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ARAŞTIRMA / RESEARCH

Association between surfactant protein B gene locus and acute bronchiolitis in infants

Bebeklerde akut bronşiolit ve surfaktan protein B gen lokusu arasındaki ilişki

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Abstract

Purpose: The aim of this study was to investigate whether there is a relationship between surfactant protein B (*SFTPB)* C1580T polymorphism and acute bronchiolitis.
Materials and Methods: The study analyzed the allele frequency and genotype distribution for the *SFTPB* C1580T polymorphism using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique in 103 acute bronchiolitis infants and 102 healthy infants.
Results: The results showed no association between *SFTPB* C1580T polymorphism and clinical characteristics of acute bronchiolitis. The distribution of the CT genotype was higher in acute bronchiolitis infants (43%) than in healthy subjects (39%) and distribution of the TT genotype was found lower in acute bronchiolitis infants (38%) than in healthy subjects (41%). No significant differences in genotype distribution and allele frequency for the *SFTPB* C1580T polymorphism were found between case group and control group
Conclusion: *SFTPB* C1580T gene polymorphism plays no important role in susceptibility to acute bronchiolitis. Further work on the relevance of *SFTPB* C1580T polymorphism in larger cohorts will require validating our results.
Keywords: Acute bronchiolitis, genetic polymorphism, *SFTPB* gene, surfactant protein

Öz

Amaç: Çalışmamızda surfaktan protein B (*SFTPB)* C1580T polimorfizmi ile akut bronşiolit arasında bir ilişki olup olmadığını araştırmayı amaçladık.
Gereç ve Yöntem: Bu çalışmada, polimeraz zincir reaksiyonu- restriksiyon parça uzunluk polimorfizmi (PZR-RFLP) tekniği kullanılarak 103 akut bronşiolitli bebekte ve 102 sağlıklı bebekte *SFTB* C1580T gen polimorfizminin alel sıklığı ve genotip dağılımı analiz edildi.
Bulgular: Bu sonuçlar *SFTPB* C1580T polimorfizmi ve akut bronşiolitin klinik özellikleri arasında bir ilişki olmadığını gösterdi. CT genotipinin dağılımı akut bronşiolitli bebeklerde (%43) kontol grubuna (%39) göre daha yüksektir ve TT genotipinin dağılımı akut bronşiolitli bebeklerde (%38) kontol grubuna (%41) göre daha düşüktür. Hasta ve kontrol grubu arasında *SFTPB* C1580T polimorfizmi için genotip dağılımı ve allel sıklığında önemli bir fark yoktur.
Sonuç: *SFTPB* C1580T gen polimorfizminin akut bronşiolite yatkınlıkta önemli bir rolü olmadığı saptanmıştır. Çalışma sonuçlarımızın doğrulanabilmesi için *SFTPB* C1580T polimorfizminin ilişkisinin ileri ve daha büyük kohort çalışmaları yapılarak değerlendirilmesine ihtiyaç vardır.
Anahtar kelimeler: Akut bronşiolit, genetik polimorfizm, *SFTPB* geni, surfaktan protein

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INTRODUCTION

Acute bronchiolitis is a common disease characterized by acute wheezing, diffuse bilateral crackles, and symptoms of respiratory infection that occur in approximately 20% of infant during first year of their life[1]. However, there is no specific treatment for this disease[2]. Respiratory syncytial virus (RSV) is a significant cause of this disease that appears more frequently in 2–5 month-old infant. Other important predisposing factors for acute bronchiolitis in infants are male sex, malnutrition, premature birth, chronic lung and congenital heart disease, low income, cystic fibrosis, immunodeficiency, and smoking of family members[3]. Recent studies suggest that infants with severe RSV bronchiolitis may be more susceptible to development of asthma and obstructive lung disease in later childhood[4]. Many genetic factors are effective in the emergence of acute bronchiolitis and pulmonary surfactant proteins are thought to be effective in the development of this disease[5].

Surfactant proteins (SPs), consist of SP-A1-A2, SP-D, SP-C, and SP-B are lipoprotein complex, have significant roles in proinflammatory cytokine production, lung function, and chemotaxis[6,7]. SP-B is encoded by the surfactant protein B *(SFTPB)* gene, which is a hydrophobic surfactant protein, increases other SPs ability to efficiently reduce surface tension[8]. The *SFTPB* gene is located on the chromosome 2p11.2 that has 11 exons[7]. The *SFTPB* gene contains many variants and mutations, and the genetic variants may replace level and functional abilities of the protein. In the *SFTPB* C1580T polymorphism, alteration from a C (Thr) to T (Ile) inhibits N-linked glycosylation site and the change affects protein processing and folding[9]. The *SFTPB* C1580T polymorphisms have been associated with lung disorders in many studies[10].

Acute bronchiolitis might associate with systemic inflammation in infants[11]. Inflammation may cause changes of SPs concentrations and previous studies also showed that there were abnormalities in quantity and/or quality of SPs in severe cases of bronchiolitis[7, 12]. In addition, use of surfactant decreased length of intensive care unit stay in patients with acute bronchiolitis[13]. Moreover, Wang et al.[14] measured the plasma concentrations of SP-A and SP-B in infants with RSV bronchiolitis using enzyme-linked immunosorbent assay. They found that plasma immunoreactive SP-B was importantly higher in the infants with RSV bronchiolitis than that in the matching controls. *SFTPB* the important candidates to study the role of genetics in acute bronchiolitis, since *SFTPB* contribute surfactant function and to innate host defense. Although there were no studies in the literature which showed the effect of *SFTPB* C1580T polymorphism on the susceptibility to acute bronchiolitis. On this basis, we hypothesised that *SFTPB* C1580T polymorphism was associated with the disease. We investigated this association between the *SFTPB* C1580T polymorphism and susceptibility to or protect against acute bronchiolitis in Turkish infants.

MATERIALS AND METHODS

Subjects

The study included 103 infants with acute bronchiolitis and 102 healthy infants from pediatric outpatient and emergency clinics in Tokat Gaziosmanpasa University hospital between January 1, 2015 and January 1, 2016. Diagnosis of the disease was based on patient clinical criteria and history and healthy subjects were consist of infants with no acute bronchiolitis, who were received pediatric outpatient and emergency clinics with other reasons.

In children <12 months, at least one of the signs of increased respiratory effort such as prolonged expiration, wheezing, rhonchi, tachypnea, subcostal or intercostal retractions as well as findings of upper respiratory tract infections such as nasal discharge, fever, and cough helped in diagnosing acute bronchiolitis[15]. Infants with fatal congenital anomalies, such as serious congenital heart diseases, diaphragmatic hernia, central nervous system malformations, and chromosome abnormalities were excluded from this study.

The blood samples were collected before initiating any treatment. A scoring system taking into account retractions, wheezing, respiratory rate per minute, and general condition of the patient was used by pediatricians to evaluate the severity of acute bronchiolitis[16].

According to this scoring system, infants with acute bronchiolitis were divided into 3 groups: mild, moderate, and severe. The present study protocol was approved by the Tokat Gaziosmanpasa University hospital's ethics committee on 3 March 2015 (15-KAEK-040), conducted in line with the principles of the Declaration of Helsinki. Written informed consent was gathered from parents of both

acute bronchiolitis infants and healthy subjects, who were acknowledged about the blood sampling for the *SFTPB* C1580T polymorphism genotype analyses in acute bronchiolitis disease. The power analysis program G*Power version 3.1.9.7 was used to determine the needed sample size. The power analysis showed that a sample size of 204 subjects was sufficient to determine the significance of the correlation with a Cohen's effect size of d= 0.350, α type I error of 5% and a power of 80%.

DNA isolation

Peripheral blood samples from both acute bronchiolitis infants and healthy subjects were collected and DNA samples were purified using an Exgene™ Blood SV DNA purification kit (GeneAll™, Korea) for genotype analyses.

Genotyping

The *SFTPB* C1580T polymorphism genotyping was examined by using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique, according to previous report[17]. For the *SFTPB* C1580T polymorphism the PCR reaction mixture was prepared in a 25 μL reaction volume containing the 1.5 μL of 25 mM $MgCl_2$, 0.25 μL of 10 mmol of dNTP, 2.5 μL of 10 x buffer, 0.5 U of *Taq* polymerase, 0.8 μL of the reverse primer, 0.8 μL of one sequence specific forward primer, 2 μL of gDNA, and Nuclease-free water. For the *SFTPB* C1580T polymorphism the PCR primers, PCR product lengths, PCR programme, restriction enzymes and restriction product size were shown in Table 1. The PCR products and restriction products were visualized by using agarose gel electrophoresis technique.

Table 1. Polymorphism, the PCR primers, PCR product lengths, PCR programme, restriction enzyme and restriction product size for *SFTPB* C1580T polymorphism

Polymorphism	Primer sequence	PCR Product Lengths	PCR programme	Restriction enzyme	Restriction product size
SFTPB C1580T	R: 5'GTGAGCTTGCAGCCCTCTCA3'	278 bp	95°C 5 min	DdeI (HpyF3I)	C allele; 164,94,20
	F 5'CTCGAATTCACTCGTGAACTCCAGCACCC3'		95 °C 45 sec } 5 cycle		T allele; 184,94
			55 °C 45 sec		
			72 °C 1 min		
			95 °C 45 sec } 35 cycle		
			57 °C 45 sec		
			72 °C 1 min		
			72 °C 10 min		

SFTPB; surfactant protein B gene R; reverse primer, F; forward primer, bp; base pair

Statistical analysis

All statistical data were analyzed by Statistical Package for Social Sciences (SPSS) software version 15.0 for Windows (SPSS Inc., Chicago, IL). Results were reported as mean ± standard deviation. The relationships among *SFTPB* C1580T polymorphism and the clinical and demographical characteristics of acute bronchiolitis patients were examined by using Chi-square test ($\chi 2$) test or analysis of variance (ANOVA) statistics. Genotype distributions of the *SFTPB* C1580T polymorphism were analyzed by $\chi 2$ test. The specific allele and genotype distributions were analyzed by using Fisher's exact test. In both the patients and the healthy controls, genotype distributions of the *SFTPB* C1580T polymorphism were examined according to the Hardy-Weinberg Equilibrium (HWE). Odds ratios (ORs) with 95% confidence intervals (CIs) were used for the assessment of risk factors. The p-values smaller than 0.05 were considered significant and all p values were two-tailed.

RESULTS

The study included 103 infants with acute bronchiolitis (1-12 months) (mean age ± SD: 7.72 ±3.03months) and 102 healthy infants (mean age ± SD: 7.63±2.85 months). In infants with acute bronchiolitis, 43 were females and 60 were males and among the healthy subjects, 44 were females and 58

were males. There was no significant gender distribution difference among infants with acute bronchiolitis and healthy subjects (p =0.84). There was also no significant mean age difference among infants with acute bronchiolitis and healthy subjects (p= 0.82). The demographical characteristics of infant with acute bronchiolitis and healthy subjects in the present study groups have been shown in Table 2. Genotype distribution for *SFTPB* C1580T polymorphism fit the HWE. The baseline clinical characterizes of acute bronchiolitis patients were demonstrated in Table 3. The results showed no association between *SFTPB* C1580T polymorphism and clinical characteristics of acute bronchiolitis. The genotype distribution of *SFTPB* C1580T polymorphism has not also been associated with severity of acute bronchiolitis in infants Table 3 (p=0.138). On the other hand, hypoxia frequencies were 10.0%, 2.3% and 17.9% for CC, CT, and TT genotypes, respectively (p=0.055). The genotypic distribution and allelic frequencies of *SFTPB* C1580T polymorphism in acute bronchiolitis patients and healthy subjects have been shown in Table 4. For *SFTPB* C1580T polymorphism, the distribution of the wild CC genotype was found similar in acute bronchiolitis infants (19%) and healthy subjects (20%). The distribution of the CT genotype was higher in acute bronchiolitis infants (43%) than in healthy subjects (39%) and distribution of the TT genotype was found lower in acute bronchiolitis infants (38%) than in healthy subjects (41%). The genotype distribution of *SFTPB* C1580T polymorphism has not been associated with acute bronchiolitis (p=0.86). The frequency of the C allele was higher in acute bronchiolitis infants (41%) than healthy subjects (39%) while was an increase in the frequency of T allele in healthy subjects (61%) when compared to acute bronchiolitis infants (59%) (p=0.75). The distribution of dominant model CC: CT+TT was not found significant between infants with acute bronchiolitis and healthy subjects (19%:81% vs. 20%:80%, respectively, p=0.97). The distribution of recessive model CC+CT:TT was also not found significant between healthy subjects and infants with acute bronchiolitis (62%:38% vs. 59%:41%, respectively, p= 0.63).

Table 2. The demographical characteristics of acute bronchiolitis patients and control subjects

		Acute bronchiolitis patients n=103 (%)	**Control patients n=102 (%)**	***p* value**
Gender	Female	43 (42%)	44 (43%)	0.84
	Male	60 (58%)	58 (57%)	
Age (months) mean ± SD		7,72±3.03	7.63±2.85	0.82

Dates were examined by analysis of variance and χ2 test, Mean ± standard deviation values are showed for age, SD: standard deviation

Table 3. Baseline clinical features of the study patients with acute bronchiolitis stratified according to *SFTPB* C1580T polymorphism

			Genotype			
Characteristic		**Total n = 103**	**CC n=20**	**CT n=44**	**TT n=39**	***p***
Hypoxia	No	93 (90.3%)	18 (90.0%)	43 (97.7%)	32 (82.1%)	0.055
	Yes	10 (9.7%)	2 (10.0%)	1 (2.3%)	7 (17.9%)	
Fever		36.69±0.52	36.67±0.47	36.63±0.50	36.78±0.57	0.442
Respiratory rate/min		43.56±9.62	41.15±10.10	42.77±9.77	45.69±8.99	0.177
spO_2		94.06±9.56	95.60±3.99	93.52±13.70	93.87±4.85	0.718
WBC/mm3		10.56±3.28	11.39±2.79	10.59±3.28	10.10±3.49	0.363
CRP (mg/dL)		4.39±5.68	3.85±3.99	4.39±6.46	4.68±5.59	0.870
Severity group	Mild	57 (55.3%)	11 (55%)	26 (59.1%)	20 (51.3%)	0.138
	Moderate	31 (30.1%)	9 (45%)	12 (27.3%)	10 (25.6%)	
	Severe	15 (11.6%)	0 (0%)	6 (13.6%)	9 (23.1%)	

Data were analyzed by χ2 test and analysis of variance, CRP: C-reactive protein; spO2: Oxygen saturation; WBC: White blood cell count.

Table 4. Genotype distributions and allele frequencies of *SFTPB* C1580T polymorphism in acute bronchiolitis patients and control subjects

	Polymorphism *SFTPB* 1580 (C/T)	Acute bronchiolitis patients n =103 (%)	Control patients n =102 (%)	*p*	OR (95% CI)
Genotypes	CC	20 (19%)	20 (20%)	0.86	
	CT	44 (43%)	40 (39%)		
	TT	39 (38%)	42 (41%)		
	CC: CT+TT	20 (19%):83 (81%)	20 (20%):82 (80%)	0.97	0.99 (0.49-1.99)
	CC+CT:TT	64 (62%):39 (38%)	60 (59%):42 (41%)	0.63	1.15 (0.65-2.02)
Alleles	C	84 (41%)	80 (39%)	0.75	1.07 (0.72-1.59)
	T	122 (59%)	124 (61%)		

SFTPB; surfactant protein B gene

DISCUSSION

Acute bronchiolitis is a common disease that causes infant morbidity and mortality, especially in temperate climates, during the winter months[18]. This disease occurs in upper respiratory tract then diffuses to lower respiratory tract[19]. RSV is an important cause of acute bronchiolitis and infants with severe RSV bronchiolitis may be more susceptible to development of asthma and obstructive lung diseases in later childhood[3,20]. Predisposing factors for acute bronchiolitis in infants are premature birth, male sex, malnutrition, chronic lung and congenital heart disease, immunodeficiency, cystic fibrosis, smoking of family members, and low income[21]. The predisposing factors are not sufficient to explain the variation in the severity of acute bronchiolitis, and certain genetic factors likely contribute to the severity of this disease. There was also a significantly higher concordance in hospitalization rates between identical twins than in fraternal twins. Additionally, genetic studies on bronchiolitis showed that RSV bronchiolitis–associated genes frequently related to immunity[22].

SP gene polymorphisms are shown to associate with pulmonary diseases such as RSV -associated disease, tuberculosis, chronic obstructive pulmonary disease (COPD), cystic fibrosis, neonatal respiratory distress syndrome, and acute respiratory distress syndrome (ARDS)[23]. SP-B is a critical protein for normal lung function and plays pivotal roles in lowering alveolar surface tension and host defense[24,25]. SP-B is encoded by *SFTPB* gene which is located on the chromosome 2p[24]. In the *SFTPB* C1580T polymorphism, alteration from a C to T changes threonine amino acid in N-linked glycosylation site and the change may affects protein processing and folding[26].

To our knowledge, this preliminary study is the first to investigate the association between the *SFTPB* C1580T polymorphism and the susceptibility and clinical findings of acute bronchiolitis. According to results of our study, the genotype distribution and allele frequency of *SFTPB* C1580T polymorphism have not been associated with the disease (p=0.86 and p=0.75, respectively). In addition to the results showed no association between *SFTPB* C1580T polymorphism and clinical characteristics of acute bronchiolitis. However, the hypoxia incidence in TT genotype carriers was found to be higher than in those carrying CT genotype. Although no significant difference between mild, moderate and severe acute bronchiolitis groups detected, there was a tendency of TT being higher at severe acute bronchiolitis and CT genotype being higher at mild acute bronchiolitis.

Ruicheng et al.[17] also found that the *SFTPB* C1580T polymorphism was an important risk factor for COPD. Lin et al. found that the C allele was a risk factor for ARDS, whereas the T allele was considered to be a protective factor against ARDS[26]. Ge et al.[27] investigated whether the *SFTPB* C1580T variants may cause differential susceptible to *Pseudomonas aeruginosa* pneumonia in humanized *SFTPB* transgenic mice carrying either the *SFTPB* 1580 C or T allele. They found that in infected mice carrying *SFTPB* 1580/C allele, minimum surface tension was higher compared to uninfected control mice and surfactant activation had been inhibited in the *SFTPB* 1580/C mice with pneumonia. They also suggested that the mice carrying *SFTPB* 1580/C allele were more susceptible to *Pseudomonas aeruginosa* pneumonia than the mice carrying *SFTPB* 1580/T allele. Some studies showed that alveolar size and total lipid level were different between the mice carrying *SFTPB* 1580/C or T alleles. In the mice carrying *SFTPB* 1580/C allele, level of total lipid was higher when

compared to the mice carrying *SFTPB* 1580/T allele and the C allele might increase susceptibility to respiratory diseases[28]. In contrast to these findings, Ezzeldin et al. [29] the homozygous alleles (C/C and T/T) were found that associated with impairment of pulmonary functions. Knockdown of *SFTPB* decreases influenza A virus replication in vitro experiment, it seems that the *SFTPB* gene is important for life cycle of the virus[30]. The *SFTPB* may affects the life cycle of RSV which is major cause of acute bronchiolitis. However, there were no studies which showed the effect of *SFTPB* polymorphisms on the life cycle of RSV.

In conclusion, the *SFTPB* C1580T gene polymorphism plays no important role in susceptibility to acute bronchiolitis. There is main one limitation in our study that is our results could not be compared with different ethnic groups because the *SFTPB* C1580T gene polymorphism has not been studied in acute bronchiolitis. Further researches with different ethnic groups are necessary to ascertain the implications of *SFTPB* polymorphisms in acute bronchiolitis.

Yazar Katkıları: Çalışma konsepti/Tasarımı: SDA, ÖA, AG, BAS; Veri toplama: SDA, SSS, BAS; Veri analizi ve yorumlama: SDA, BAS; Yazı taslağı: SDA; İçeriğin eleştirel incelenmesi: SDA, ÖA, SSS, AG, ST, BAS; Son onay ve sorumluluk: SDA, ÖA, SSS, AG, ST, BAS; Teknik ve malzeme desteği: AG, BAS, ST; Süpervizyon: SDA, ÖA, BAS, AG; Fon sağlama (mevcut ise): yok.
Etik Onay: Çalışmamız 03.03.2015 tarihinde Gaziosmanpaşa Üniversitesi yerel Etik Kurulu tarafından onaylanmıştır (Sayı: 15-KAEK-040). Akut bronşiyolit hastalığında SFTPB C1580T polimorfizm genotip analizleri için kan örneklemesi konusunda hem akut bronşiyolitli bebeklerin hem de sağlıklı deneklerin ebeveynlerinden yazılı bilgilendirilmiş onam alındı.
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Ethical Approval: Our study was approved by the local Ethics Committee of Gaziosmanpasa University, Turkey on 3 March 2015 (Number: 15-KAEK-040). Written informed consent was gathered from parents of both acute bronchiolitis infants and healthy subjects, who were acknowledged about the blood sampling for the SFTPB C1580T polymorphism genotype analyses in acute bronchiolitis disease.
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