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Assessment of SNPs (rs4774, rs6498122) of *CIITA* Gene in Buccal Swabs and Blood of Oral Lichen Planus Patients in Iraq

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ABSTRACT

Background: *Oral lichen planus (OLP) is a common autoimmune inflammatory disorder that is difficult to cure, and its pathogenesis is still unknown. The major histocompatibility complex (MHC) class II transactivator (CIITA) gene has been reported to be an important candidate in some classical autoimmune diseases, and certain single nucleotide polymorphisms (SNPs) in CIITA gene have been confirmed to be associated with susceptibility to some autoimmune diseases. This research was conducted to investigate the existence of any correlation between OLP and SNPs (rs4774, rs6498122) in CIITA gene separately and in combination method by using (buccal swabs and blood sample), and to compare their existence with that of apparently healthy group. The validity of DNA extraction method from buccal swabs as convenient, painless, less risk of diseases transition and cost-effective alternatives way to blood sampling for participants and researchers was also assessed in this study.* ***Materials and Methods:*** *A case-control study was performed to genotype detection of 2 SNPs in the CIITA gene from buccal swab and blood sample from OLP patients as patient group, and buccal swab and blood sample from healthy people as control group; this was carried out by qRT-PCR.* ***Results:*** *The SNP rs4774 variant in exon 11 (G/C, Gly500Ala) of CIITA gene is significantly associated with OLP in heterozygous frequency, SNP rs6498122 variant in intron 5 (A/G) of CIITA gene is significantly associated with OLP in mutant frequency, and at allele frequency the two SNPs is significant. When the two SNPs analyzed in genotypes combination method for SNPs (rs4774, rs6498122) between healthy and OLP patient groups, significant result was shown when the rs4774/rs6498122 be wild/mutant and hetero/hetero. Also, the result of DNA extraction for SNPs analyses of buccal swab is the same of blood sample.* ***Conclusion:*** *Data was shown that the two SNPs rs4774 and rs6498122 at CIITA gene are associated with OLP and could also indicate the autoimmune characteristics of OLP. Buccal swab instead of blood sample in SNPs genetic research can be used.*

Keywords: Oral lichen planus, *CIITA* gene, SNPs rs4774 and rs6498122, Buccal swabs

INTRODUCTION

Oral lichen planus (OLP) is a common chronic inflammatory disorder that usually affects the stratified squamous epithelium of mucosa. Approximately 1-2% of the adult population (mainly middle-aged and elderly subjects) suffer from this disease and the morbidity in women is higher than in men [1,2]. Although the etiology of OLP is still unclear, it is generally believed that, immunologic, and environmental factors are involved [3]. Also, angiogenesis may be an integral component associated with the development of the OLP [4].

Oral lichen planus presents clinically with some features of autoimmune disease, including chronicity, adult onset, female predilection, autocytotoxic T cells, and a cyclic nature, indicating that autoimmunity could be a possible cause of OLP [5].

The major histocompatibility complex (MHC) class II molecules sit at the heart of autoimmune processes and are under control of the MHC class II transactivator (*CIITA*, also called MHC2TA) [6]. The *CIITA* gene spans 47.8 kb on chromosome 16p13 and has four alternate first exons (named I-IV) in the promoter region [6].

Recently, a growing number of researchers have focused on the association between single nucleotide polymorphisms (SNPs) in *CIITA* gene and susceptibility to certain kinds of autoimmune diseases. One of them found that SNPs (rs4774, rs6498122) has a real associated with oral lichen planus in Chines people [7]. Other study found that a missense variant SNP (rs4774) in *CIITA* was associated with the risk of systemic lupus erythematosus (SLE) [8]. In a separate

study, a newly identified polymorphism in an intronic region at nucleotide 485 in *CIITA* gene was also found to have a probably important role in the pathogenesis of SLE [9]. Major histocompatibility complex genes, particularly human leukocyte antigen (HLA) class II, are strongly associated with the risk of developing rheumatoid arthritis (RA). Based on the strong biological relationship between *CIITA* and HLA class II genes, a comprehensive investigation of *CIITA* variation in RA was conducted, which included an assessment of haplotypes; however, the results did not provide evidence that common variation in *CIITA* gene had a role in susceptibility to RA [10]. In contrast, in Scandinavian populations, 2 SNPs were found to be significantly associated with RA [11]. Recent genome-wide association (GWA) studies have identified *CIITA* gene and the extended gene region (*CIITA*-CLEC16A-SOCS1) as a susceptibility locus for celiac disease. Given its pivotal role in MHC class II regulation, *CIITA* gene was considered to be an important candidate gene in autoimmune-related diseases.

Based on the autoimmune-related characteristics of OLP and the importance of *CIITA* gene in autoimmune regulation. This research was conducted to investigate the existence of any correlation between OLP and SNPs (rs4774, rs6498122) in *CIITA* gene in Arab/Iraqi patients.

MATERIAL AND METHODS

Subjects

The patient group comprised from 30 OLP patients and 30 apparently healthy subjects as control group, with no evidence of severe chronic periodontitis or autoimmune diseases such as SLE and RA. Oral lichen planus was diagnosed by clinical examination (Figure 1), Subjects were excluded if they were edentulous, pregnant, have any other autoimmune diseases, were took medications, or were receiving operative treatment.

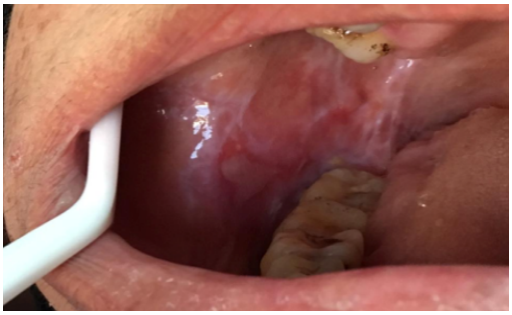

Figure 1 Clinical appearance of oral lichen planus. Typical interlacing white keratotic lines with erosive area

All subjects were Arab/Iraqi and were recruited from the Merjan Hospital, Dermatology Department, Babil, Iraq, between January 5, 2017 to May 1, 2017. Buccal swab from all 60 individuals was obtain and peripheral blood from all 60 individuals was anticoagulated with EDTA, then all sample was refrigerated in deep freeze, Genomic DNA was extracted using WizPrep g DNA Mini Kit (WizeBio solution, South Korea).

TaqMan fluorescent oligonucleotide probes and primers sequences were synthesized by Alpha DNA Ltd. (Canada) and stored lyophilized at (-23°C). Primer and probe sequence were matched by the bioinformatics programs NCBI [1,12] (Table 1). Informed written consent was obtained from all participants involved in this study.

Table 1 The sequences of (reverse and forward of primer) and (Vic and Fam for Probes) for SNPs rs4774 and rs6498122

rs4774		
Primers	Forward	5'--TCCCCTGCCATTGCTTGAAC--3'
	Reverse	5'--AACCTCGGAGCAGCTTCTTCT--3'
Probes	Fam	ACGGCTTCGAGGAGC
	Vic	AGACGCCTTCGAGGA
rs6498122		
Primers	Forward	5'--AAGCGAGCTCCTGTATTTGC--3
	Reverse	5'--GCTGCTGAAGATAGGACCGT--3
Probes	Fam	TCACATTCCATATTATTGAGG
	Vic	AGGTCACGTTCCATATTATTGA

SNP Selection

Potential functional SNPs of *CIITA* gene were selected from published association studies and from the dbSNP database of the National Center for Biotechnology Information. In order to capture the greatest degree of genetic variation in the *CIITA* gene.

Genotyping

Single nucleotide polymorphism genotyping was performed using the quantity real time PCR. Approximately 100 ng of genomic DNA was used to genotype each sample. The primer and probe sequences are summarized in (Table 1). The DNA samples were extract by WizPrep g DNA Mini Kit (WizeBio solution, South Korea). Real time PCR machine used is (Rotor-Gene Q, USA).

The resulting products were desalted and transferred to photo fluorescence signal. Allele detection was performed using matrix mix (WizPure q PCR Master (PROBE) kit, WizeBio solution, South Korea).

Statistical Analysis

Genotype frequencies were checked for consistency amongst controls with those expected from the Hardy Weinberg equilibrium (HWE). The genotype distributions were compared between patients and controls using a Statistical Analysis System-SAS (2012) to measure the effect of different factors in studying the parameters. Similar tests were also used to assess allele frequencies, The P-values, ORs and 95% CI were calculated. $P<0.05$ was considered statistically significant.

RESULTS

Result Between Blood Sample and Buccal Swab Genetically By DNA Extraction

The result of DNA extraction from buccal swab of 60 sample (30 from patient group, and 30 from control group) have the same results of blood for 60 sample (30 from patient group and 30 from control group).

Result of Genotype and Allele Frequency for SNPS rs4774, rs6498122

Total 2 SNPs (rs4774, rs6498122) were shown in the *CIITA* gene for genotype and allele detection. The genotype frequencies of the 2 SNPs in the *CIITA* gene were confirmed to be in Hardy-Weinberg equilibrium ($P>0.05$), and the Wild type for rs4774, rs6498122 were taken as reference, Table 2.

There were significant differences between patients and healthy groups in the Hetero genotype distributions (P=0.01, OR=5.69, 95% CI=1.59-20.33), and non-significant differences in the Mutant genotype distributions (P=0.11) of the SNP rs4774 (G/C, Gly500Ala), (Table 2).

There were non-significant differences between patients and healthy in the Hetero genotype distributions (P=0.514, OR=1.63,95%, CI=0.41-6.47), and significant differences in the mutant genotype distributions (P=0.0009, OR=12.25, 95% CI=2.46-60.91) of the SNP rs6498122 (A/G), (Table 2).

Allele frequencies for the 2 SNPs are shown in Table 2. The frequencies of the rs4774 mutant C-allele and the rs6498122 mutant G-allele were significantly higher in the patient than in healthy groups (P=0.0001, OR=8.11, 95% CI=2.59-25.4 and P=0.0001, OR=8.5, 95% CI=3.45-20.96, respectively).

Table 2 The genotype and allele frequencies of the 2 SNPs in the *CIITA* gene

SNP		Type	OLP patient group (n=30)	Healthy group (n=30)	P-value	OR 95%CI
rs4774	Genotype frequencies	GG (wild)	40.0% (n=12)	86.6% (n=26)	-	1.00 (Reference)
		GC (hetero)	46.7% (n=14)	13.3% (n=4)	0.01*	5.69 (1.59-20.33)
		CC (mutant)	13.3% (n=4)	0.00	0.11^{NS}	-
	Number	-	(n=60)	(n=60)	-	-
	Allele frequencies	G (wild)	63.3% (n=38)	93.4% (n=56)	-	1.00 (Reference)
		C (mutant)	36.6% (n=22)	6.6% (n=4)	0.0001***	8.11 (2.59-25.4)
Number		-	(n=30)	(n=30)	-	-

rs6498122	Genotype frequencies	AA (wild)	33.3% (n=10)	80.0% (n=24)	-	1.00 (Reference)
		AG (hetero)	20.0% (n=6)	13,3% (n=4)	0.514 NS	1.63 (0.41-6.47)
		GG (mutant)	46.6% (n=14)	6.6% (n=2)	0.0009***	12.25 (2.46-60.91)
	Number	-	(n=60)	(n=60)	-	-
	Allele frequencies	A (wild)	43.3% (n=26)	86.6% (n=52)	-	1.00 (Reference)
		G (mutant)	56.6% (n=34)	13.3% (n=8)	0.0001***	8.5 (3.45-20.96)
NS: Non-significant; *Statistical Significant; *** Very Highly Statistical Significant						

Result of Genotypes Combination Method for SNPs (rs4774, rs6498122) between OLP Patient Groups and Healthy Group

SNPs-genotypes combination method is used to know the effect of gene's SNPs on concentration of the gene product in clinical samples [13].

The results of genotype combination frequencies shown in Table 3 reveal that the wild type of rs4774/rs6498122 were taken as reference. The relation between 2 SNPs is significant in GG/GG,GC/AG as P=0.002 for GG/GG and P=0.015, OR=3.5, 95% CI=0.65-18.98 for GC/AG respectively.

Table 3 The result of genotypes combination method for SNPs rs4774, rs6498122

***CIITA* polymorphisms**	**Frequencies (%)**		**P-value**	**Odd Ratio (95% CI)**
Genotype rs4774/ rs6498122	**OLP Patient group (n=30)**	**Healthy group (n=30)**		
Wild/Wild GG/AA	13.32% (n=4)	80.0% (n=24)	-	1 (Reference)
Wild/Hetero GG/AG	0.00	6.63% (n=2)	0.49 NS	-
Wild/Mutant GG/GG	26.6% (n=8)	0.00	0.002**	-
Hetero/Wild GC/AA	13.32% (n=4)	0.00	0.112 NS	-
Hetero/Hetero GC/AG	20.0% (n=6)	6.63% (n=2)	0.015**	3.5 (0.65-18.98)
Hetero/Mutant GC/GG	13.32% (n=4)	6.63% (n=2)	0.67 NS	2.15 (0.36-12.76)
Mutant/Wild CC/AA	6.63% (n=2)	0.00	0.491 NS	-
Mutant/Hetero CC/AG	0.00	0.00	1.00 NS	-
Mutant/Mutant CC/GG	6.63% (n=2)	0.00	0.491NS	-
NS: Non-significance; **: Highly statistical significant				

DISCUSSION

Based on the statistical results of this study, there was an unquestionable association between certain SNPs in the *CIITA* gene and OLP.

According to the literature, *CIITA* gene activates the expression of MHC class II, which is involved in antigen processing. Therefore, *CIITA* gene is a master switch of antigen presentation in antigen-presenting cells (APCs) [14]. Professional APCs in *CIITA*-deficient mice do not express MHC class II, which leads to severe immunodeficiency and insufficiency in mature CD4+ T-cell, in the peripheral lymphatic system [15].

Sugerman, et al. reported that an early event in the formation of an OLP lesion may be the presentation of MHC class II antigen to CD4+ T cells, followed by keratinocyte apoptosis triggered by CD8+ cytotoxic T cells [5]. Transcription of the human *CIITA* gene is controlled by four alternative promoters: promoter I (pI) is responsible for constitutive *CIITA* gene expression in dendritic cells, Promoter II (pII) was not clear by now. promoter III (pIII) is responsible for constitutive *CIITA* expression in B cells, and promoter IV (pIV) becomes activated by IFN-c activation in non-professional APCs [16].

In previous research, the frequency of this SNPs was also reported to be significantly difference between patient and healthy groups in many diseases such as RA, multiple sclerosis (MS) and SLE [17]. The amino acid substitution was predicted to have a tolerable effect on protein function [18]. However, the exact functional consequences are not known.

rs4774 SNP (located in exon 11) was shown on this study as a very important polymorphism related to OLP by genotype and allele analyses.

Also, significant differences between OLP patients and healthy subjects were observed in the genotype and allele frequency of the rs6498122 SNP (located in intron 5).

The association between these two SNP and OLP was reported by one study only when We, et al. make a study on Chinese people using blood sample only [7]. The different allele frequency in this SNP might influence the regulation of the *CIITA* gene and further trigger the abnormal expression of MHC II molecules.

SNPs-genotypes combination method is used to know the effect of gene's SNPs on concentration of the gene product in clinical samples [13]. There is significant relation between these two SNPs at GG/GG,GC/AG. This study considered as the first study about the combination between these two SNPs at *CIITA* gene.

CONCLUSION

In conclusion, data suggest an association between OLP and two SNPs (rs4774 and rs6498122) in the *CIITA* gene. Buccal swab instead of blood sample in SNPs genetic research.

Further replication studies in more individuals with OLP and with different genetic backgrounds are needed to fully elucidate the role of *CIITA* in OLP.

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