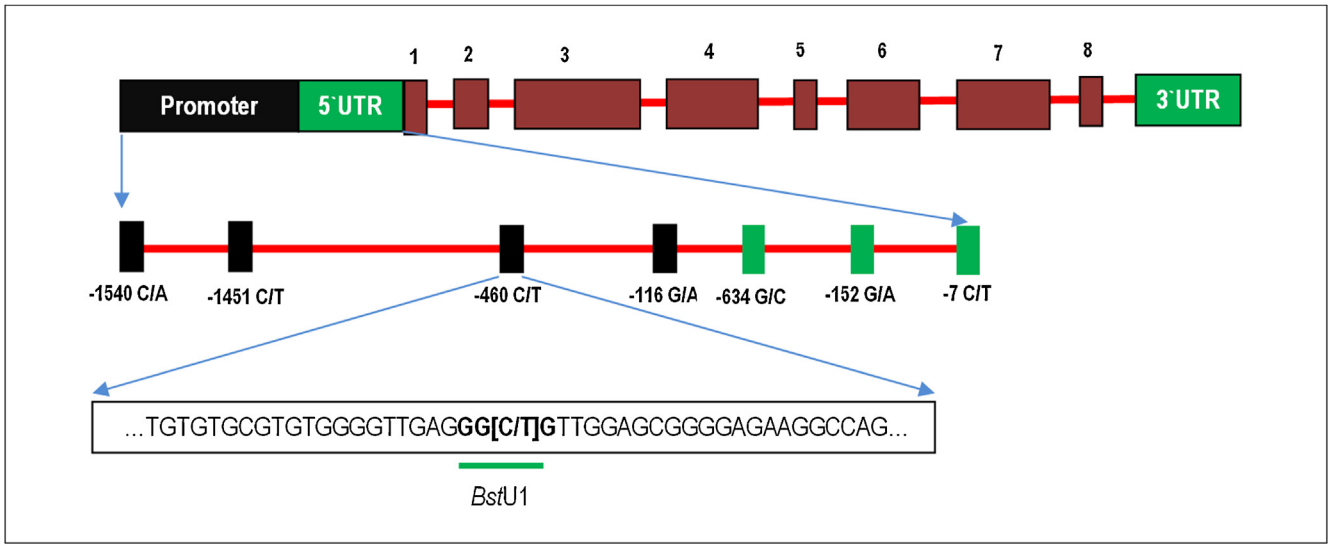

Fig. 1 – The location of VEGF −460C>T and other polymorphisms located in VEGF gene promoter and 5′-UTR. Brown boxes are 8 exons; black and green boxes are the location of some of the common polymorphisms in VEGF gene promoter and in 5′-UTR, respectively. The bold sequence represent the exact sequence of VEGF −460C/T polymorphism and the recognition site for BstU1 restriction enzyme.

support previous findings that sexual intercourse frequency, marital status and intrauterine device contraception were correlated with RUTI. In this study, the age between case and control groups was not matched properly; therefore, age seems as an important risk factor. However, further analysis found that age had strong correlation with marital status (analysis not shown). Therefore, the independent risk factor for UTI in this study was marital status whereas age was a cofounder only.

Interestingly, a previous study found that two of the independent risk factors for RUTI were age at first UTI and UTI history in the mother.[3] These risk factors are suggesting that genetic factors might predispose to RUTI like other infectious diseases susceptibility that are influenced by genetic factors.[30–33] Afterwards, various studies have been conducted to investigate genetic factors of RUTI susceptibility in humans and a review study found that several genes in humans such as heat shock 70 kDa protein 1B (HSPA1B), chemokine receptors (CXCRs), toll-like receptor 2 (TLR2), TLR4, and transforming growth factor beta (TGF-β1) were associated with RUTI susceptibility.[5]

Table 2 – Distribution of genotype and allele frequencies of VEGF −460C>T between uncomplicated RUTI group (n = 34) and control group (n = 34).

VEGF −460C>T genotype and allele	Group		P
	RUTI, n (%)	Control, n (%)	
Genotype			0.486
CC	25 (73.5)	29 (85.3)	
CT	9 (26.5)	5 (14.7)	
TT	0 (0)	0 (0)	
Dominant model			
CC	25 (73.5)	29 (85.3)	0.368
CT + TT	9 (26.5)	5 (14.7)	
Allele			0.398
C	59 (86.8)	63 (92.6)	
T	9 (13.2)	5 (7.4)	

Abbreviations: RUTI – recurrent urinary tract infection; VEGF – vascular endothelial growth factor.

In this study we tried to investigate the role of VEGF gene, one of the most controversially genes, in UTI. VEGF gene expression is controlled at many levels including transcription, mRNA stability and mRNA translation via internal ribosome entry site (IRES) sequences present in the 5′-UTR.[12] One of the factors that influence the level of VEGF transcription is polymorphisms that are located in VEGF gene promoter. Several polymorphisms have been identified in the VEGF promoter and in the 5′-UTR (Fig. 1). One of the most important polymorphisms that is located in VEGF gene promoter is VEGF −460C>T. Previous study found that this polymorphism altered VEGF protein expression in individuals.[15] The association of VEGF −460C>T with diseases related to UTI has been investigated in a couple of studies.[24,25] One study found that there was an association between CC and CT genotypes and total UTI case (with or without VUR complication).[24] However, where multistage analysis was conducted, the result revealed that VUR likely had a role as confounding factor because when all UTI cases with VUR complication were excluded from analysis, there was no association of CC and CT genotypes with UTI. Excluding VUR, they found that there was no difference in genotype frequencies between UTI cases (CC 11.1%, CT 27.8%, TT 61.1%) and controls (CC 4.3%, CT 41.4%, TT 54.3%). Also, there was no association of allele frequencies between UTI cases (C 0.75, T 0.25) and controls (C 0.75, T 0.25).

Another study also found that there was no difference in genotype frequencies between UTI (CC 26%, CT 35.6%, TT

Table 3 – The summary of the role of VEGF −460C>T polymorphism in UTI.

Group comparison	Genotypes			Allele	References
	CC	CT	TT	C:T	
Control vs. UTI	±	±	–	–	Yim et al.[24], Hussein et al.[25]
Control vs. UTI with VUR	+	±	–	–	Yim et al.[24], Hussein et al.[25]
Control vs. UTI without VUR	–	–	–	–	Yim et al.[24], Hussein et al.[25]
Control vs. UTI with renal scarring	+	–	–	–	Hussein et al.[25]
Control vs. UTI without renal scarring	–	–	–	–	Hussein et al.[25]
Control vs. RUTI without VUR and renal scarring	–	–	–	–	Present study

Note: –, no association; +, there is an association; ±, conflicting results between Yim et al. and Hussein et al. study. *Abbreviations*: RUTI – recurrent urinary tract infection; UTI – urinary tract infection, VUR – vesicoureteral reflux.

38.4%) and control (CC 15.4%, CT 45.3%, TT 39.3%).[25] Also, there was no association of allele frequencies between UTI (C 0.438, T 0.562) and control (C 0.381, T 0.619). The summary of the results from previous studies is presented in Table 3. In Table 3, it is clear that there is a robust correlation between VEGF −460 CC genotype and UTI with VUR complication or between VEGF −460 CC genotype and UTI with renal scaring complication but there is no association between VEGF −460 C>T polymorphism (genotypes or alleles) and uncomplicated UTI (UTI without VUR and renal scaring complications). In addition, our study found that there was no association between all types of VEGF −460 C>T genotypes and alleles and uncomplicated RUTI.

This present study is the first study that was conducted to evaluate the association between VEGF −460 C>T polymorphism and uncomplicated RUTI among young women. Yim et al.[24] evaluated the correlation of this polymorphism among childhood UTI with and without VUR complication, while Hussein et al.[25] analyzed the role of this polymorphism in childhood UTI with or without renal scaring. However, there are some limitations of this present study. First, the socioeconomic status between case and control groups was not measured. Although there is no study that reveals the association between socioeconomic status and RUTI; studies found that low socioeconomic increased the prevalence of UTI among pregnant women.[34,35] Second, the sample size was relatively small. However, the genotype distributions among subjects of this study had no HWE deviation. Third, serum VEGF protein concentrations or VEGF gene expression in peripheral blood mononuclear cells was not measured. The VEGF concentrations are important because some studies found that VEGF −460C>T genotypes have conflicting results in VEGF concentration in circumstantial conditions.[15,33,36]

6. Conclusions

This present study found that there was no association between VEGF −460C>T polymorphism of VEGF gene promoter and uncomplicated RUTI among young women.

Conflict of interest

There is no conflict of interest.

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