• Clinical Research •

Association of the macrophage migration inhibitory factor promoter polymorphisms with benign lymphoepithelial lesion of lacrimal gland

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Abstract

• AIM: To identify the association of the macrophage migration inhibitory factor (MIF) gene polymorphism with the susceptibility of benign lymphoepithelial lesions (BLEL) of the lacrimal gland.

• METHODS: A total of 40 BLEL of lacrimal gland cases were matched with 40 healthy subjects (HS). Extraction the plasma and whole blood DNA of patients of lacrimal gland BLEL and HS. Elisa and polymerase chain reaction was used to determine in plasma contents of MIF and MIF gene SNP-173G>C and STR -794 $CATT_{(5-8)}$ polymorphism, respectively.

• RESULTS: The MIF levels in plasma were significantly higher in patients with lacrimal gland BLEL versus HS (*P*<0.001). The -173 G>C MIF polymorphism was significantly associated with lacrimal gland BLEL, with a significantly higher frequency of the C allele in lacrimal gland BLEL patients compared with HS (OR=2.38, 95% CI=1.07-5.31, *P*=0.032), and the -173 C/x is more frequent in patients than in HS, *P*=0.037. Besides, we found that the carriage rate of the MIF -173C/x is associated with higher plasma levels of MIF in the BLEL of lacrimal gland.

• CONCLUSION: MIF -173G/C variants play an insidious role in susceptibility of BLEL of lacrimal gland. Otherwise, there is no statistically significant correlation exists between MIF-794 $CATT_{(5-8)}$ and BLEL of lacrimal gland.

• **KEYWORDS:** benign lymphoepithelial lesion; lacrimal gland; macrophage migration inhibitory factor; gene polymorphism
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INTRODUCTION

Benign lymphoepithelial lesion (BLEL)[1], also referred to as Mikulicz disease[2], is a relatively rare disease, with the major clinical manifestations being symmetrical and painless enlargement of the bilateral lacrimal glands and/or the salivary glands[3-4]. The cause and pathogenesis of BLEL remain unclear. Clinically, BLEL can be treated with glucocorticoid therapy, but glucocorticoid resistance is a frequent occurrence[5].

Macrophage migration inhibitory factor (MIF) is a pro-inflammatory cytokine first identified in 1966 during studies of the delayed-type hypersensitivity reaction and characterized as a soluble product of activated T lymphocytes that inhibits macrophage migration *in vitro*[6-7]. MIF has been shown to act as a critical mediator of host defence with a role in septic shock and chronic inflammatory and autoimmune diseases[8-9]. Furtherly, MIF has the unique ability to override the inhibitory effects of glucocorticoid on the immune system[10-11].

MIF gene is located in chromosome 22 (22q11.2) and contains two clinically relevant polymorphisms within the promoter region that have been associated with susceptibility to several diseases[12-16]. A short tandem repeat (STR) polymorphism is located at locus -794 (rs5844572), which is a microsatellite repetition of cytosine-adenine-thymine-thymine (CATT), and the repeat length (5 to 8 repetitions) which correlates with increased gene expression and with circulating MIF levels[12]. Likewise, the single nucleotide polymorphism (SNP) -173 G>C MIF (rs755622) has been found at location -173 of the MIF gene with a change from guanine (G) to cytosine (C). Similar to the functions of STR polymorphism above, this -173*C

allele is also reported to associated with mRNA expression and circulating MIF levels[17]. More fundamentally, some studies indicated that MIF-173G/C gene polymorphism may increase the risk of glucocorticoid resistance in a series of diseases including juvenile arthritis, nephrotic syndrome and colitis[18-20].

In this study, we evaluated the association of the -794 $CATT_{(5-8)}$ and -173G>C MIF polymorphisms with glucocorticoid susceptibility to lacrimal gland BLEL.

SUBJECTS AND METHODS

Participants or Samples A total of 40 patients with lacrimal gland BLEL were registered and treated at the Beijing Tongren Hospital between September 2013 and April 2016. The study population comprised 11 males and 29 females, the ages ranged from 23 to 63y with a median age of 47y. The enlargement of the lacrimal in patients with BLEL was found to be uncongested and symmetric or unilateral as well as asymptomatic and nontender to palpation. All BLEL diagnosis was confirmed by post-surgical histological examinations. Forty subjects with healthy subjects (HS) were recruited as a control group comprised of 20 male and 20 female subjects, aged between 29 and 64y.

Peripheral blood samples of 40 patients with lacrimal gland BLEL and 40 HS from Beijing Tongren Hospital and University Hospital of Beijing University of Technology are prepared for this trial. The study protocol was approved by the Ethics Committee of Beijing University of Technology and adhered to the tenets of the Declaration of Helsinki. Written informed consent was obtained from all participants before their enrollment (Table 1).

Pretreatments Whole blood was centrifuged at 2000 rpm for 10min; the upper layer was then carefully removed into a clean tube and stored at -20℃ ; whole blood DNA was at last extracted from the left blood cells by using Genomic DNA Extraction from blood system (TIANGEN® China).

Genotyping of the SNP-173G>C and STR -794 $CATT_{(5-8)}$ Polymorphism To analyze the SNP-173G>C MIF polymorphism, we amplified polymorphic fragments by conventional polymerase chain reaction (PCR). Amplification of a 497 bp fragment was completed using the primers as follows: forward primer 5'-CCCCGC CCC ATC TCA AAC ACA-3' and reverse primer 5'-CCGCCG CTG AGC TAC GTG CC-3'. Cycling conditions were as follows: initial denaturing at 94℃ for 5min followed by 30 cycles of 30s at 94℃ , 30s at 58℃ , and 30s at 72℃ and then a final extension of 5min at 72℃ .

The -794 $CATT_{(5-8)}$ MIF polymorphism was analyzed by conventional polymerase chain reaction (PCR) and amplification of a 346 bp fragment was completed using the primers as follows: forward primer 5'-TGCAGGAACCAATACCCATAG G-3' and reverse primer 5'-AATGGTAAACTCGGGGAC-3'. Of 30 cycles and an annealing temperature of 58℃ were used.

Table 1 Clinical characteristics in the study population

Groups	Age (a)	Gender		Affected eyes		
		M	F	L	R	Bilateral
BLEL (*n*=40)	47 (23-63)[a]	11	29	6	10	24
HS (*n*=40)	49.2 (29-64)[a]	20	20			

[a]Minimum-maximum.

Amplification products were sequenced by Sangon Biotech (Shanghai, China), and the sequential peaks showed genotyping results of the SNP-173G>C MIF and STR -794 $CATT_{(5-8)}$ MIF polymorphism.

Enzyme-linked Immunosorbent Assay for Macrophage Migration Inhibitory Factor The determination of MIF plasma levels was performed by commercial ELISA Kits (RayBio® USA) according to manufacturer's instructions. The sensitivity of MIF detection was 6 pg/mL.

Statistical Analysis Data analysis was performed using IBM SPSS Statistics ver.20, GraphPad Prism6 software and Revman 5.3. Student's *t*-test for parametric variables (data presented as mean±SD), and Mann-Whitney *U* test for nonparametric variables (data presented as median and 5^{th} to 95^{th} percentiles). Genotype and allele distribution in the study groups was determined by direct counting and was expressed as frequencies with standard errors (SE), and their association with the disease was studied using odds ratios (OR) and 95% confidence intervals (95%CI). The genotype and allele frequencies were calculated by the Chi-square test. $P<0.05$ was considered statistically significant.

RESULTS

We analyzed the association of the SNP-173 G>C MIF and STR -794 $CATT_{(5-8)}$ MIF polymorphism with the susceptibility to BLEL of lacrimal gland. The -173 G>C MIF polymorphism was significantly associated with lacrimal gland BLEL, with a significantly higher frequency of the C allele in lacrimal gland BLEL patients (22/80; 27.50%) compared with HS (11/80; 13.75%) (OR=2.38, 95%CI=1.07-5.31, $P=0.032$). Furthermore, we found that the G/G genotype was more frequent in HS (30/40, 75.00%) than in patients (21/40, 50.25%); thus, the C/x was more frequent in patients (47.50%) than in HS (25.00%) ($P=0.037$). Otherwise, there was no statistically significant correlation existed between MIF-$CATT_{(5-8)}$ and the morbidity risk rate of lacrimal gland BLEL (Table 2).

The MIF level in plasma was significantly higher in patients of lacrimal gland BLEL (mean 11.07 ng/mL, range 2.01-33.41 ng/mL) versus HS (mean 1.71 ng/mL, range 0.98-2.71 ng/mL) ($P<0.001$; Figure 1). As shown in Figure 2, a total of 40 patients with lacrimal gland BLEL were genotyped for the -173 polymorphism of the MIF gene and evaluated for MIF levels in plasma. We found that patients carrying the MIF-173*C allele had higher MIF levels of serological MIF, which were significantly higher than those of patients with the GG genotype ($P=0.0041$). Although the plasma level of

Table 2 Distribution of genotypes at -794 and -173 loci of MIF gene in patients and HS

Parameters	Patients, *n*=40 (%)	HS, *n*=40 (%)	OR (95%CI)	*P*
SNP-173				
Genotype				0.002
G/G	21	30	-	-
G/C	16	9	2.07 (0.78-5.51)	NS
C/C	3	1	3.16 (0.31-31.78)	NS
Allele				
G	58	69	-	-
C	22	11	2.38 (1.07-5.31)	0.032
Do				
GG	21	30	-	-
CC+GC	19	10	2.71 (1.05-7.00)	0.037
STR-794				
Genotype				0.900
5/5	3	4	0.73 (015-3.49)	NS
5/6	20	22	0.82 (0.34-1.97)	NS
5/7	2	2	1.00 (0.13-7.47)	NS
6/6	11	8	-	-
6/7	3	4	0.73 (0.15-3.49)	NS
7/7	1	0	3.08 (0.12-77.80)	NS
Allele				0.691
5	28	32	0.81 (0.43-1.53)	NS
6	45	42	-	-
7	7	6	1.18 (0.38-3.69)	NS
Do				1.000
5/5+5/6+6/6	34	34	-	-
7/x	6	6	1.00 (0.31-3.24)	NS

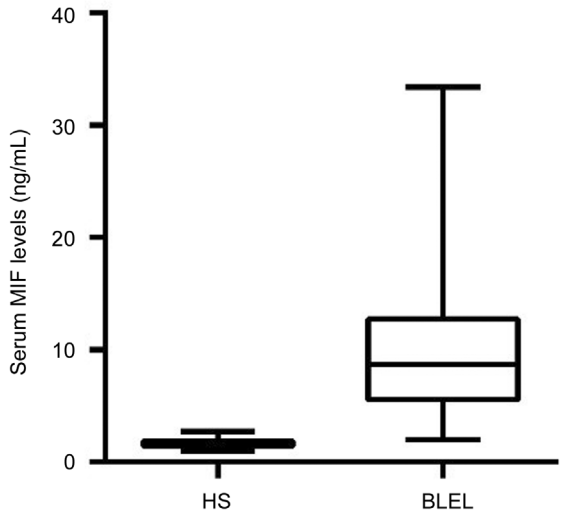

Figure 1 Plasma levels of macrophage MIF in healthy subjects and lacrimal gland BLEL Note that the lacrimal gland BLEL subjects had a significantly increase in MIF concentration of plasma when compared with healthy subjects. Comparison among groups was performed using Mann-Whitney *U* test; *P*<0.001.

MIF in patients with MIF-794 $CATT_{(7/x)}$ was elevated, but no significant difference was observed.

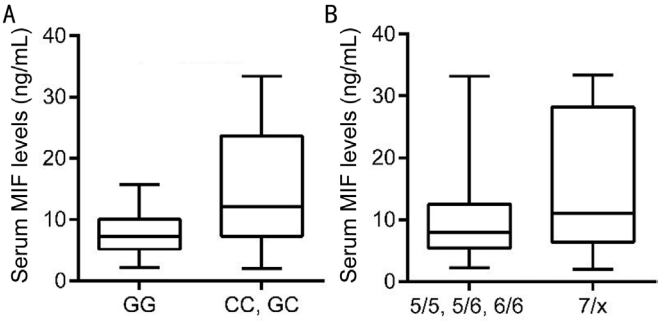

Figure 2 Plasma levels of macrophage MIF in patients with lacrimal gland BLEL A: According to the carriage of only the MIF-173*G allele (GG) or to the carriage of the MIF-173*C allele (GC or CC), *P*=0.0041; B:According to the allele frequencies of MIF-794 $CATT_{(5/5, 5/6, 6/6)}$ or to the allele frequencies of MIF-794 $CATT_{(7/x)}$, *P*>0.05. Comparison among groups was performed using Mann-Whitney *U* test.

DISCUSSION

Lacrimal glands BLEL is characterized by unilateral or symmetric bilateral swelling of the lacrimal glands, the etiology and pathogenesis of which, remain ill defined, is relatively less studied over the last decade.

In this study, we investigated the association of -173G>C MIF and -794 $CATT_{(5-8)}$ MIF polymorphism with the risk of BLEL of lacrimal gland in Beijing population. The patients with BLEL in this study were mainly middle-aged females with a male-to-female ratio of 1:3. The median age was 47y (range 23-63y). The enlargement of the lacrimal in patients with BLEL was found to be uncongested as well as asymptomatic and nontender to palpation. We found that -173 G to C mutations located in promoter region might be a potential risk factor. However, we did not find a significant association between -794 $CATT_{(5-8)}$ MIF polymorphism with the risk of BLEL of lacrimal gland.

MIF-173 G to C mutations are increasingly recognized causes of immune-system disorders, including acute myeloid leukemia, erythema nodosum and psoriatic arthritis[21-23]. We found the similar situation in BLEL of lacrimal gland. Besides, we found that the carriage rate of the MIF -173C/x was related to higher plasma levels of MIF in the BLEL of lacrimal gland. There were already massive evidences that elevated MIF overcomes the inhibitory effects of glucocorticoids on TNF-alpha, IL-6 and IL-8 production, restores IL-2 and IFN-gamma production, and antagonizes the glucocorticoid inhibition of the production of several enzymes and cell surface molecules[24]. However, glucocorticoid therapy is the main method of drug treatment in the BLEL of lacrimal gland. In line with this, higher carried allele MIF -173C and higher plasma levels of MIF in patients with BLEL of lacrimal gland were consistent with poorer response to glucocorticoid treatment, with a higher risk of local recurrence. Thereby, the detection of MIF -173G/C polymorphism could be a good index that can determine the

curative effect of glucocorticoid therapy in BLEL of lacrimal gland.

In summary, we investigated for the first time the association between the functional MIF polymorphisms and BLEL of lacrimal gland. Our results suggested that MIF-173 G to C mutations played an insidious role in susceptibility of BLEL of lacrimal gland, and plasma MIF expression. Further studies are still needed to deeply reveal the mechanism of its mighty function.

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