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Original Article

Relationship between occurrence of postoperative acute respiratory distress syndrome of esophageal carcinoma and genetic polymorphism of angiotensin-converting enzyme as well as its protein expression

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Abstract: Objective: To investigatethe relationship between angiotensin-converting enzyme (ACE) and onset of acute respiratory distress syndrome (ARDS) after esophageal cancer radical surgery. Method: After radical surgery of esophageal cancer, 108 patients with ARDS were studied, and 110 patients without ARDS were selected as control. ACE gene polymorphism was detected using polymerase chain reaction (PCR), and serum ACE level was analyzed in the meantime. Results: Compared to control group, frequency of ACE genotype DD (47.2% vs. 23.6%, P=0.004) and D allele (0.57 vs. 0.48, P=0.002) among ARDS patients was significantly higher than that in control group. ARDS patients carrying II genotype had shortest duration of mechanical ventilation and stay length in ICU compared with DD and ID carriers, with significant difference (P<0.05). The frequencies of ACE genotype and allele distribution between ARDS survival subgroup and death subgroup also had significant differences (P<0.05). Serum ACE level of ARDS patients was significantly lower than that in control group, with significant difference (P<0.05). Conclusion: ACE genetic polymorphism may be a significant genetic factor which determines the onset of ARDS following esophageal cancer surgery.

Keywords: Acute respiratory distress syndrome, angiotensin-converting enzyme, genetic polymorphism, esophageal cancer, surgery

Introduction

Surgery removal is currently considered the most effective treatment of esophageal carcinoma [1]. Acute respiratory distress syndrome (ARDS) is the main complication with a high mortality. Thus, exploration of the pathogenesis of ARDS as well as the effective therapeutic approaches has become a critical research area in critical care medicine. In recent years, a number of researchers pointed out that the occurrence and progression of diseases were regulated by genetic factors and genetic polymorphism resulted in various susceptibility and prognosis in different patient populations [2-5]. It has been suggested that I/D polymorphism of angiotensin-converting enzyme (ACE) is an important genetic factor determining ARDS susceptibility and prognosis [6, 7]. However, the research of ARDS polymorphism is difficult due to complex pathogenesis of ARDS, acute onset, quick progression, wide differences in patient age, immunity, nutritional status and health conditions, and the lack of family history.

This study was conducted on esophageal cancer patients to assess the relationship between ACE polymorphism and the occurrence and prognosis of post-operational ARDS, which may contribute to the further exploration of the pathogenesis of ARDS, identification of susceptible populations, and exploration of therapeutic target and provide basis for the future interference gene therapy.

Material and method

Study subjects

According to ARDS Diagnostic Criteria defined by the 1994 Consensus Conference of Ame-

Table 1. The characteristics of participants

Group	N	Age (Year)	Sex (M/F)	CRP (mmol/L)	WBC ($\times 10^9$)	Death (n, %)
ARDS group	108	60.2±10.3	87/21	15.7±4.4	14.4±4.5	22, 20.37%
Control group	110	60.3±9.4	89/21	8.2±2.3	5.4±1.5	-

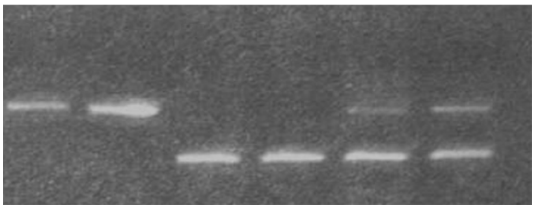

Figure 1. The genotyping results of ACE gene. 1, 2: II genotype; 3,4: DD genotype; 5,6: ID genotype.

rican College of Chest Physicians/Society of Critical Care Medicine (ACCW/SCCM), as well as the American-European Consensus Conference Criteria, 108 ARDS patients after radical surgery of esophageal cancer who were admitted to ICU in our hospital during January 2009 to January 2015 were selected for the study. These patients were aged between 45 to 71 years, and the mean was 60.2±10.3 years. There were 87 male and 21 female. 110 patientswithout ARDS after radical surgery of esophageal cancer were classified into control group. There were no significant differences in age and gender between the two groups. Informed consent was obtained from all subjects prior to the study.

Methods

Whole blood genomic DNA extraction: 5ml venous blood was collected under fasting and added by EDTA as anticoagulant. Genomic DNA was extracted using blood genomic DNA extraction kit provided by TIANGEN (Beijing, China).

Detection of ACE genetic polymorphism

The target sequence with polymorphism in intron 16 of ACE gene was amplified using polymerase chain reaction (PCR). Primers were designed as previously described in the literature [8]. Upstream primer: 5'-CTGGAGACCACTCCCATCCTTTCT3'; Downstream primer: 5'-GATGTGGCCATCACATTCGTCAGAT-3'.

Primers were synthesized by Shanghai Bioengineering Co., Ltd (Shanghai, China). PCR kit was bought from Promega (USA). PCR system and proceduresfollowedthose previously described in the literature [8].

Detection of plasma ACE level

Serum ACE level was detected using ELISA. ELISA kit was purchased from R&D Co. (USA) and the assay was performed according to the manufacture's protocol.

Statistical analysis

Statistical analysis was performed using SPSS22.0 software. Data were expressed as mean ± standard deviation (mean ± SD). Variable data were analyzed using two sample t-test and one-way analysis of variance (one-wayANOVA) to compare the two means. Attribute data were analyzed by multiple sample chi-squared test. Hardy-Weinberg equilibrium was analyzed by chi-squared test. $P<0.05$ indicated that the difference was statistically significant.

Results

General information

108 ARDS patients, aged between 45 to 71 years with a mean age of 60.2±10.3 years, included 87 male and 21 female. The inpatient mortality was 20.37% (22/108). Mean age of the subjects among control group (110 patients) was 60.3±9.4 years. There were 89 male and 21 female, and all survived. General information of the two groups is listed in **Table 1**.

ACE genotype test

Human ACE gene is located on chromosome 17 and there is an insertion or deletion of 287 base pair (bp) fragment. The PCR amplification generated three types of the products, DD, ID and II. As shown in **Figure 1**, II homozygotewas located on a 490 bp fragment, DD homozygote was located on a 190 bp fragment, and ID heterozygote was located on a 490 bpfragment and a 190 bp fragment (**Figure 1**).

ACE genotype distribution

The genotype distribution of control group was in accordance with Hardy-Weinberg equilibri-

Table 2. The distribution of genotype in ARDS group and control group

Group	N	Genotype (n, %)			Allele	
		DD	ID	II	D	I
ARDS group	108	51 (47.2)	22 (20.4)	35 (32.4)	124 (0.57)	92 (0.43)
Control group	110	26 (23.6)	54 (49.1)	30 (27.3)	106 (0.48)	114 (0.52)
P value		0.004			0.002	

Table 3. Effect of ACE genotype on the mechanical ventilation time and ICU length of stay in patients with ARDS between different genotype

Genotype	N	Time of Mechanical Ventilation (d)	*P* value	ICU stay time (d)	*P* value
DD	51	10.4±4.2	<0.001	11.5±3.5	<0.001
ID	22	11.5±4.1		12.4±3.8	
II	35	4.5±1.5		5.8±2.4	

Table 4. Relation between ACE genotype and prognosis of ARDS patients

Group	N	Genotype (n, %)			Allele (n, %)	
		DD	ID	II	D	I
Survival	86	39 (45.3)	14 (16.3)	33 (38.4)	92 (0.53)	80 (0.47)
Death	22	12 (54.5)	8 (36.4)	2 (9.1)	32 (0.72)	12 (0.28)
P value			0.002		<0.001	

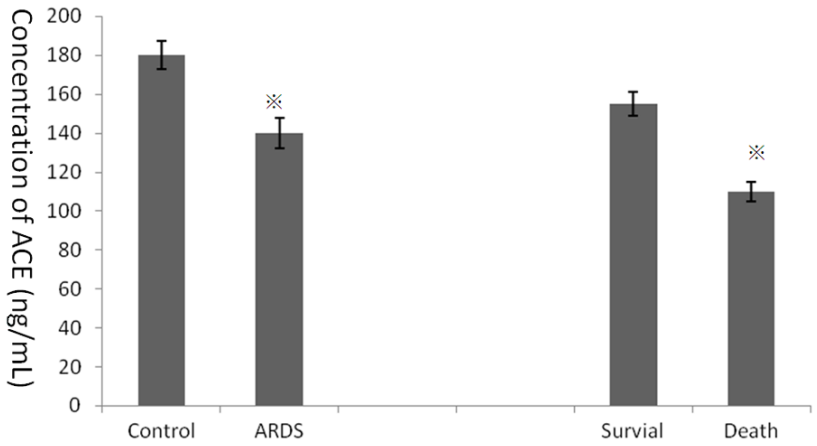

Figure 2. The serum ACE concentration in different groups. Compared to control group (or survial group), *P<0.05.

um, indicating that the samples were from the same population, which could represent the population.

ACE genotype distribution

The genotype and allele distribution of the two groups are shown in **Table 2**. ACE genotype distribution between the two groups revealed significant difference (P=0.004). Frequency of DD genotype in ARDS patients was significantly higher than that in control group. Esophageal cancer patients who carried ACE DD genotype were more susceptible to ARDS after radical surgery, compared to patients with ID or II genotype, with relative hazard ratio of 5.12 (95% CI: 2.45-8.13) and 2.33 (95% CI: 1.02-4.34), respectively. The frequency of allele D in ARDS group was 0.57, significantly higher than that of control group (0.48, P=0.002).

Influence of ACE genotype on ARDS patient duration of mechanical ventilation and stay length in ICU

As shown in **Table 3**, ARDS patients carrying ACE II genotype had a significant shorter duration of mechanical ventilation and stay length in ICU, compared with ARDS patients with ID or DD genotype. The difference was statistically significant (P<0.001).

Influence of ACE genotype on ARDS prognosis

As shown by **Table 4**, within ARDS patients, frequency of ACE genotype and allele exhibited significant difference between survival subgroup and death subgroup (P>0.05), indicating that D allele carriers have more risk than I allele carriers.

Serum ACE level in ARDS and control group

As shown in **Figure 2**, the serum ACE level in ARDS group was significantly lower than that in control (P<0.001). Also, within ARDS group,

there was significant difference between survival subgroup and death subgroup (P>0.05).

Discussion

ARDS is a severe complication after esophageal cancer surgery with a mortality rate up to 50% [9]. A number of domestic and foreign studies have demonstrated that occurrence and prognosis of ARDS are likely to be influenced by genetic factors [10-14]. ACE converts angiotensin I to angiotensin II. The latter is the key enzyme in degradation of Bradykinin, which participates in the regulation of vasodilation and cardiac function. ACE is mainly expressedin endothelial cells, while lung is the organ containing the largest area of endothelial cells. It has been demonstrated that the lung renal-angiotensin system (RAS) regulates ARDS through the regulating vascular permeability, vascular tone, fiber activity, as well as inhibition of production of alveolar endothelium [15]. ACE can act on pulmonary capillary endothelial cells, epithelial cells and fibroblasts. Through RAS, ACE is also able to change pulmonary vascular tone and permeability as well as the coagulation fibrinolysis system and pulmonary fibrosis, therebyaffecting the occurrence, prognosis and outcomes of ARDS.

It has been reported that ACE polymorphism is a significant genetic factor determining ARDS susceptibility and prognosis. Marshall et al. discovered that allele D was correlated with ARDS susceptibility and prognosis, and that ARDS patients carrying II homozygote had a significant low mortality in contrast to ID heterozygote or DD homozygote carriers. Frequency of DD genotype is significantly high in ARDS patients. Recently, Japanese researchers analyzed the ACE polymorphism from 44 severe ARDS patients and found that the D allele frequency was significantly higher in severe hypoxemia patients compared to patients without hypoxemia. However, different view is also proposed. In the study conducted by Chan et al., it was revealed that ACE polymorphism was uncorrelated with the severity of ARDS.

Our study showed that DD genotype and D allele exhibited high frequency in ARDS group. For esophageal cancer patients carrying DD phenotype, the relative risk of post-operational ARDS was 5 and 2 times of that among ID and II carriers, respectively. The relative risk of post-operational ARDS for D allele carriers was 1.8 times of that among I allele carriers, supporting the theory that ACE polymorphism might influence ARDS occurrence. In addition, II allele carriers in our study showed reduced duration of mechanical ventilation and stay length in ICU, indicating that ACE polymorphism may affect ARDS progression. Furthermore, we also found that there was significant difference indistribution of ACE genotypes and alleles between survival subgroup and death subgroup of ARDS, indicating that mortality rate was affected by ACE genotypes.

It has been reported that in acute pulmonary injury, SACE activity is significantly reduced, and the association between the decline and disease severity may be used to predict prognosis. The more severe the pulmonary damage, the lower the SACE level. Sustained reduction of SACE is an indication of pulmonary endothelial cell damage or sustained lesion [16, 17]. In this study, ELISA was used to detect ACE activity, and the results showed that the serum ACE level of ARDS patients was reduced compared to control group, consistent with previous literatures. The reason might be associated with the pulmonary vascular endothelial cell damage, destruction and reduction of ACE release: 1) hypoxia leaded to the damage of pulmonary vascular endothelial cells, resulting in decreased production of ACE; 2) hypoxia caused pulmonary capillary vascular constriction, reduced blood flow through pulmonary microcirculation, and thus decreased serum ACE level; 3) hypoxia stimulated sympathetic nerves, constricted renal arteries, decreased renal blood flow, stimulated renin-angiotensin system, and enhanced ACE substrates level, thereby consuming and reducing serum ACE. Therefore, the change of SACE activity reflected the integrity and damage of pulmonary endothelial cells. Monitoring on the dynamic activity of SACEamong ARDS patients can predict the occurrence of ARDS and aid clinicians' judgment of disease situation, guiding treatment and implying prognosis. However, there was no significant difference between the survival and death subgroup of ARDS patients in ACE activity, possibly due to alternative collection time of blood samples at the diagnosis of ARDS caused bythe smaller sample size and varied time of admission to ICU.

In conclusion, our study reveals that ACE generic polymorphism affects the onset of post-operational ARDS of esophageal carcinoma and the

prognosis to a certain degree, contributing to further understanding of ARDS pathogenesis, identification of susceptible population, exploration of therapeutic target and providing guidance to the future individualized immunotherapy and genetic interference. However, for such a complex disease as ARDS, it remains a long way to go in the immunogenic research.

Disclosure of conflict of interest

None.

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