RESEARCH ARTICLE

The Impact of eNOS Gene Polymorphism (rs1799983 T>G) in the Malar Rash, Sex and Treatment Types of Systemic Lupus Erythematosus

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ABSTRACT

The study aims to detect Endothelial Nitric Oxide Synthase gene polymorphism (rs1799983 T>G) in the systemic lupus erythematosus (SLE) in some Iraqi cases, tetra ARMS-PCR was used to detect T>G substitution mutation, the results showed that About 80% of patients didnot suffer from malar rash while 20% were with malar rash and patients used three types of drugs (Rituximab, Endoxan and methylperdnisolon). The rs1799983 T>G genotyping showed three types of genotypes; GT, GG and TT. Non-significant differences were observed for all genotypes. The GG frequent percent was 50% in SLE and 56% in control group (p 0.641), GT was more frequent in SLE than control (47.36% and 40%), respectively (p 0.688), and low percent's of TT in both group (2.63% and 4%). Low T allele percentage in SLE and control, high frequent of G allele in both groups. According to malar rash, a high percent of GT frequency in the patients with malar rash (71.42%) than non malar rash group (41.93%) there was non-significant association of all genotypes with malar rash (p = 0.223 and p = 0.906) for GG, GT and TT, respectively. The allele frequency shows significant association (p = 0.000), G was more frequent in malar rash than non-malar rash group (0.64), while T low percentage in malar rash than another group (0.35). belong to sex, significant association was observed in GG with female and GT with male (p = 0.0002), the TT non-significant association with sex, the allele frequency showed non-significant association with sex despite of the high percent of G allele in both male and female, The genotyping frequency distribution according to SLE treatment, in the Rituximab the GG and GT was more frequent, then in the endoxan that the GG and GT have a same frequent, the methylperdnislon was little used as treatment and has same frequent of GG and GT. The current study concluded that no association between eNOS rs1799983 T>G and SLE, malar rash and weak association with SLE treatment, but significant association was observed in GG with female.

Keywords: eNOS, Gene polymorphism, Malar rash, (rs1799983 T>G), SLE treatment, Systemic lupus erythematosus.

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INTRODUCTION

One of the chronic autoimmune diseases is SLE its inflammatory disorder that contributed the connective tissue, organs such as lung, kidney, blood vessels, muscles, and skin,[1-4] also different genetic factors associated with SLE etiology.[4,5] The prevalence of SLE is higher in women than men,[6-8] with an age range (15–40) years.[9] Among Asian populations Investigations reported the prevalence rate (30–50) cases per every 100,000, while the incidence rate is (0.9–3.1)% among every 100,000 per year.[10] The SLE symptoms are weight loss, fever, alopecia, glomerulonephritis, vesiculobullous lesions and rash.[11] The SLE characterized by auto production of antibody that led to enhance innate immunity against tissue.[1]

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The role of oxidative stress in the SLE represented by the reduction-oxidation (redox) signaling process which have criticized in the many innate Immune responses, in this process enzymes function were alterations by ROS.[2] the Nitric oxide (NO) is one of the membrane-permeable free radicals formed by three nitric oxide synthase (NOS) isoforms by arginine and oxygen as substrates.[3] The NO involved in some physiological processes according to the level of NO production with other reactive intermediates.[11] other studies found that the dysfunction of eNOS phenotype was associated with eliminated of endothelium-dependent vasodilation in SLE that found in the kidney biopsy of SLE patients represented by low level of eNOS,[6-8] other eNOS functions have been detected like vital role in endothelial cell physiology, blood presser maintenances, coagulation and adhesion of leukocyte.[10] In addition to its role in the activation and clearance of T cell, other evidences reported that eNOS-NO contributed in the mitochondrial membrane hyperpolarization and biogenesis, elevation in the [Ca^{2+}] in T cell cytosol and mitochondria, and recapitulates the enhanced CD3/CD28-induced Ca^{2+} fluxing of lupus T cells.[12] The role of eNOS in SLE still unclear and still under investigations.[13] The present study aims to detect the Nitric Oxide Synthase gene polymorphism (rs1799983 T>G) in SLE patients in middle Euphrates cities.

METHODOLOGY

A case control study was conducted to deals with SLE patients that attended to a chronic disease clinic from different provinces, all patients' diagnosis by a specialist physician by clinical biomarker and symptoms, and treated by three types of drugs including (Rituximab, Endoxan and methylperdnisolon). Blood samples were collected from 38 patients and 29 healthy individuals as a control group, DNA was isolated from each sample, then PCR-ARMS technique was used by tetra primer for eNOS rs1799983 detection F1: 5'-AGCCTCGGTGAGATAAAGGATG-3' R1: 5'-CCTGGACCTGCTCTGATTGTC-3' F2: 5'-GCTGCTGCAGGCCCCAGATAAG-3' R2:5' GCAGAAGGAAGAGTTCTGGGAGA-3' the amplification products were G allele (475bp) T allele (271 bp) Control band (701 bp)[14] at annealing Tm 60ºC and extended for 40 seconds. products were separated by agaros gel electrophoresis. Homozygotes and heterozygotes analyzed by odd ratio (CI95%) at *p-value* less than 0.05, other results represented as mean ± SD.

RESULTS AND DISCUSSION

The our results show that the age mean of patients was (31.47 ± 9.59) year and the control group was (26.80 ± 4.79) years, the duration of disease was (8.87 ± 6.08) years, according to sex the females percent was more than male in patients (90, 10)%. Belong to occupy about 75% of patients was a housewife, 15% students and 10% employments, while all control individuals were employment. About 80% of patients didn't suffer from malar rash while 20% were with malar rash.

The rs1799983 T>G genotyping was detected in the present study using PCR-ARMS, the results show GT, GG and TT genotypes (Figure 1), non-significant differences were observed for all genotypes. the GG frequent percent was 50% in SLE and 56% in the control group (OR 0.7857, P 0.641), GT more frequent in SLE than control group (47.36 and 40%) respectively (OR 1.80, P 0.688) and low percents of TT in both groups (2.63% and 4%). Low T allele frequent in SLE and control group, high frequency of G allele in both groups also, according to hardy-Weinberg law (Table 1).

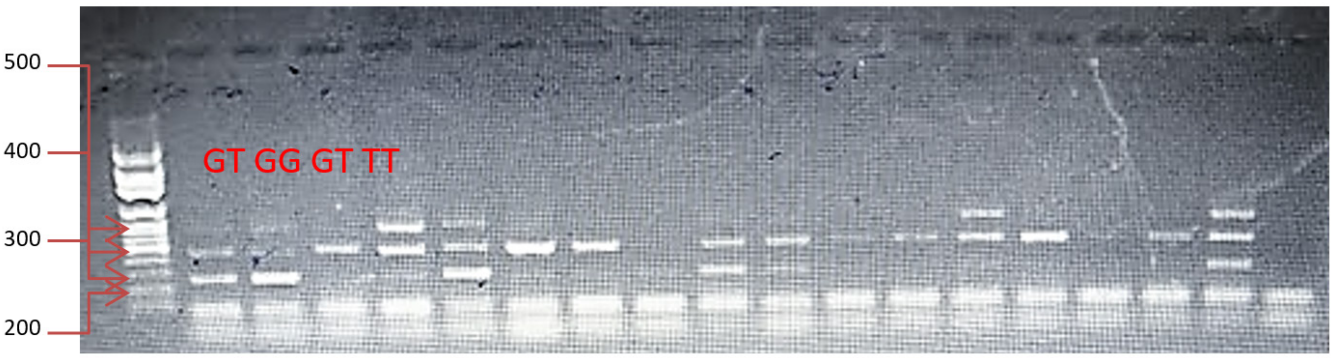

Figure 1: Agaros gel electrophoresis of eNOS rs1799983 T>G in SLE patients using PCR-ARMS. GT, GG and TT genotypes with control band. DNA ladder (100-1000bp).

Table 1: Association the eNOS rs1799983 T>G genotype with SLE patients and control groups using PCR-ARMS.

rs1799983 T>G	*SLE*	*Control group*	*Odd ratio (CI95%)*	*Sig*
GG	19 (50%)	14(56%)	0.7857 (0.2851–2.1657)	0.6411
GT	18(47.36%)	10(40%)		
TT	1(2.63%)	1(4%)	1.8000 (0.1013 to 31.9899)	0.6889
T	0.26	0.24	1.1279 (0.5941 to 2.1413)	0.7130
G	0.73	0.76		

Table 2: Association of the eNOS rs1799983 T>G genotype with malar rash in SLE patients.

rs1799983 T>G	*Malar rash*	*Non malar rash*	*Odd ratio (CI95%)*	*Sig*
GG	2(28.57)	17(45.82%)	3.0357	0.2230
GT	5(71.42)	13(41.93%)	0.5089 to 18.1082	
TT	0	1(3.22%)	0.8182	0.9066
			0.0287 to 23.3371	
T	0.35	0.75	5.7143	0.0001
G	0.64	0.24	3.0822 to 10.5939	

Table 3: Association of the eNOS rs1799983 T>G genotype with SEX in SLE patients.

rs1799983 T>G	*SLE group*		*Odd ratio (CI95%)*	*Sig*
	Male	*Female*		
GG	25%	51.42%	3.1875	0.0002
GT	75%	45.71%	(1.7490–5.8092	
TT	0	2.85%	0.0861	0.1075
			(0.0043–1.7052)	
T	0.37	0.25	0.5661	0.0673
G	0.62	0.74	(0.3078–1.0413)	

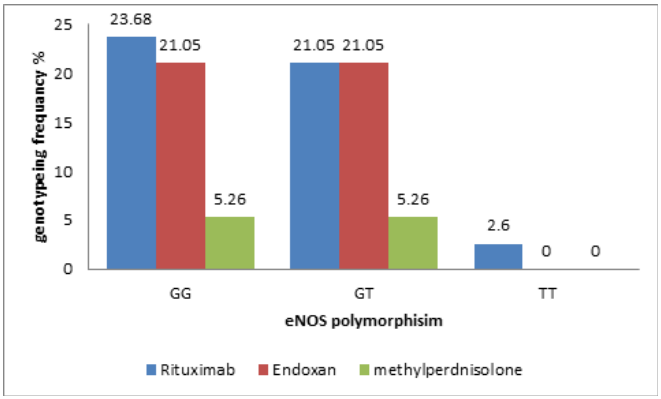

Figure 2: The genotyping frequency distribution according to SLE treatment.

According to malar rash, which is one of the clinical manifestation of SLE disease a high percent of GT frequent in the patients with malar rash (71.42%) than non malar rash group (41.93%) there was non-significant association of all genotypes with malar rash (p = 0.223 and p = 0.906) for GG, GT and TT, respectively. The allele frequency shows significant association, G was more frequent in malar rash than non-malar rash group (0.64), while T was low observed (0.35) in malar rash than another group (Table 2).

The association of rs1799983 T>G with sex was showed in the Table 3, significant association was observed in GG with female and GT with male (p 0.0002), TT non-significant association with sex, the allele frequency showed non-significant association with sex despite of the high percent of G allele in both male and female. The association between sex and eNOS gene polymorphism was studied in other disease like arteriosclerotic and myocardial infarction.[15,16] No clear results about related sex with eNOS, the eNOS gene located within the chromosome 7 thus there wasn't direct associated with sex, the association of GG with female may be because high percent of female that has high prevalence of SLE as a previous reported.[6,8,17]

The eNOS gene polymorphism proved to be associated with different diseases, its located on the chromosome 7q36.1 consists of 26 exons.[18-20] The current result didn't find an association between SLE and rs1799983 T>G and this didn't agree with Serrano *et al*.,[21] that found the eNOS polymorphism influences SLE predisposition, they found that intron 4bb genotype responsible for eNOS higher level synthesis and intron 4 ab genotype is associated with lower synthesis, also another study was seen that the a/b eNOS gene intron 4 a/b VNTR polymorphism represents a severity rather than a susceptible genotype for SLE.[22] The present results agree with Alfadhli *et al*.,[23] who observed that polymorphisms of eNOS probably do not exert a major influence on susceptibility to SLE, but they have significant effects when combined within a specific haplotype. The role of eNOS gene production and immune dys-regulation in the thyroid gland was reported by Varul *et al*.[24] That may be effected on the immune response in the SLE patients, The synthesis of nitric oxide by eNOS may produce a protective or anti- inflammatory function.[25] On the other hand, the endothelial cell activation is One of the SLE characteristic features which was a source of the excess NO production.[26] The high level of nitric oxide production may modification complement mediated clearance of apoptotic cells in SLE patients, involved in autoimmunity.

However, Oxidative stress was reported to be increased in SLE and it reflects disease activity which related with eNOS gene polymorphism.[27]

The genotyping frequency distribution according to SLE treatment showed that in the Rituximab the GG and GT was more frequent, then in the endoxan the GG and GT has the same frequent, the methylperdnislon was little used as treatment and has same frequent of GG and GT (Figure 2).

Studies found that the Rituximab can be stimulated ROS generation when used as anti-cancer drugs.[28] The Endoxan and methylprednisolone also enhanced free radicals generation.[29,30] The association of these drugs with eNOS gene polymorphism didn't reported in SLE, in present study the effect of drugs that used in the SLE treatment in the eNOS gene polymorphism show weak association with Rituximab and Endoxan, however it needs more studies. The current study didnot find an association between malar rash and eNOS rs1799983 T>G), the previous studies didn't document this relationship, thus it needs more investigations to explain role of eNOS in malar rash in SLE patients. The current study concluded

that no association between eNOS rs1799983 T>G and malar rash. Weak association with SLE treatment, And significant association of GG with female in SLE patients.

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