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Association of cytokine gene polymorphisms with susceptibility to invasive candidiasis

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ABSTRACT. The aim of this study was to investigate the role of cytokine genes in the susceptibility to *Candida* infection. A total of 275 consecutive patients diagnosed with *Candida* infection were selected between May 2010 and May 2011, along with 305 uninfected controls. Genotyping of the IL-1β gene polymorphisms (*IL1β*) rs1143634, *IL1β* rs16944, *IL8* rs4073, *IL10* rs1800872, and *IL10* rs1800896 was carried out using a 384-well plate format on the Sequenom MassARRAY platform. Patients with invasive *Candida* infections were more likely to have had an immunocompromised state, hematopoietic stem cell transplantation, solid organ transplant, solid tumor, chemotherapy within the past three months, neutropenia, surgery within the past 30 days, acute renal failure, liver failure, and/or median baseline serum creatinine. Conditional logistic regression analyses found that individuals with the rs1800896 GG genotype were associated with a higher risk of invasive *Candida* infections than those carrying the AA genotype (odds ratio = 0.61, 95% confidence interval = 0.37-0.94). From the results of this case-control study, we suggest that the cytokine

IL-10 gene rs1800896 polymorphism might play a role in the etiology of invasive *Candida* infections.

Key words: Cytokine gene; IL-1β; IL-8; IL-10; Polymorphism; Invasive *Candida* infection

INTRODUCTION

Invasive candidiasis is a pervasive nosocomial infection. Patients with invasive candidiasis might have complications such as endocarditis, abscesses, or chronic-disseminated candidiasis. Previous studies have reported that persistent fungemia is one of the complications found in patients with candidemia, occurring in over 10% of the patients (Da Matta et al., 2010). Some studies have shown that the immune response can influence the infection, comorbidities, and efficacy of pharmacologic therapy of patients with invasive candidiasis (Garey et al., 2006).

A previous study has further found that both the innate and adaptive immune mechanisms play an important role in the host defense against *Candida* species (Romani, 2004), and that the immune system can defend against fungal pathogens through the phagocytosis and killing of invading pathogens and through the activation of adaptive immunity by antigen presentation and secretion of proinflammatory cytokines (Netea et al., 2008). It has been reported that adaptive fungal immunity stimulates host responses to pathogens through protective cellular T-helper 1 (Th1) cytokines such as interferon-c (IFNc), as well as through humoral Th2 responses that might exert maladaptive anti-inflammatory effects by the release of interleukin (IL)-4 and IL-10. One study found that polymorphisms in *IL10* and *IL12β* that result in low production of proinflammatory cytokines were associated with persistent fungemia in patients with candidemia (Johnson et al., 2012). However, few studies have investigated the role of cytokine gene variants in the susceptibility to *Candida* infection. Therefore, we aimed to investigate the role of cytokine genes in the susceptibility to *Candida* infection in patients with candidemia.

MATERIAL AND METHODS

Patients, treatments, and clinical variables

A total of 275 consecutive patients diagnosed with *Candida* infection were selected from the Weihai Municipal Hospital between May 2010 and May 2011. All patients were identified by more than one positive blood cultures for *Candida* species in the clinical microbiology laboratory. Blood samples were obtained from all patients. The controls consisted of 305 subjects who were randomly selected from individuals who came to the Health Check Center; they were not infected with invasive candidiasis and did not carry any other invasive fungal infection. Written informed consent was provided by all subjects. Our study was approved by the Ethics Committee of the Weihai Municipal Hospital.

The basic clinical and microbiological variables were selected from medical records, and assessed for their relationship with the clinical outcome of the patients. The clinical data included gender, age, and immunocompromised host status. When patients

showed the presence of *Candida* species at normally sterile sites outside the bloodstream (excluding the urine), the disease was regarded as disseminated, and then further defined as acute or chronic. Persistent fungemia was defined as ≥5 days of persistently positive blood cultures for the same *Candida* species. All infected patients were followed for three months to assess their clinical outcome.

Genotyping

All patients were asked to provide 5 mL venous blood, and their genomic DNA was isolated using a Qiagen Blood Kit (Qiagen, Chastworth, CA, USA) according to manufacturer instructions. Genotyping of the IL-1β gene (*IL1β*) rs1143634, *IL1β* rs16944, *IL8* rs4073, *IL10* rs1800872, and *IL10* rs1800896 polymorphisms was carried out using a 384-well plate format on the Sequenom MassARRAY platform (Sequenom, San Diego, CA, USA). The Sequenom Assay Design 3.1 software (Sequenom) was conducted to design primers for polymerase chain reaction amplification and single base extension assays. For the rs1143634, the forward and reverse primers were 5'-TGCTCCACATTTCAGAACCTATCTTCGTC-3' and 5'-CTTGTTGC TCCATATCCTGTCCCTGGAGG-3', respectively. For rs16944, the forward and reverse primers were 3'-CAGAGGCTCCTGCAATTGACA-5' and 3'-GGTCTCTACCTTGGGTGCTGT TC-5', respectively. For rs4073, the forward and reverse primers were 5'-CTAGAAATAAA AAAGCATACAT-3' and 5'-CTAGAAATAAAAAAGCATACAA-3', respectively. For rs1800872, the forward and reverse primers were 5'-GGTAAAGGAGCCTGGAACACA TC-3' and 5'-GCCCTTCCATTTTACTTTCCAGAGA-3', respectively. For rs1800896, the forward and reverse primers were 5'-ACACACACAAATCCAAGACAACACT-3' and 5'-GCTGGATAGGAGGTCCCTTACTTT-3', respectively. Polymerase chain reaction (PCR) amplification was performed in a 25 μL total volume containing 50 ng genomic DNA, 0.1 μL dNTPs, 1.25 U Taq DNA polymerase (Promega Corporation, Madison, WI, USA), and 21 μL forward and reverse primers. The cycling program involved preliminary denaturation at 95°C for 2 min, followed by 45 step cycles of denaturation at 95°C for 30 s, annealing at 56°C for 30 s, extension at 72°C for 60 s, and a final extension at 72°C for 5 min. The PCR products were analyzed by 1.0% agarose gel electrophoresis. For quality control, 10% of the subjects were randomly selected for repeated sample genotyping, the results of which showed 100% concordance.

Statistical analysis

Continuous variables are reported as means ± standard deviation (SD), while categorical variables are reported as frequencies and percentages (%). The odds ratios (ORs) and corresponding 95% confidence intervals (CIs) were calculated by unconditional logistic regression analysis and utilized to assess the potential association between *IL-1β* rs1143634, *IL-1β* rs16944, *IL-8* rs4073, *IL-10* rs1800872, and *IL-10* rs1800896 polymorphisms and the risk of *Candida* infection. The homozygote for the most frequent allele was regarded as the reference group. SPSS statistical package, version 11.0 (SPSS Inc., Chicago, IL, USA) for Windows was used for statistical analyses. All P values were two-tailed, and a difference was considered to be statistically significant when $P < 0.05$.

RESULTS

There were 169 men and 106 women among the patients with invasive *Candida* infection, and there were 171 men and 134 women among the control subjects (Table 1). The mean age of patients and controls were 51.3 ± 15.3 and 52.4 ± 14.9 years, respectively. Of the patients with invasive *Candida* infection, 107 (38.91%) were infected with *Candida albicans*, 85 (30.91%) with *Candida glabrata*, 39 (14.18%) with *Candida parapsilosis*, 30 (10.91%) with *Candida tropicalis*, 11 (4.00%) with *Candida krusei*, and 3 (1.09%) with other *Candida* species. Patients with invasive *Candida* infections were more likely to have an immunocompromised state, had hematopoietic stem cell transplantation, solid organ transplant, solid tumor, chemotherapy within the past three months, neutropenia, surgery within the past 30 days, acute renal failure, liver failure, and/or median baseline serum creatinine.

Table 1. Baseline characteristics of included subjects and controls.

Variable	Infected subjects N = 275	%	Controls N = 305	%	*t* or χ^2 value	P value
Gender						
Male	169	61.45	171	56.07		
Female	106	38.55	134	43.93	1.73	0.19
Age, years (mean ± SD)		51.3 ± 15.3		52.4 ± 14.9	0.79	0.22
Immunocompromised state	158	57.45	37	12.13	133.11	<0.001
Active malignancy	78	28.36	11	3.61	68.23	<0.001
Hematopoietic stem cell transplantation	4	1.45	0	0.00	4.47	0.05
Solid organ transplant	6	2.18	0	0.00	6.72	0.01
Solid tumor	9	3.27	6	1.97	0.98	0.32
Leukemia	2	0.73	2	0.66	0.01	0.92
Lymphoma	6	2.18	1	0.33	4.17	0.04
Chemotherapy within past 3 months	31	11.27	4	1.31	25.31	<0.001
Neutropenia (ANC, 500 cells/mm^3)	17	6.18	1	0.33	16.48	<0.001
Surgery within past 30 days	97	35.27	33	10.82	49.72	<0.001
Acute renal failure	78	28.36	0	0.00	99.95	<0.001
Liver failure	59	21.45	0	0.00	72.85	<0.001
Median baseline serum creatinine (mg/dL)	3	1.09	0	0.00	3.34	0.11
Median baseline WBC count (cells/mm^3)	26	9.45	4	1.31	19.55	<0.001
Candida species						
Candida albicans	107	38.91				
Candida glabrata	85	30.91				
Candida parapsilosis	39	14.18				
Candida tropicalis	30	10.91				
Candida krusei	11	4.00				
Other *Candida* species	3	1.09				

SD = standard deviation; ANC = absolute neutrophil count; WBC = white blood cell

The genotype distributions of *IL-1β* rs1143634, *IL-1β* rs16944, *IL-8* rs4073, *IL-10* rs1800872, and *IL-10* rs1800896 polymorphisms were in Hardy-Weinberg equilibrium in the control subjects. Conditional logistic regression analyses found that individuals with the rs1800896 GG genotype were associated with an increased risk of invasive *Candida* infections compared to those with the AA genotype (OR = 0.61, 95%CI = 0.37-0.94) (Table 2). However, we did not find significant association between *IL-1β* rs1143634, *IL-1β* rs16944, *IL-8* rs4073, or *IL-10* rs180087 and risk of *Candida* infection.

Table 2. Association of *IL-1β* rs1143634, *IL-1β* rs16944, *IL-8* rs4073, *IL-10* rs1800872, and *IL-10* rs1800896 with susceptibility to invasive *Candida* infections.

Polymorphism	Infected subjects (N)	%	Controls (N)	%	OR (95%CI)	P value
IL-1β rs1143634						
GG	169	61.45	181	59.34	1.0 (Ref.)	-
GA	97	35.27	106	34.75	0.98 (0.68-1.41)	0.91
AA	9	3.27	18	5.90	0.54 (0.21-1.30)	0.13
IL-1β rs16944						
AA	30	10.91	46	15.08	1.0 (Ref.)	-
GA	130	47.27	136	44.59	1.47 (0.85-2.56)	0.15
GG	115	41.82	123	40.33	1.43 (0.82-2.52)	0.18
IL-8 rs4073						
AA	57	20.73	56	18.36	1.0 (Ref.)	-
TA	143	52.00	163	53.44	0.86 (0.55-1.36)	0.50
TT	75	27.27	86	28.20	0.86 (0.51-1.43)	0.53
IL-10 rs1800872						
GG	158	57.45	190	62.30	1.0 (Ref.)	-
TG	94	34.18	99	32.46	1.14 (0.79-1.65)	0.46
TT	23	8.36	16	5.25	1.73 (0.84-3.63)	0.11
IL-10 rs1800896						
AA	78	28.36	70	22.95	1.0 (Ref.)	-
GA	127	46.18	144	47.21	0.79 (0.52-1.21)	0.25
GG	70	25.45	91	29.84	0.61 (0.37-0.94)	0.10

OR = odds ratio; CI = confidence interval.

DISCUSSION

This study was the first to investigate the role of cytokine genes in the risk of *Candida* infection among patients in a Chinese population. To our knowledge, only one study has reported on the association between cytokine genes and the susceptibility to invasive *Candida* infections (Johnson et al., 2012); this reported that variants of the genes *IL12β* and *IL10* are correlated with systemic *Candida* infection persistence (Johnson et al., 2012). Our study showed that the *IL10* rs1800896 polymorphism was associated with a risk of invasive *Candida* infections, which is consistent with the results of the previous study (Johnson et al., 2012).

Previous studies have shown that polymorphic variants of the genes for IL-12β and IL-10 are correlated with persistent systemic *Candida* infection, and that polymorphisms in other key innate immunity genes such as *TLR4*, *TLR2*, or *TLR1* can play important roles in the risk of invasive *Candida* infections (Van der Graaf et al., 2006; Woehrle et al., 2008; Johnson et al., 2012). Johnson et al. (2012) conducted a cohort study with 338 patients with candidemia and 351 noninfected controls, and investigated single nucleotide polymorphisms (SNPs) in six cytokine genes and one cytokine receptor gene, including *IFNG*, *IL10*, *IL12β*, *IL18*, *IL1β*, and *IL8* as well as *IL12RB1*. This study found that polymorphisms in *IL10* and *IL12β* could cause low production of proinflammatory cytokines, and were correlated with persistent fungemia in patients with candidemia. Fatahinia et al. (2012) investigated the efficacy of propolis on cytokine levels in systemic candidiasis in mice. They found that IL-10 counterbalanced the role of proinflammatory cytokines, including IFN-c. The *IL10* rs1800896 polymorphism modified IL-10 secretion and was associated with reduced IL-10 production, and might therefore be predicted to influence the outcomes in several disease states (Stanilova et al., 2006; Zeng et al., 2009). The *IL-10* rs1800896 polymorphism was shown to modify the response to sepsis caused by many microorganisms, and was associated with an increased rate of persistence of the infections (Hunninghake et al., 2008). Our study found that the *IL-10* rs1800896 polymor-

phism was associated with the risk of invasive *Candida* infections, and showed that high IL-10 production was a predisposing factor for prolonged candidemia.

This study has a few limitations. First, the patients and controls were selected from two hospitals, which might not be representative of other populations. However, the controls consisted of a random sample from a pool of individuals who came to receive a health check-up, and could therefore potentially be representative of the general population. Second, because of the rarity of invasive *Candida* infections, the sample size of the patient group is relatively small, which could limit the statistical power to find an association between groups. Third, the risk of invasive *Candida* infections could be modified by many genetic factors other than cytokine genes. Therefore, further studies with more subjects are needed to confirm the association between cytokine gene polymorphisms and the risk of invasive *Candida* infections found in this study.

In summary, the results of this case-control study suggest that the *IL-10* rs1800896 polymorphism, among cytokine gene variants, might play a role in the etiology of invasive *Candida* infections. Further large sample studies are needed to confirm this association.

Conflicts of interest

The authors declare no conflict of interest.

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