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Short Communication

Single-nucleotide polymorphism of interleukin-10 promoter (*IL-10* −819C/T) in leprosy patients with and without erythema nodosum leprosum, and healthy household contacts

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Abstract

Leprosy, caused by *Mycobacterium leprae,* is a chronic infectious disease that impacts the skin and peripheral nerves, causing long-term disability. The invasion of *M. leprae* into the body triggers immunologic responses and single single-nucleotide polymorphisms in cytokine-encoding genes may influence predisposition and susceptibility, possibly predicting the incidence of leprosy reactions. The aim of this study was to assess the gene polymorphism of interleukin-10 promoter *IL-10* −819C/T in leprosy patients, leprosy patients with erythema nodosum leprosum (ENL) reaction, and household contacts. A total of 54 individuals were included, with 18 in each group. Skin smear and histopathologic examinations were used to confirm the diagnosis of leprosy and ENL. The polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) technique was used to determine the polymorphism. The results confirmed the presence of polymorphism of which all TT, CT, and CC genotypes presented. The TT genotype was most prevalent in household contacts (94.4%) followed by ENL (50%), and leprosy patients (44.4%). The CT genotype was most frequently detected in leprosy patients (50%), followed by ENL cases (44.4%), and household contacts (5.56%). In contrast, CC was mostly presented in ENL cases (5.56%), only 1% in leprosy patients, and absent among household contacts. Although the most prevalent allele in all three groups was the T allele, the C allele presented in 27% and 30% of ENL and leprosy patients, respectively and only 5% in household contact individuals. This study suggests that the polymorphism variations of *IL-10* −819C/T are higher in leprosy and ENL patients compared to household contacts. Since this data is preliminary, larger studies are needed.

Keywords: Polymorphism, *IL-10* −819C/T, leprosy, ENL, rs1800871

Introduction

Leprosy is a persistent infection that affects the skin and peripheral nerve system caused by *Mycobacterium leprae* and is associated with significant disability and disfigurement [1-5]. As of 2021, leprosy continues to be a significant health problem in Indonesia with a prevalence rate of 0.78 per 10,000 population [6]. The high incidence and prevalence of multibacillary leprosy in in Indonesian in particular in South Sulawesi province have significant epidemiological consequences on public health, as these patients continuously spread the infection [4,7,8].

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Based on the immunological system, leprosy is characterized as a clinicopathological spectrum that includes tuberculoid leprosy (TT), borderline tuberculoid (BT), borderline (BB), borderline lepromatous (BL), and lepromatous leprosy (LL) [3,9-11]. The World Health Organization (WHO) classified leprosy into paucibacillary (PB) and multibacillary (MB) for treatment purposes [12]. The clinical manifestations of spectrum leprosy are highly dependent on how the individual immune system responds to the presence of *M. leprae* [4,11], when cellular immune response against *M. leprae* decreases, clinical manifestation occurs and the infection can develop over time. During the chronic progression of leprosy, patients may experience leprosy reactions, which are acute episodes of immune-mediated inflammatory episodes [8,13]. Leprosy reactions can be classified into two types: type 1 reaction or reversal reaction occurring frequently in unstable borderline leprosy patients (BT, BB, BL) and LL patients; and type 2 reaction or erythema nodosum leprosum (ENL) occurring typically in BL and LL patients [8,14]. These reactions could exacerbate the clinical condition and lead to permanent nerve damage and disability, of which ENL causes nerve damage more rapidly compared to type 1 reaction [4].

Cellular mediated immunity (CMI) is an essential component of individual resistance to *M. leprae* which is considered a strong CMI regulation through the balance of type 1 cytokines including interleukin (IL-2), interferon-gamma (IFNγ), tumor necrosis factor-alpha (TNF-α), and IL-12 and type 2 cytokines such as IL-4, IL-6 and IL-10 [15]. Different variations in cytokine genes are associated with risk or protection against leprosy [16-18]. One cytokine that has a crucial role in the pathogenesis of leprosy is IL-10 [19]. Elevated production of IL-10 might affect the decreased activity of macrophages in the eradication of bacteria. The genes that are responsible for encoding a cytokine that regulates IL-10 production [20] and the involvement of genes related to anti-inflammatory cytokine, significantly contribute to nerve damage in leprosy [7].

Some studies have investigated the variations in cytokine genes among leprosy patients, which might affect propensity, susceptibility, and anticipation of leprosy reaction occurrence [18,20-22]. The polymorphism of *IL-10* gene at position −819 C/T (rs1800871) within the promoter region is associated with leprosy susceptibility [16,18]. Other studies found that polymorphisms of *IL-10* promoter at positions −819 C/T (rs1800871) as well as at positions −1082 G/A and −592 C/A were associated with resistance against leprosy in Brazilian, Indian, and Columbian populations [17,20,23,24]. According to a meta-analysis study, polymorphism of *IL-10* −819C/T is associated with leprosy susceptibility in Brazilians; and −819T allele is associated with leprosy susceptibility in the Asians group [17]. These findings suggested that *IL-10* variations could control the immune responses and potentially the course of leprosy [17].

Studies on polymorphism *IL-10* −819C/T as a risk factor for developing leprosy ENL reaction and becoming symptomatic in those having contact or household contacts are still limited. The aim of this study was to assess the distribution of genotypes and alleles of *IL-10* −819C/T in leprosy with ENL, leprosy patients without ENL and among those who had household contact with leprosy cases in South Sulawesi, Indonesia.

Methods

Study setting

A cross-sectional study was conducted of which 54 individuals were included consisting of 18 leprosy cases without ENL, 18 leprosy with ENL, and 18 household contacts with leprosy patients. All leprosy individuals with and without ENL were selected from those who visited the Dermatology Service Hospital in South Sulawesi, Indonesia, within the period of six months (January and June) in 2019. The blood samples were collected to assess the distribution of genotypes and alleles of *IL-10* −819C/T polymorphism.

Sample and inclusion criteria

The leprosy individuals without ENL included in the multi-drug-treatment (MDT) multibacillary type according to WHO criteria such as more than five anesthesia skin lesions and more than two peripheral nerves involved. The leprosy patients with ENL were included in the MDT multibacillary type according to WHO with tenderness erythema nodules. All of them were

confirmed with an examination of skin smear and histopathology. Household contacts included healthy adult individuals (more than 16 years old) who have been living with individuals with leprosy with or without ENL for more than five years.

Blood collection

A total of 2 ml of venous blood samples was collected from each individual for DNA extraction, and then tested for the 819 *IL-10* gene using the polymerase chain reaction (PCR) method followed by a PCR-restriction fragment length polymorphism (PCR-RFLP) method.

Polymerase chain reaction (PCR)

Amplification of the *IL-10* gene promoter fragment using PCR was conducted using primers: sense *IL-10 –819* NF; 5' GTT ATT TCA ACT TCT TCC ACC C 3' and antisense *IL-10 –819* R; 5' TTT ATA GTG AGC AAA CTG AGG CAC AGA CAT 3'. The product was 244 base pair DNA fragments of the amplicon. The process of PCR used PCR Thermal Cycler ABI 9700 (ABI System, USA), with a volume of 50 µL containing Tris-HCl 20 mM pH 8.4, KCl 50 mM, MgCl 1.5 mM, dNTP 200 M, sense and antisense primers each 400 nM, 1-unit Taq platinum polymerase DNA (Invitrogen, USA) and 5 µL of DNA extraction. The reaction was run at a denaturation temperature of 94°C for 30 s, an annealing temperature of 53°C for 30 s, and an elongation temperature of 72°C for 1 min, for 35 cycles. The amplicons of the PCR results were run through the electrophoresis agarose and visualized using GelDoc (Biorad, USA).

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)

To determine a change of the *IL-10* –819C/T, 10 µL of result of the 244 bp PCR amplification was digested using 5 units of the NlaIII restriction enzyme (ThermoFisher Scientific, Singapore) and incubated at 37°C overnight. These results were then electrophoresed on 2% agarose gel. If the DNA band was not cut or the size of the DNA was 244 bp, the sample has a T base at position *IL-10* –819. However, if the DNA band was digested, two DNA fragments were produced (217 bp and 27 bp) and the sample has C base. The sample that has three sizes of DNA bands (244, 217, and 27 bp) indicated heterozygous mutations. Schematically, it is presented in **Figure 1**.

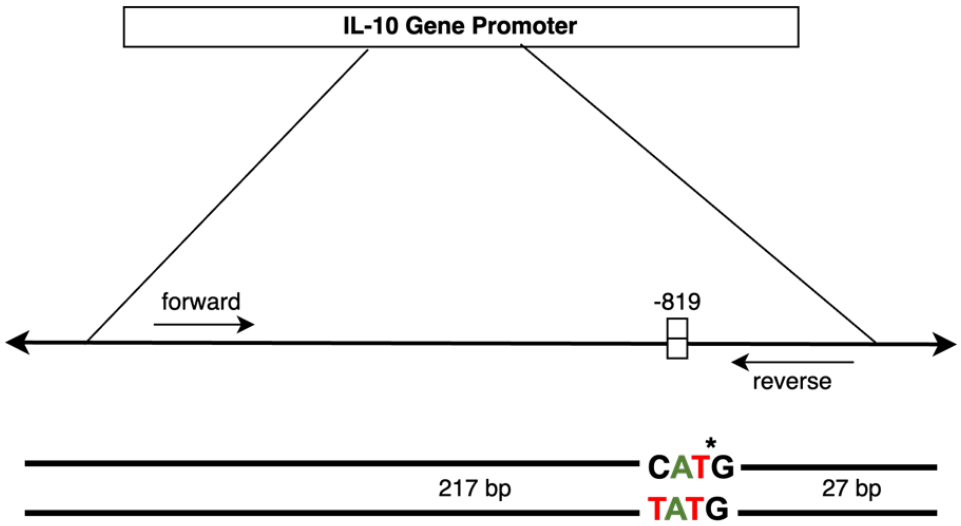

Figure 1. Schematic of *IL-10* –819C/T gene polymorphism.

Results

Patients' characteristics

The characteristics of the individuals included in this study are presented in **Table 1**. The majority of the leprosy patients (with and without ENL) were within productive ages and most were between 21–30 years old. Males and females had similar proportions (**Table 1**).

Table 1. Characteristics of the leprosy patients with and without erythema nodosum leprosum (ENL) and household contact individuals

Characteristic	Leprosy with ENL		Leprosy without ENL		Household contacts	
	Frequency	%	Frequency	%	Frequency	%
Age (year)						
≤ 20	0	0.0	1	5.5	5	27.7
21–30	6	33.3	8	44.4	2	11.1
31–40	5	27.7	2	11.1	4	22.2
41–50	5	27.7	5	27.7	4	22.2
50–60	2	11.1	2	11.1	3	16.6
Gender						
Men	9	50.0	10	55.5	10	55.5
Women	9	50.0	8	44.4	8	44.4
Duration of leprosy (month)						
<6	6	18.0	10	55.5	0	0.0
6–12	7	33.3	5	27.7	0	0.0
12–24	5	27.7	3	16.6	0	0.0

Distribution of genotypes and alleles of *IL-10* –819C/T polymorphism

Our data confirmed the presence of polymorphism of *IL-10* –819C/T in all groups of which all genotypes of TT, CT, and CC presented in the leprosy group with and without ENL. The TT genotype was (50%) in ENL, 44.4% in leprosy, and 94.4% in household contacts. The CT genotype (heterozygote) was 44.4% in ENL patients, 50% in leprosy, and 5.5% in household contacts. The wild-type genotype (CC) was 5.56% in ENL, 1% in leprosy patients, and 0% in household contact. The genotype and allele distributions of *IL-10* –819C/T are presented in **Table 2** and **Figure 2**.

Table 2. Distribution of genotypes and alleles of *IL-10* –819C/T among leprosy patients with and without erythema nodosum leprosum (ENL) and household contact individuals

	Variations	Leprosy with ENL		Leprosy		Household contacts	
		n	%	n	%	n	%
Genotype	TT	9	50.00	8	44.44	17	94.44
	CT	8	44.44	9	50.00	1	5.56
	CC	1	5.56	1	5.56	0	0
Allele	T	26	72.22	25	69.44	36	94.74
	C	10	27.78	11	30.56	2	5.26

The percentage of genotypes and alleles showed that the T allele was higher than the C allele in all groups, either ENL (72.2%), leprosy (69.4%), or in household contacts (94.7%) (**Figure 2**).

Discussion

The *IL-10* gene in humans is positioned on chromosomes 1q31-q32 and encodes for five exons. The *IL-10* gene promoter region contains three single nucleotide polymorphisms: G/A at –1082, C/T at –819, and C/T at –592. Studies assessing the role of *IL-10* –819 gene polymorphisms and leprosy susceptibility have been conducted in Brazilian [25,26], Indian [27], Mexican [22], Colombian [23], and Chinese populations [5], but research focusing in household contacts are still limited. A study in Indonesia, which assessed the association *IL-10* gene promoter polymorphism with leprosy susceptibility in South Sumatra, reported no association between *IL-10* –819C/T and leprosy susceptibility [28]. A study found that *IL-10* –819C/T allele C was more predominant in all types of leprosy than in controls [25]. In this study, different results were found of which T allele was more dominant in household contacts. A previous study that found association between *IL-10* –819C/T and susceptibility to leprosy or with disease development [29] and TT was prevalent in leprosy patients as compared to healthy controls [29].

The role of *IL-10* –819C/T on leprosy are conflicting. According to a meta-analysis study showed that the 819T allele was related to leprosy susceptibility [29]. A study that included the Mexican population showed that *IL-10* –819C/T polymorphism was not associated with LL patients [22]. Another study conducted in Columbian population found that the CC and CT genotypes were associated with leprosy [23]. In this present study we found a high percentage of TT genotype in leprosy with ENL and without ENL than CT or wild type genotype (CC). We also found that the highest frequency of –819TT was observed in healthy close contacts.

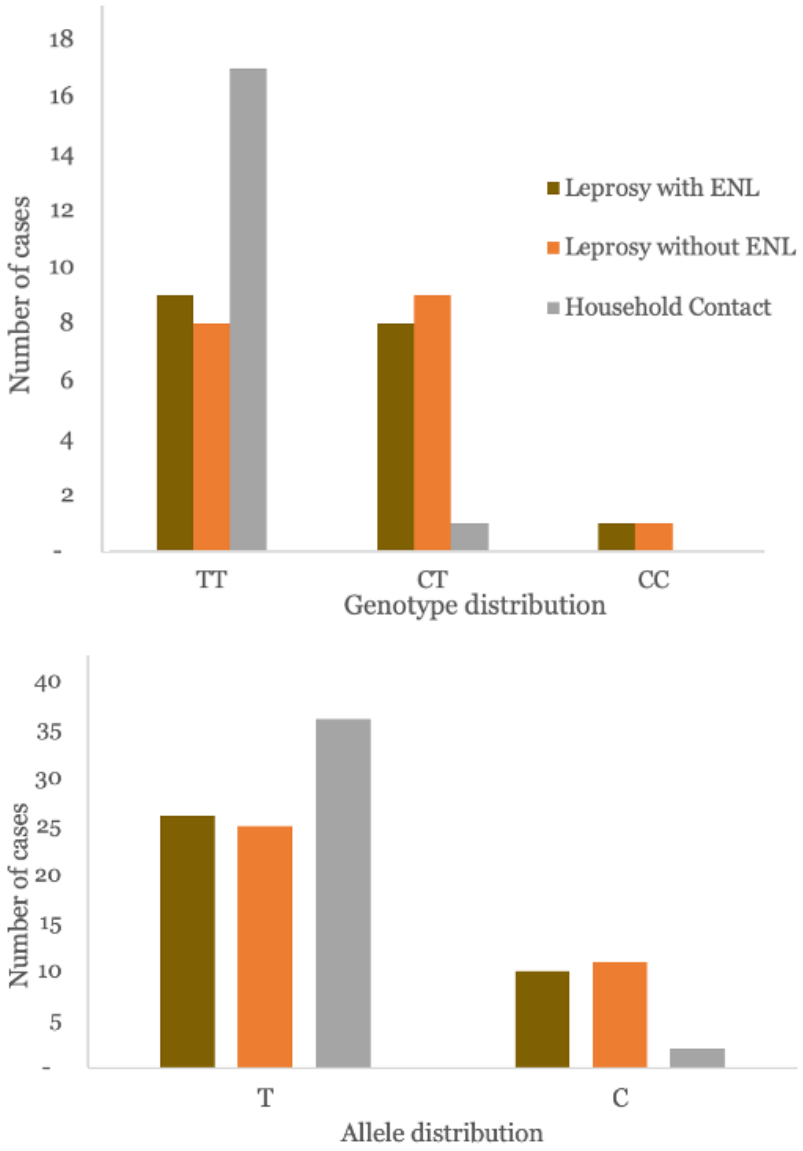

Figure 2. Distribution of genotypes and allele of *IL-10* -819C/T polymorphism among leprosy patients with and without erythema nodosum leprosum and household contacts.

IL-10 is a vital cytokine and plays a role in the early and late stages of *M. leprae* immunity. However, its role may be different at each stage [29]. A study assessing the expression of IFNγ, IL-10, toll-like receptor (TLR) 1 and 2, and their potential effects on downgrading leprosy reaction, found an increase of IL-10 in this reaction which may be explained by its ability to stimulate the proliferation and differentiation of B cells, which then secrete immunoglobulins in their membranes and subsequently, activate components in the formation of the immune complexes [30]. In terms of the relationship between *IL-10* gene polymorphisms and *IL-10* production, individuals with C genotype produce higher *IL-10* levels compared to other genotypes [16,20,30,31].

According to the concept that polymorphisms are changes or mutations in genes that do not induce alterations in protein structure but merely generate differences in protein function, polymorphisms can arise in household contacts without clinical symptoms. Therefore, polymorphisms do not manifest clinically but can determine the risk and susceptibility to disease, including leprosy [33,33]. The other risk factors of leprosy include race, age, bacterial load, and length of exposure, all of which may cause individuals to present the clinical sympthoms. To discuss, extensive research with a sufficient number of subjects is required.

Conclusion

Our results suggest that the *IL-10* -819 gene polymorphism can be present in individuals with leprosy, those experiencing reaction leprosy type 2 (ENL), and household contacts. TT genotype and T allele are more prevalent in household contact individuals when compared to leprosy and ENL patients. However, further investigation is necessary to understand the potential association of this polymorphism with the incidence or prediction of leprosy or ENL.

Ethics approval

This study was conducted after obtaining approval from the Ethics Committee of the Faculty of Medicine, Universitas Hasanuddin, Makassar, with registration number UH 12100225.

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Competing interests

The authors declare that there is no conflict of interest

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Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

How to cite

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