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Original article

Vascular endothelial growth factor G1612A (rs10434) gene polymorphism and neuropsychiatric manifestations in systemic lupus erythematosus patients

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ABSTRACT

Aim: To investigate the relation between vascular endothelial growth factor (VEGF) gene polymorphism in systemic lupus erythematosus (SLE) patients and lupus related neuropsychiatric manifestations.

Patients and methods: Sixty adult SLE patients recruited from the Rheumatology and Neurology departments of Cairo University hospitals were classified into two groups; Group A: 30 patients with neuropsychiatric manifestations (NPSLE) and Group B: 30 patients without. For both groups the SNP G1612A (rs10434) of the VEGF gene was genotyped by real time polymerase chain reaction (RT-PCR).

Results: Statistically significant difference was found in genotype and allele frequencies between both groups (AA [70% vs 13.3%, $p < 0.001$] and GG [10% vs 66.7%, $p < 0.001$]).

Conclusion: Polymorphism in the gene coding for VEGF may be associated with increased incidence of neuropsychiatric lupus in SLE patients.

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Polimorfismo genético do fator de crescimento vascular endotelial G1612A (rs10434) e manifestações neuropsiquiátricas em pacientes com lúpus eritematoso sistêmico

Palavras-chave:
LES
VEGF
Gene G1612A (rs10434)
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Manifestações neuropsiquiátricas

RESUMO

Objetivo: Investigar a relação entre o polimorfismo genético do fator de crescimento vascular endotelial (VEGF) em pacientes com lúpus eritematoso sistêmico (LES) e manifestações neuropsiquiátricas relacionadas com o lúpus.
Pacientes e métodos: Foram recrutados 60 pacientes adultos com LES nos departamentos de Reumatologia e Neurologia de hospitais universitários do Cairo e classificados em dois grupos; grupo A: 30 pacientes com manifestações neuropsiquiátricas (LESNP) e grupo B: 30 pacientes sem manifestações neuropsiquiátricas. Genotipou-se o SNP G1612A (rs10434) do gene VEGF em ambos os grupos por reação em cadeia da polimerase em tempo real (RT-PCR).
Resultados: Foi encontrada diferença estatisticamente significativa nas frequências genotípicas e alélicas entre os dois grupos (AA [70% vs. 13,3%, $p < 0,001$] e GG [10% vs. 66,7%, $p < 0,001$]).
Conclusão: O polimorfismo no gene que codifica o VEGF pode estar associado ao aumento na incidência de lúpus neuropsiquiátrico em pacientes com LES.

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease,[1] with both genetic and environmental factors playing significant roles in its pathogenesis.[2] As a consequence of its complex immunopathology, involving the production of autoantibodies and immune complex vasculitis with endothelial cell damage,[3] different organs and blood vessels may be affected by chronic inflammation.[4]

The damage and activation of vascular endothelial cells are the initiating factors in the pathogenesis of SLE.[5] Vascular endothelial growth factor (VEGF) is a key modulator of angiogenesis, endothelial cell proliferation and migration, chemotaxis, and capillary hyper-permeability,[6] and was found to be upregulated in a number of collagen diseases including SLE.[7] Furthermore, it has been reported that high VEGF levels may be associated with the disease activity in SLE.[3,4] Also polymorphism in VEGFR2 gene has already been correlated with vascular diseases and may influence endothelial integrity, repair and function.[8]

In addition to association of VEGF levels with disease activity in SLE, it was also found to be correlated with other disease manifestations as lupus nephritis, and with higher mean carotid intima media thickness,[9,10] pulmonary hypertension,[11] and inversely correlated to platelet count.[7] Although it was reported that anti-ribosomal P antibody may influence the pathology of neuropsychiatric lupus through the elevation of VEGF production from monocytic cells,[12] however, the association between neuropsychiatric lupus and VEGF remains unclear.

This study aims to investigate the relation between VEGF single nucleotide polymorphism (SNP) G1612A (rs10434) gene polymorphism in SLE patients and lupus related neuropsychiatric manifestations.

Subjects and method

This is a cross-sectional study that included sixty patients who fulfilled the updated ACR revised criteria for the classification of SLE.[13] Thirty patients with neuropsychiatric manifestations of SLE (NPSLE) (Group A) were recruited from Rheumatology and Neurology departments of Cairo University Hospitals from May 2013 to May 2015. Neuropsychiatric manifestations of SLE (NPSLE) were defined by the presence of current or past stroke, transient ischemic attack, psychosis, seizure disorder, confusional state, and/or cognitive dysfunction. Another 30 consecutive patients without NP involvement (Group B) were selected to match the same number of patients with NP involvement. Informed consents were taken from the patients and the study was approved by the local ethics committee. Full history taking, thorough clinical examination with special emphasis on neuropsychiatric involvement was performed for all patients. Patients suffering from active renal disease, pulmonary hypertension, hypertension, active arthritis and thrombocytopenia were excluded from the study.

The SNP G1612A (rs10434) in the 3′ untranslated region (3′-UTR) of VEGF gene was genotyped by real time polymerase chain reaction (RT-PCR) in all patients.

DNA isolation

Genomic DNA was isolated from white blood cells (WBCs) of peripheral blood using High Pure PCR Template Preparation Kit (Roche Diagnostics GmbH, Roche Applied Science, 68298 Mannheim, Germany). Isolation was carried out according to the manufacturers' instructions.

VEGF genotyping by RT-PCR

Real time PCR was performed by the Carousel-Based System, Lightcycler 2.0, using the master mix kit, Lightcycler fast start Master Hybprobe kit (Roche Diagnostics GmbH, 68298 Mannheim, Germany), together with the LightSNiP rs10434 VEGFA kit (TIB MOLBIOL GmbH – Eresburgstrasse 22-23, D-12103 Berlin, Germany) that contained the primers necessary for the reaction.

The Lightcycler fast start Master Hybprobe kit is a Hot Start Reaction Mix for PCR using HybProbe probes as detection format. The LightCycler HybProbe format is based on the principle of fluorescence resonance energy transfer (FRET), where two sequence specific oligonucleotide probes, labeled with different dyes (donor and acceptor), were added to the reaction mix in addition to PCR primers. The amount of fluorescence generated by the tag on the probe is directly proportional to the amount of target DNA generated during the PCR process. The PCR reaction was carried out according to the manufacturers' instructions and was followed by melting curve analysis.

Statistical analysis

Data were statistically described in terms of mean ± standard deviation (±SD), median and range, or frequencies (number of cases) and percentages when appropriate. Chi-square test was used to compare qualitative variables. Correlation between parameters was performed using Pearson correlation coefficient. Differences between groups were considered statistically significant with p values less than 0.05 and highly significant if less than 0.01. All statistical calculations were done using computer programs SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 16 for Microsoft Windows.

Results

Demographic data and laboratory parameters of the present study are presented in Table 1. Both groups were age and sex matched with no significant differences in disease duration ($p = 0.9$).

Neuropsychiatric manifestations in Group A (NPSLE) patients were as follows; 9 (30%) had psychosis, 8 (26.7%) had seizures, 5 (16.7%) had transient ischemic attacks (TIAs), 4 (13.3%) had stroke, 3 (10%) had transverse myelitis (TM) and one patient (3.3%) had depression. Clinical manifestations in Group B included: mucocutaneous manifestations, arthralgia, leucopenia, hemolytic anemia and serositis.

Laboratory features including the genotypic distribution of the study population are shown in Table 2.

Table 1 – Demographic features and disease duration in SLE patients Group A (NPSLE) and Group B without neuropsychiatric manifestations.

	Group A ($n = 30$)	Group B ($n = 30$)	p
Age			
Range	19–52	21–55.0	0.3
Mean ± SD	31.9 ± 7.6	34.3 ± 8.6	NS
Sex			
Male	1 (3.3)	3 (10)	0.6
Female	29 (96.7)	27 (90)	NS
Disease duration			
Range	1.0–12	1.0–11	0.9
Mean ± SD	5.5 ± 3.5	5.5 ± 3.3	NS

Discussion

SLE is a multi-system autoimmune disease.[14] In lupus chronic systemic inflammation leads to activation of vascular endothelial cells which in turn leads to a substantial increase in angiogenic factors which play a significant role in vascular permeability, vascular growth, and inflammatory response leading to blood vessel destruction and serious internal organ dysfunction.[15]

VEGF is also involved in kidney and lung function as well as serving as a survival factor for neuronal cells.[16] Polymorphisms in angiogenesis-regulating genes may affect the response to an angiogenic stimulus and thereby affect susceptibility to and/or the progression of angiogenesis-dependent disease. In previous gene studies, polymorphisms in VEGF and vascular endothelial growth factor receptor 2 (VEGFR2) were clearly associated with the development of angiogenesis-dependent disease.[17]

Circulating VEGF levels are highly heritable,[18] however, VEGF gene is highly polymorphic, with hundreds of polymorphisms currently annotated in the Single Nucleotide Polymorphism database (dbSNP). It includes at least three polymorphisms that are relatively common and may affect VEGF expression. The insertion/deletion polymorphism (I/D) at the −2549 position of the promoter region and the −634G/C (rs2010963) polymorphism located in the 5′-UTR have been considered to be associated with increased VEGF expression.[19] Also the 936C/T (rs3025039) polymorphism located in the 3′-UTR is associated with substantially increased serum VEGF levels.[20]

Owing to the implication of VEGF as a modulator of angiogenesis, endothelial cell proliferation and migration, the aim of the current study was to investigate the possible relationship between polymorphisms in the gene coding for VEGF and neuropsychiatric manifestations in SLE patients.

In our study, we found a statistically significant difference in genotype and allele frequencies between patients in Group A (those with neuropsychiatric manifestations) and patients in Group B (those without neuropsychiatric involvement) (AA [70% vs 13.3%, $p < 0.001$] and GG [10% vs 66.7%, $p < 0.001$]).

Although none of the previous studies focused on VEGF gene polymorphism and its association with NPSLE, the relation of such polymorphism to other lupus related manifestations were studied; the significant relation of (*VEGF*) gene +405GG to lupus nephritis was reported.[21] Similarly, relationship between 6 SNPs of VEGF gene (rs2010963, rs3024994, rs3025000, rs3025010, rs3025035 and rs833070) and SLE susceptibility in Northern China has been investigated in 44 patients.[22] They reported that the frequency of rs833070 A allele was significantly higher in SLE than in the controls and

Table 2 – Comparison between laboratory data in SLE patients Group A (NPSLE) and Group B without neuropsychiatric manifestations.

	Group A ($n=30$)	Group B ($n=30$)	p
ANA			
+ve	28 (93.3)	25 (83.3)	0.4
−ve	2 (6.7)	5 (16.7)	NS
DNA			
+ve	11 (36.7)	10 (33.3)	1
−ve	19 (63.3)	20 (66.7)	NS
VEGF genotype			
AA	21 (70)	4 (13.3)	<0.001
AG	6 (20)	6 (20)	1
GG	3 (10)	20 (66.7)	<0.001
ESR	48.8 ± 32.3 (5–12)	48.2 ± 34.9 (12–150)	0.9
Hb	12.3 ± 1.4 (9.5–15.3)	12.3 ± 1.5 (9.3–15.3)	0.9
WBC	8.4 ± 2.5 (3.8–16)	7.6 ± 1.5 (3.8–9.5)	0.1
PLT	246.2 ± 77.4 (80–466)	229.8 ± 42.3 (130–321)	0.3

ANA, antinuclear antibodies; VEGF, vascular endothelial growth factor; ESR, erythrocyte sedimentation rate; Hb, hemoglobin; WBC, white blood cells; PLT, platelet.
Results are presented as number (%) or mean ± SD (range).

rs833070 GG decreased the susceptibility of arthritis in the SLE patients.

Limitations

The small number of patients included in the study and the absence of similar studies focusing on the relation of VEGF gene polymorphism and lupus related neuropsychiatric manifestations are the most important. However this study can be considered as a pilot study; which may open the door in the future, for studies conducted on large populations to establish the real impact of VEGF gene polymorphism in pathogenesis or in the clinical characteristics of neuropsychiatric manifestations of SLE. Also further studies with assessment of VEGF serum levels in patients with neuropsychiatric manifestations could be beneficial.

Key message

The SNP G1612A (rs10434) of VEGF gene may represent an increased susceptibility to neuropsychiatric involvement in patients with SLE.

Conflict of interest

The authors declare no conflicts of interest.

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