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Original Article

Relationship of interferon gamma gene polymorphisms with the burn-induced sepsis susceptibility in Chinese Han population

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Abstract: Background: Interferon gamma (IFN-γ), the only member of type II interferon, it played an important role in immune systems. Objective: This study was aimed to explore the relationship of *IFN-γ* polymorphisms (rs2430561 and rs2069705) with sepsis susceptibility in sever burn patients in China. Methods: 92 patients and 108 controls were enrolled in present study. All of the participators were Chinese Han population, and had no genetic relationship with each other. Controls were matched with the cases in age and gender. Odds ratios (OR) and corresponding 95% confidence intervals (CIs) were calculated and used to represent the relevant risk of sepsis. Results: AA genotype and A allele of rs2430561 polymorphism were positively associated with the risk of sepsis in burn patients (*P*=0.035, OR=4.000, 95% CI=1.014-15.781; *P*=0.007, OR=1.957, 95% CI=1.192-3.213). But the genotypes and alleles of rs2069705 polymorphism had no significant association with the risk of sepsis (*P*>0.05). Besides, serum IFN-γ concentration was distinctly decreased in case group, and it was significantly lower in AA genotype respectively compared with TT and TA genotypes in case group (*P*<0.001). Conclusion: AA genotype of rs2430561 significantly decreased the serum IFN-γ concentration, and then leads to the onset of sepsis. It still needs further studies to verify our results.

Keywords: *IFN-γ*, polymorphisms, sepsis

Introduction

Sepsis is a kind of systemic inflammatory response syndrome (SIRS) which is caused by infection [1]. Sepsis is a life-threatening complication that arises from many locations of the body including lungs, brain, abdominal organs, urinary tract, and skin [2-4]. Risk factors of sepsis include very young or old age, weakened immune system from some diseases, disorders, and severe trauma or burns [5]. But it is mainly caused by the immune response begot by infection [6]. The infection usually triggered by bacteria, but can also be by fungi, viruses, or parasites [7]. Further development of sepsis could lead to severe sepsis, septic shock and multiple organ dysfunction syndrome (MODS). It is one of the leading causes of death in clinical critically ill patients. With quickly progress and poor prognosis, it is difficult to treat the sepsis patients. In order to find out an effective treatment method, it is necessary to certify the mechanism of sepsis.

Interferon gamma (IFN-γ), a dimerized soluble cytokine, is the only member of type II interferon. IFN-γ is firstly find as a product of human leukocytes [8], and then find in lymphocytes [9]. Recently, it has been confirmed that IFN-γ is mainly synthesized by activated $CD4^+$Th1, $CD8^+$T and natural killer (NK) cells. IFN-γ plays an important role in innate and adaptive immune systems [10]. IFN-γ could against the infections from viral, some bacterial and protozoal, but the mainly biological activity of it is immune regulation [11, 12]. It has been found that IFN-γ closely relate to the development of sepsis [13, 14]. Human *IFN-γ* gene is located in 12q14, and contains 4 exons [15].

Polymorphims in *IFN-γ* gene might affect the expression and function of the encoded pro-

tein, and then lead to some immune disorders. Recent years the single nucleotide polymorphism (SNP) has become the new biomarker in the pathological studies. Therefore, we performed this case-control study to explore the potential role of *IFN-γ* gene SNPs (rs2430561 and rs2069705) in the development of sepsis in burned patients.

Materials and methods

Study objects

Ethic committee of The Chinese People's Liberation Army Eighty-eight Hospital approved this case-control study. All of the participants realized this study and signed the informed consent. Baseline information and the clinical characteristics were collected and recorded by the same well trained nurse. Cases and controls matched with each other in age and gender in frequencies. All of the participants were admitted to hospital within 8 h after injury. They had no blood relationship.

These patients were diagnosed by two independent physicians in The Chinese People's Liberation Army Eighty-eight Hospital from January 2013 to July 2015. 92 patients, who were diagnosed according to the stands of sepsis [1], included 64 males and 28 females aged 33.9±10.5. Controls had no sepsis and included 74 males and 34 females with the mean age of 34.2±10.3. Patients who had the following conditions were excluded from this study: ① patients with chemical burn and poisoning; ② allergic constitution; ③ had severe combined injury; ④ had history of diabetes and abnormal glucose metabolism; ⑤ had severe heart, liver, kidney failure and any other systemic diseases; ⑥ women during pregnancy and lactation; ⑦ lack of information.

Sample collection

5 ml fasting peripheral blood was collected in an Heparin vacutainer from every patient on the first day after injury. Leukocyte and serum were separated from peripheral blood using a centrifuge, and then stored in -70°C up to use.

Genotyping method

Genomic DNA was extracted from the leukocyte using a TIANamp Blood DNA Kit (TIANGEN, China). Genotypes of *IFN-γ* rs2430561 and rs2069705 polymorphisms were detected by ABI 3730XL genetic analyzer (Applied Biosystems, USA) following the manufacture's introduction.

IFN-γ concentration

Serum IFN-γ concentration was inspected by sandwich ELISA method [16], using a Human γ-IFN ELISA kit (CWBIO, China). Detection process complied with the instructions.

Statistical analysis

PASW 18.0 was used to perform the calculations. Significant level is set to two-side 0.05. Significant level of multiple comparisons was adjusted by Bonferroni method. The data which accorded with normal distribution were analyzed by t test, while the data which did not conform to the normal distribution were assessed by non-parametric test. Representativeness of the subjects was detected by Hardy-Weinberg equilibrium (HWE) test. Genotype and allele frequencies were calculated by direct counting. The differences of genotypes and alleles between the case and control groups were evaluated by Chi-square test. Association strength between *IFN-γ* polymorphism and sepsis risk was denoted by odds ratios (ORs) and 95% confidence intervals (CIs).

Results

Characteristics of participants

Characteristics, such as burn size, APACHE-II score, inhalation injury, hypovolemic shock, thrombocytopenia duration, blood cultures and IFN-γ concentration, were significantly different between the sepsis group and non-sepsis group in burn patients (**Table 1**, $P<0.05$). But the other features had no obvious differences between the two groups.

Association between the IFN-γ polymorphisms and the sepsis risk in burn patients

Genotype and allele distributions of the two SNPs (rs2430561 and rs2069705) were accorded with the HWE test both in case and control groups. Frequencies of TA and TT genotypes were higher in cases than in controls, but only AA genotype had significant association

Table 1. Characteristics of the participants

	Case n=92 (%)	Control n=108 (%)	*P*
Baseline characteristic			
Age			0.985
>18	64 (69.57)	75 (69.44)	
Gender			0.873
Male	64 (69.57)	74 (68.52)	
Female	28 (30.43)	34 (31.48)	
BMI (kg/m^2)			0.932
≤24	44 (47.83)	51 (47.22)	
>24	48 (52.17)	57 (52.78)	
Burn cause			0.844
Flame 270 (83%)	69 (75.00)	81 (75.00)	
Scald 17 (5%)	9 (9.78)	12 (11.11)	
Electricity 22 (7%)	2 (2.17)	4 (3.70)	
Other 15 (5%)	12 (13.04)	11 (10.19)	
Burn size (%TBSA)			0.001
<15	12 (13.04)	22 (20.37)	
15-29	21 (22.83)	45 (41.67)	
>29	59 (64.13)	41 (37.96)	
Just admitted to hospital			
APACHE-II score	2.75±3.08	17.44±3.92	<0.001
Inhalation injury			<0.001
No	47 (51.09)	81 (75.00)	
Yes	45 (48.91)	27 (25.00)	
Hypovolemic shock			<0.001
No	19 (20.65)	53 (49.07)	
Yes	73 (79.35)	55 (50.93)	
Others			
Thrombocytopenia duration (d)	5.53±2.23	2.83±1.42	<0.001
Surgery			0.853
No	27 (29.35)	33 (30.56)	
Yes	65 (70.65)	75 (69.44)	
Blood cultures			<0.001
Negative	18 (19.57)	86 (79.63)	
Positive	74 (80.43)	22 (20.37)	
IFN-γ concentration (pg/mL)	74.05±8.15	88.30±8.05	<0.001

Notes: BMI, Body mass index; TBSA, total body surface area.

with the risk of sepsis (**Table 2**. *P*=0.035), which indicating that AA genotype might increase the risk of sepsis (OR=4.000, 95% CI=1.014-15.781). Meanwhile, the A allele of rs2430561 also had a positive association with the incidence of sepsis (*P*=0.007, OR=1.957, 95% CI=1.192-3.213). For the rs2069705 polymorphism, although CT and TT genotypes were had high frequencies in case group, the differences were not significant (*P*>0.05).

Serum IFN-γ concentration in different genotypes of rs2430561 SNP

In order to evaluate the association of rs2430561 polymorphism with the susceptibility of sepsis, we analyzed the alteration of serum IFN-γ concentration in different genotypes of rs2430561 SNP (**Table 3**; **Figure 1**). Serum IFN-γ concentration was obviously higher in controls than in cases (**Figure 1A**, *P*<0.001). Besides, AA genotype had significantly lower IFN-γ level respectively compared with TT and TA genotypes in case group (**Table 3**; **Figure 1B**, *P*<0.001). In control group, although the IFN-γ level was decreased in AA genotype, but the difference was not significant. So we conjectured that AA genotype increased the sepsis risk in burn patients via decreasing the serum concentration of IFN-γ.

Discussion

Sepsis includes the infection of microorganism and the immune response of host. Sepsis is the most common complication in many diseases, and it is the leading cause of death in burn patients. Recent years the therapy method of sepsis acquires many progresses. Many studies have shown that the degree of sepsis is related to many cytokines, such as TNF-α, IL-6 and IFN-γ [17, 18]. IFN-γ is one of the early pro-inflammatory mediators. It is expressed by many immune cells and involve in immune response. One of the INF-γ function is to active macrophage, and then refer to the antigen processing and presentation pathways through up-regulating the expression of the major histocompatibility complexes (MHC) [19]. INF-γ could induces the products of IgG2a and IgG3 from the activated plasma B cells [20, 21]. Besides, INF-γ participate in the JAK/STAT1 signaling pathway [22]. Therefore, it plays an important role in the immune regulations.

Table 2. Association between the *IFN-γ* polymorphisms (rs2430561 and rs2069705) and the sepsis risk in burn patients

SNP	Case n=92 (%)	Control n=108 (%)	*P*	OR (95% CI)
rs2430561				
TT	52 (56.52)	78 (72.22)		
TA	32 (34.78)	27 (25.00)	0.68	1.778 (0.956-3.308)
AA	8 (8.70)	3 (2.78)	0.035	4.000 (1.014-15.781)
T	136 (73.91)	183 (84.72)		
A	48 (26.09)	33 (15.28)	0.007	1.957 (1.192-3.213)
rs2069705				
CC	39 (42.39)	61 (56.48)		
CT	44 (47.83)	40 (37.04)	0.069	1.721 (0.956-3.095)
TT	9 (9.78)	7 (6.48)	0.193	2.011 (0.692-5.841)
C	122 (66.30)	162 (75.00)		
T	62 (33.70)	54 (25.00)	0.056	1.525 (0.988-2.353)

Table 3. Serum IFN-γ concentration in rs2430561 polymorphism

Genotype	Case n=92 (%)	Control n=108 (%)
TT	79.42±7.15	89.96±7.67
TA	70.35±8.26*	84.57±7.31*
AA	57.73±5.09*,#	78.87±8.54

Notes: *compared with TT genotype, #compared with TA genotype, $P<0.016$.

Previous studies showed that polymorphisms of the genes might alter the expression and function of the encoding proteins, and associated with many diseases. *INF-γ* gene polymorphisms is associated with immunosuppressive therapy of aplastic anemia [23] and hepatitis B virus (HBV) infection [24]. These studies showed that the polymorphisms of *INF-γ* gene might associated with the infection in sepsis patients. However, the detailed pathogenesis of sepsis is still unknown. As the two widely researched SNPs in *INF-γ* gene, rs2430561 (+874A/T) and rs2069705 (-1616T/C) have been found that correlated with many diseases [25, 26].

In present study, we found that AA genotype of rs2430561 SNP significantly increased 4.000 times risk of sepsis, meanwhile A allele might associated with 1.957 times increased risk of sepsis. Our results were different from the previous study which showed that AA genotype of rs2430561 had no significant association with the susceptibility of Pneumonia-induced sepsis [27]. However, a meta-analysis showed that TT genotype and T allele of rs2430561 polymorphism persistently reduce the risk of HBV infection [28]. Besides Allam et al. found that AA genotype of rs2430561 associated with increased risk of neonatal infections [29]. +874A/T SNP also associated to severe liver fibrosis in chronic HBV infection [30]. Although variant allele carriers of rs2069705 were higher in case group, but they had no significant association with the risk of sepsis. It was reported that rs2069705 had no significant association with all-cause mortality [31]. But TT genotype of rs2069705 could retard the severity of Pneumonia-induced sepsis [27] and the development of HBV infection [26]. Another study suggested that rs2069705 associated with the susceptibility of complicated skin and skin structure infections (cSSSIs) [32]. These inconsistent results may be caused by pathogenic bacteria, ethnicity, region and other factors.

In order to certify the pathogenesis of burn sepsis, we analyzed the serum IFN-γ concentration in different genotypes of rs2430561 SNP in burn patients. Afterwards, we found that IFN-γ concentration was higher in non-sepsis burn patients. AA carriers had the lowest IFN-γ concentration in burn sepsis patients, while the difference in non-sepsis burn patients was not significant. These results might indicate that rs2430561 SNP reduce the serum IFN-γ level, then give rise to the growth of pathogenic bacteria, and finally lead to the onset of sepsis in burn patients. To our knowledge, this is the first research focused on the relationship between polymorphism and the serum concentration of IFN-γ in Chinese Han burn patients.

However, because the little sample size and the unadjusted result, current results need to be verified by further studies. There still many advantages existed in our study. The genotypes and alleles were accorded to the HWE test indicated the study objects could represent the general population. In addition, the matched age and gender between case and control

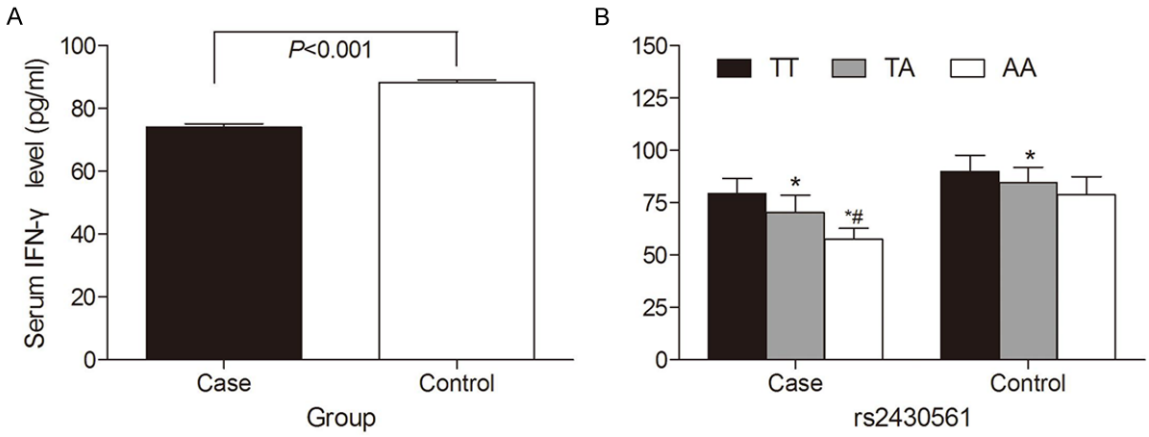

Figure 1. Serum IFN-γ concentration respectively in the genotypes of rs2430561 SNP. *, compared with TT genotype; #, compared with TA genotype; *P*<0.016.

groups showed the comparability of the two groups.

Disclosure of conflict of interest

None.

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