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Original Article

SCL11A1 Gene Polymorphism and susceptibility to Cutaneous Leishmaniasis in Al-Najaf province-Iraq

Luma H. Ali*, Saleem Khuteer Al-Hadraawy

Department of biology, faculty of science, University of kufa, Najaf, Iraq.

Correspondence: Luma H. Ali, Department of biology, faculty of science, University of kufa, Najaf, Iraq. E mail: h_luma@yahoo.com

ABSTRACT

In many regions of Iraq, cutaneous leishmaniasis (CL) is endemic and the number of patients has been increased during the last 10 years. *SLC1A11* gene (Solute Carrier family A 11 member 1 protein), formerly called *NRAMP1* (Natural Resistance-Associated Macrophage Protein 1 gene) has an essential role in susceptibility to CL and the disease pathology. This study was aimed to investigate the polymorphisms of SLC1A11 gene and detect a relationship between gene variants and susceptibility to CL infection in Iraqi population/AL-Najaf province. The blood samples were collected from (60) patients with CL and (30) apparently healthy controls. Polymorphism of SLC1A11 575-18G/A was detected in control and patients groups by PCR-RFLP technique. The results were as follows: There was not any significant frequency (p=0.67) in the genetic variations of patients and controls; the frequency of allele G in controls was more than the patients, and the genetic variations of 575-18 G/A are not associated with susceptibility to CL infection.

Keywords: Cutaneous Leishmaniasis, *SLC1A111,* polymorphisms, 575-18G/A, Al-Najaf province, Iraq.

Introduction

Cutaneous Leishmaniasis is a vector-borne disease transmitted by biting of the fly, it is an awesome health problem in many countries and endemic in most regions of Iraq [1]. Both *L. major* and *L. tropica* are the causative agents of cutaneous Leishmaniasis in Iraq [2]. SLC1A11 gene Solute Carrier family 11 A member 1 protein, formerly called NRAMP1: Natural Resistance-Associated Macrophage Protein 1 [3] has an essential role in the disease pathology and susceptibility to CL [4]. It is localized in the phagosome membrane and is involved in the transport of divalent cations [5].

During an intracellular infection, SLC11A1 transports vital elements (Co^{2+},Fe^{2+},Mn^{2+}) for survival of the parasite, from the phagolysosome into the cytosol and so restricts their growth [6], gene expression regulation is necessary for biological processes including cell growth, development, differentiation and adaptation to environmental changes [7]. Multiple genetic polymorphisms have been identified within the human homologue SLC11A1 [8], and these genetic variations have been studied as for susceptibility to intracellular Leishmania protozoa among various areas around the world [9]. In the present study, the associations of the mentioned polymorphisms with susceptibility to CL infection were assessed.

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Material and Methods

Subjects and study design

A total of 30 apparently healthy people and 60 patients with cutaneous Leishmaniasis were included in this study during the period between August / 2018 to January/ 2019 in the out-patients clinic of the dermatology department in Al-Hakim and Al-Manathira hospitals, Al-Najaf Province in Iraq. Cases were diagnosed clinically by a special dermatologist as cutaneous Leishmaniasis and confirmed as CL patients based on clinical symptoms and parasitological parameters.

575-18G/A (rs3731864) Typing

Genomic DNA was extracted from the blood samples by using the Macrogen DNA extraction kit (Whole Blood), according to the manufacturers' instructions. Genotyping for 575-18G/A

polymorphisms was performed by PCR-RFLP with two primers as described by [8] and provided from Magrogen Company (Table 1). The total volume of PCR reaction for polymorphisms was 20 µl, including (1X) Pre master mix (Bioneer, Korea), 1 µL of each primer (10 pm/µl), 5 µl of genomic DNA (ng/µL) and the volumes were completed with nuclease-free water. Thermocycler condition was as follows: 95 °C for 5 minutes then 35 cycles at 95 °C for 30 seconds, 58 °C for 30 seconds, and 72 °C for 30 seconds followed by 5-minute extension at 72 °C. Then the products of PCR were analyzed by (1%) agarose gel electrophoresis, which was stained with (10 mg/ml) ethidium bromide. The PCR products were digested by using restriction enzyme *mspl*. Then RFLP-PCR was done according to the instructions of the company (Biolabs/U.K). After that, RFLP-PCR product was analyzed by (2.5 %) agarose gel electrophoresis.

Table 1: The sequence of primers, restriction enzyme, and DNA Fragments size

Name	Primer sequence	Restriction Enzyme	Genotype	Products
575-18G/A	F:5'-AGGAGGCCAGATTCCTGTCT-3 R:5'-TTCGATGTCAGAGCCCTTCT-3	*mspl*	GG	166,74bp
			G/A	240,166 and 74bp
			AA	240 and 74bp

Statistical analysis

The result was analyzed by using the statistical software package SPSS 23. The frequency of allele and genotyping of 575-18 G/A were performed for each polymorphism among the patient and control group by using χ2-test and odds ratio with (95%) confidence [10]. The probability of (P≤ 0.05) was considered statistically significant.

Results

In total, 60 CL cases and 30 controls were genotyped; equilibrium of Hardy-Weinberg was used in both cases and controls. Figure (1) and Table (2) shows the distribution of 575-18G/A alleles and genotypes among cases and controls, detected by PCR-RFLP technique. There were three genotypes at this locus; GG, G/A, and AA with band sizes of 166/74 bp, 240/166/74 bp, and 240/74 bp respectively. There was not any significant difference in genotype distribution, recorded between CL cases and controls (p=0.674). Also, there was a significant difference in G and A allele between patients and controls. However, data in the present study shows a high frequency of allele G in the CL patients more than controls (34.2% and 28.4% respectively).

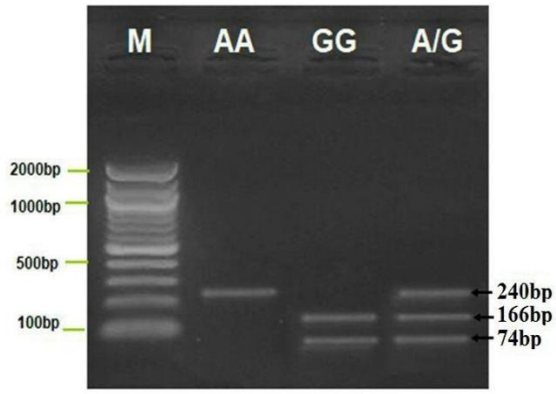

Figure 1: Agarose gel electrophoresis result that shows the RFLP-PCR product analysis of *SCL1A11* (575-18 G/A) gene by using the *MSPI* restriction enzyme. lane (GG) homozygote product digested by the restriction enzyme at 166 bp and invisible 74 bp bands. Lane (G/A) heterozygote, the product digested by the restriction enzyme into 240 bp, 166 bp, and invisible 74 bp bands. Lane (AA) homozygote, the product digested by the restriction enzyme into 240 bp and invisible 74 bp bands, M: DNA ladder (1000-50bp).

Table 2: Distribution of SCL1A11 (575-18 G/A) genotypes and alleles.

Variable		Patients (n=60)	Controls (n=30)	*P value*	Odd ratio	95% Confidence Interval	
		No. (%)	No. (%)			Lower	Upper
Genotype	A/G	19(31.1)	9(30)	0.87	0.76	0.31	1.8
	AA	29(48.3)	17(56.6)	0.45	1.08	0.41	2.8
	GG	12(20.6)	4(13.4)	0.13	1.45	0.42	5.03
Overall		X2=0.790 (NS) P value =0.674					
Allele frequency							
A		79(65.8)	43(71.6)	0.42	0.76	0.38	1.49
G		41(34.2)	17(28.4)	0.42	1.31	0.66	2.58
Overall		X2=0.623 (NS) P value =0.42					

X2 represent chi-square value
NS : non-significant differences (P >0.05).

Discussion

Genetic factors are crucial in resistance or susceptibility of the host to infectious diseases. Variations within the *SCL1A11* gene were evaluated in this study with respect to cutaneous Leishmaniasis in Al-Najaf province of Iraq, which has not been reported before from this region.

In the current study which showed the effects of (575-18 G/A) polymorphisms on susceptibility to cutaneous leishmaniasis, there was not any significant difference in frequency of alleles and genotypes 575-18 G/A (RS3731864) between the controls and patients. The results showed that there is no relationship between the genetic variations of RS3731864 and susceptibility to cutaneous leishmaniasis infection, and the allele G frequency in patients was more than controls: 34.2% and 28.4% respectively. The current findings were in agreement with [11] in

Pakistan, who showed that there was not any association between cutaneous leishmaniasis infection and rs3731864 polymorphisms, where allele A was higher in patients with INS (OR= 2.447, p = 0.127). The current data finding was also consistent with [1] in Al-Muthana province/Iraq who showed that there is no significant difference of alleles between cutaneous Leishmaniasis patients and controls. The present study refers to no association between cutaneous Leishmaniasis and 575-18 G/A polymorphisms, and to that the allele G frequency in patients was more than controls, in another hand [12] in Iran found an association between CL infection and the G/A genotype.

However, other factors should be taken into account. For example, infecting *Leishmania* species are not the same in all cases. There is a high percentage of *Leishmania* responsible for most infections in the areas of Iraq, and also different vector and animal host species found in the areas of this country [13]. Moreover, the host genetic factors play an important role in the host susceptibility, and presumably more SNPs other than 575-18 G/A were included the inefficiency of SCL1A11.

Conclusion

The results of the present study indicated that the genetic variations in *SCL1A11* (575-18G/A) gene do not associate with susceptibility to CL infection in the sample population from Iraq.

Conflict of interest

There was no conflict of interest in this study.

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