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Serum Chitotriosidase Activity in Bronchopulmonary Dysplasia: A Cross-Sectional Study

Bronkopulmoner Displazide Serum Kitotriozidaz Aktivitesi: Kesitsel Bir Çalışma

Fatma Tuba Eminoğlu[1], Elif Keleştemur[2], Özlem Doğan[3], Engin Köse[1], Zehra Şule Haskoloğlu[4], Nazan Çobanoğlu[5]

[1]*Ankara University Faculty of Medicine, Department of Pediatrics, Department of Pediatric Metabolism, Ankara, Turkey*
[2]*Ankara University Faculty of Medicine, Department of Pediatrics, Ankara, Turkey*
[3]*Ankara University Faculty of Medicine, Department of Biochemistry, Ankara, Turkey*
[4]*Ankara University Faculty of Medicine, Department of Pediatrics, Division of Pediatric Allergy and Immunology, Ankara, Turkey*
[5]*Ankara University Faculty of Medicine, Department of Pediatrics, Division of Pediatric Pulmonology, Ankara, Turkey*

Abstract

Amaç: Serum kitotriozidaz (*CHIT1*), hava yolu ve akciğer hastalıkları dahil olmak üzere enflamatuvar hastalıklarda kullanılan yeni bir biyobelirteçtir. Bugüne kadar, bronkopulmoner displazi (BPD) hastalarında enflamasyonu belirlemek için serum *CHIT1* aktivitesini değerlendiren bir çalışma yoktur. Bu çalışmanın amacı, serum *CHIT1* aktivitesinin BPD hastalarında enflamasyonu belirlemede yararlı bir belirteç olup olmadığını araştırmaktır.

Gereç ve Yöntem: Bu kesitsel çalışmada, BPD'li hastalarda (n=21) ve sağlıklı kontrollerde (n=19) demografik veriler ve serum *CHIT1* aktivitesi karşılaştırıldı. Tedaviye göre BPD hastaları inhale steroid tedavisi almayan (n=13) ve inhale steroid tedavisi alan (n=8) olmak üzere iki gruba ayrıldı ve yukarıda belirtilen tüm veriler bu iki grup arasında da karşılaştırıldı.

Bulgular: Laboratuvar parametrelerinin karşılaştırılmasında, sağlıklı grup ile BPD grubu arasında C-reaktif protein (CRP), albümin ve CRP/albümin oranı (CAO) açısından anlamlı fark bulunmadı. BPD grubunda [16,4±13,0 nmol/mL/h] sağlıklı gruba [12,1±8,3 nmol/mL/h] göre daha yüksek *CHIT1* aktivitesi saptanırken, bu fark istatistiksel olarak anlamlı değildi (p=0,456). Öte yandan, inhale steroid içermeyen BPD hastalarında *CHIT1* aktivitesi inhale steroid tedavisi alanlara göre anlamlı olarak daha yüksekti (p=0,025). Tüm çalışma popülasyonunda yaş ile serum *CHIT1* (r=0,26, p=0,025) arasında pozitif bir korelasyon vardı. Öte yandan laboratuvar parametrelerinin korelasyon analizinde tüm çalışma popülasyonunda *CHIT1* düzeyi ile CRP, albümin ve CAO arasında anlamlı bir ilişki yoktu.

Sonuç: Bu çalışma, inhale steroid tedavisinin BPD'de serum *CHIT1* aktivitesini azaltabileceğine dair bazı kanıtlar sağlasa da, daha fazla hasta içeren ileri çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Bronkopulmoner Displazi, Kitotriozidaz Aktivitesi, enflamasyon

Öz

Objectives: Serum chitotriosidase (*CHIT1*) is a novel and promising biomarker of inflammatory diseases including airway and lung diseases. To date, there are no studies regarding serum *CHIT1* activity in bronchopulmonary dysplasia (BPD) patients for determining inflammation. The aim of this study was to investigate whether serum *CHIT1* activity is a useful marker for determining inflammation in BPD patients.

Materials and Methods: In this cross-sectional study, demographic data and serum *CHIT1* activity in patients with BPD (n=21) and in healthy controls (n=19) were compared. According to therapy, BPD patients were divided into two groups: inhaled steroid-free (n=13) and inhaled steroid-positive (n=8), and all the aforementioned data were compared between these two groups also.

Results: In the comparison of laboratory parameters, no significant differences were in C-reactive protein (CRP), albumin and CRP/albumin ratio (CAR) between healthy group and BPD group. While higher *CHIT1* activity was detected in BPD group [16.4±13.0 nmol/mL/h] compared to healthy

Address for Correspondence/Yazışma Adresi: Fatma Tuba Eminoğlu
Ankara University Faculty of Medicine, Department of Pediatrics, Department of Pediatric Metabolism, Ankara, Turkey
Phone: +90 505 249 95 72 **E-mail:** tubaeminoglu@yahoo.com **ORCID ID:** orcid.org/0000-0002-5880-1113
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group [12.1±8.3 nmol/mL/h], this difference was not statistically significant. (p=0.456). On the other hand, *CHIT1* activity was significantly higher in inhaled steroid-free BPD patients (p=0.025) than inhaled steroid-positive ones. There was a positive correlation between ages and serum *CHIT1* (r=0.26, p=0.025) in all subjects. On the other hand, in the correlation analysis of laboratory parameters, there was no correlation between *CHIT1* level and CRP, albumin and CAR in all subjects.

Conclusion: Although this study provided some evidence that inhaled steroid treatment might decrease serum *CHIT1* activity in BPD, further studies with more patients are necessary.

Key Words: Bronchopulmonary Dysplasia, Chitotriosidase Activity, Inflammation

Introduction

The lining of the digestive tracts of many insects, the exoskeleton of crustaceans (crabs, shrimp, etc.) and insects, the cell walls of bacteria and fungi, mushrooms, and the microfilariae sheath of parasitic nematodes contain chitin (1-3).

Hosts produce the chitinases, the key degrading enzymes to against to organisms include chitin (4). Although they do not have chitin, a wide range of organisms from prokaryotes to eukaryotes, including mammals, express chitinases (5,6). Humans have acidic mammalian chitinase and chitotriosidase (*CHIT1*) (6).

Gaucher cells, neutrophils, and lung macrophages express *CHIT1*. While, *CHIT1* expression in monocyte-derived macrophages is stimulated by proinflammatory cytokines and lipopolysaccharide, interferon-γ and interleukin-4 inhibit *CHIT1* expression (7,8). Increased *CHIT1* expression have been described not only in lysosomal storage disorders but also in bacterial and fungal infections, chronic inflammation such as atherosclerosis and sarcoidosis, and neurodegenerative diseases (9,10).

YKL-40 is a chitinase-like protein and the dysregulation of YKL-40, seen in acute or chronic inflammation and tissue remodeling such as asthma, cystic fibrosis (CF) and bronchopulmonary dysplasia (BPD) (11-13).

As inflammation has a key role in the pathogenesis of BPD, measurement and then suppression of inflammation is important for this disease. Therefore, we aimed in this study to investigate whether serum *CHIT1* activity is a useful marker for determining inflammation in BPD patients. To the best of the authors' knowledge, there are no studies regarding serum *CHIT1* activity in BPD patients for determining inflammation.

Materials and Methods

Study Population and Study Design

In this cross-sectional study, BPD patients who were admitted to the pediatric pulmonology outpatient clinic and age-sex matched healthy control children who were admitted to the general outpatient clinic of our hospital, were consecutively enrolled to the study between September 2018 and September 2019.

Mild and moderate BPD diagnosed patients were enrolled in study. According to therapy, BPD patients were divided into two groups: inhaled steroid-free and inhaled steroid-positive. All patients treated with steroid were administered prophylactic inhaled budesonide (125 microgram twice daily) to avoid asthma and chronic obstructive lung disease. Inclusion criteria were as follows: a) For all cases, to be younger than 6 years old; b) For BPD patients, to be without any acute infection; c) For the control group, not to have any chronic diseases and to be without any acute infection.

Demographic data (age, gender), physical examination findings [weight (kg) Height (cm), Body mass index (BMI) Z score] and blood samples drawn for serum *CHIT1* activity and serum C-reactive protein (CRP) levels, and serum CRP and albumin ratio (CAR) - a biomarker to assess the inflammation and oxidative stress in many diseases including restless leg syndrome (14), Crohn's disease (15), and to predict mortality and morbidity in patients on parenteral nutrition (16) - were collected from children in the groups. Serum samples were stored at -80 °C until the analysis of *CHIT1* activity. Collected data were compared among groups.

Chitotriosidase Assay

Fasting venous blood was collected in the 8.5 mL plastic non-anticoagulant evacuated tubes (BD Vacutainer Systems, BectonDickinson, Plymouth, UK). The sample tubes were left in upright position for 30 min at room temperature for complete clot formation. All were then centrifuged at 2500 x g for 10 minutes (according to the instruction of the tube manufacturer). Serum samples were checked visually for hemolysis and lipemia for possible interferences. Serum samples were aliquoted into 1.5 mL Eppendorf (Eppendorf, Milano, Italy) tubes. Serum samples were stored -80 °C about 9 months (until chitotriosidase assay).

The *CHIT1* was analyzed essentially as described by Hollak et al. (17). Briefly, 5 µL of plasma was incubated with 100 µL of 22 µmol/L 4-methylumbelliferryl-β-D-N-N'-N''-triacetylchitotriosidase (Sigma-Aldrich ChemieGmBH, Taufkirchen, Germany) in phosphate-citrate buffer; pH=5.2, for 1 hr at 37.0 °C in darkness. The reaction was stopped by the

addition of 1 mL 0.25 mol/L glycine-NaOH. In the quantitative method, the fluorescence of 4-methylumbelliferone was measured in a Microfluor plate by a fluorometer (Thermo-Fisher Varioskan, US) at excitation and emission wavelengths of 360 nm and 450 nm, respectively. CHT activity was expressed as nanomoles of substrate hydrolyzed per milliliter per hour (nmol/mL/h). In the study 10 serum samples activity mean was 10.06, standard deviation was 1.53 and intraassay coefficients of variation were 15.22% (17,18).

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Ethical approval was obtained from Ankara University Clinical Research Ethics Committee (approval no: 21.311.17, date: 25.12.2017). Written informed consent was obtained from parents of the children enrolled to the study.

Statistical Analysis

Categorical variables were expressed as number and percentage (%) and assessed by means of the chi-square test. Continuous variables [mean ± standard deviation (minimum-maximum)] were assessed with Mann-Whitney U test. Spearman's correlation coefficient was used to analyze the associations between continuous variables (age, *CHIT1*, albumin, CAR levels). The Statistical Package for the Social Sciences (SPSS) (version 21.0; SPSS, Chicago, IL) computer software was used for data analysis. A two-tailed p-value <0.05 was considered significant.

Results

Forty subjects were enrolled in this cross-sectional study. Of these 21 were BPD (21 female) and 19 were healthy children (13 female). The mean age of study population was 34.9±16.1 (6-67) months. There were no significant differences between BPD diagnosed patients and healthy subjects in terms of age and gender (p=0.056 and p=0.055) (Table 1).

While the weight and height of healthy group were found to be higher than BPD group (p<0.0001 and p=0.001), no significant difference in BMI z score was determined between two groups (p=0.062) (Table 1).

In the comparison of laboratory parameters, no significant differences were in CRP, albumin and CAR between healthy group and BPD group (Table 1). While higher *CHIT1* activity was detected in BPD group [16.4±13.0 (0-52.8) nmol/mL/h] compared to healthy group [12.1±8.3 (0-37.4) nmol/mL/h], this difference was not statistically significant (p=0.456) (Table 1).

In BPD diagnosed patients, while 8 were treated with inhaled steroid and 13 of them were not treated with inhaled steroid (Table 2). In the comparison of BPD diagnosed patients treated with inhaled steroid and inhaled steroid free patients, there were no significant differences in terms of age, gender, weight, height and Z score. Serum *CHIT1* activity was significantly lower in patients treated with inhaled steroid BPD patients [9.1±6.8 (0.001-0.24) nmol/mL/h] than inhaled steroid-free patients [20.9±14.2 (3.9-52.8) nmol/mL/h] (p=0.014) (Table 2).

In the comparison of inhaled steroid free BPD patients and healthy controls, there was almost statistically significant

Table 1: Demographic and laboratory findings of control group and BPD group

Parameters	Total (n=40)	Healthy group (n=19)	BPD group (n=21)	p-value
Age (month), mean ± SD (min.-max.)	34.9±16.1 (6-67)	40.3±16.2 (15-67)	30.2±15.0 (6-61)	0.056
Gender (F/M), n (%)	21 (52.5)/19 (47.5)	13 (68.4)/6 (31.6)	8 (38.1)/13 (61.9)	0.055
Weight (kg), mean ± SD (min.-max.)	12.9±3.8 (3.6-19.6)	15.2±3.0 (10.4-19.6)	10.8±3.3 (2.6-17.0)	<0.0001
Height (cm), mean ± SD (min.-max.)	89.9±14.4 (47-114)	97.6±10.1 (77-114)	83.0±14.3 (47-102)	0.001
BMI Z score mean ± SD (min.-max.)	-0.77±1.71 (-6.04-2.84)	-0.16±0.02 (-2.34-2.84)	-1.31±1.85 (-6.04-2.1)	0.062
CRP (mg/dL), mean ± SD (min.-max.)	1.4±2.3 (0.2-11.2)	1.2 ±1.4 (0.2-5.81)	1.7±2.9 (0.2-11.2)	0.588
Albumin (g/L), mean ± SD (min.-max.)	44.9±2.7 (35.4-57.8)	45.8±2.6 (40.7-49.6)	44.2±4.4 (35.4-57.8)	0.072
CAR, mean ± SD (min.-max.)	0.03±0.06 (0.003-0.27)	0.026±0.10 (0.01-1.39)	0.039±0.01 (0.01-0.27)	0.524
CHIT1 (nmol/mL/h), mean ± SD (min.-max.)	14.3±11.2 (0-52.8)	12.1±8.3 (0-37.4)	16.4±13.0 (0-52.8)	0.456

BPD: Bronchopulmonary dysplasia, CAR: CRP/albumin ratio, CHIT1: Chitotriosidase, CRP: C-reactive protein, F: Female, M: Male, min.-max.: Minimum-maximum, SD: Standard deviation

difference in terms of *CHIT1* activity [20.9±14.2 (3.9-52.8) vs 12.1±8.3 (0-37.4)] (p=0.065).

There was a positive correlation between ages and serum *CHIT1* (r=0.26, p=0.025) in all subjects. On the other hand, in the correlation analysis of laboratory parameters, there was no correlation between *CHIT1* level and CRP, albumin and CAR in all subjects. Furthermore, no significant correlation between *CHIT1* levels and CRP and CAR was determined in BPD group, and subgroups (inhaled steroid treated patients and inhaled steroid free patients). A positive correlation between *CHIT1* and albumin levels were detected in BPD diagnosed patients and patients treated with inhaled steroid (r=0.458, p=0.037; r=0.731, p=0.04).

Discussion

In CF, YKL-40 bronchoalveolar lavage fluid levels consist with the inflammation of airway and the infection of lung disease in early CF and inversely correlate with lung function (11). In another study, the systemically increase of YKL-40 levels in CF patients was defined (19). These findings suggest that YKL-40 may be a reliable biomarker to assess disease severity, chronic airway inflammation and airflow obstruction in CF patients. Similar relations in patients with chronic obstructive pulmonary disease, asthma and smokers were detected with the activity of *CHIT1* compared to healthy control subject (20-22). To the best of the authors' knowledge, there are no studies regarding serum *CHIT1* activity in BPD patients for determining inflammation. Although the results of this study did not support our hypothesis that serum *CHIT1* activity is a useful biomarker in the detection of inflammation in BPD patients, this is the first reported study evaluating serum *CHIT1* activity in BPD. However, we believe that this hypothesis should be evaluated in larger population.

It is well known that due to the exogenous chitin from fungi or dust mites, an accumulation of chitin could be present in the lungs which could initiate an exaggerated pro-inflammatory response without active *CHIT1*. In a healthy population, *CHIT1* activity is very low and originates in the circulating polymorphonuclear cells (23). The *CHIT1* gene is localized in 1q31-q32 chromosome, which includes 12 exons, and covers 20 kb of DNA (18). The complete lack of *CHIT1* activity is presented in individuals with homozygous for a relatively common 24-bp duplication in exon 10 of *CHIT1* (rs3831317) (23). *CHIT1* deficiency in humans appears as an autosomal incompletely dominant disorder. While, no activity is detected in homozygous subjects for the defective allele, almost half-normal activities in heterozygous subjects are revealed. In fact, a high incidence of *CHIT1* gene mutations was found in different populations (23). James et al. (24) analyzed the effect of common polymorphisms in the *CHIT1* gene and their results supported that *CHIT1* activity is subject to genetic regulation. They have demonstrated that *CHIT1* genes strongly affect serum *CHIT1* levels. In the present study, we did not evaluate *CHIT1* gene mutations of the patients and the control group, an important factor that might influence the results.

Table 2: Demographic and laboratory findings of BPD patients treated and not treated with inhaled steroid

Parameters	BPD group (n=21)		p-value
	Inhaled steroid treated patients (n=8)	Inhaled steroid-free patients (n=13)	
Age (month), mean ± SD (min.-max.)	24.0±12.3 (6-48)	34.0±15.7 (8-62)	0.095
Gender (F/M), n (%)	1 (12.5)/7 (87.5)	7 (53.8)/8 (46.2)	0.074
Weight (kg), mean ± SD (min.-max.)	10.9±3.0 (6.8-17.0)	10.7±3.6 (3.6-17.0)	0.772
Height (cm), mean ± SD (min.-max.)	81.3±13.7 (58-101)	84.1±15.1 (47-102)	0.468
BMI Z score mean ± SD (min.-max.)	-1.02±1.4 (-3.88-0.89)	-1.49±2.1 (-6.04-0.21)	0.664
CRP (mg/dL), mean ± SD (min.-max.)	1.9±3.2 (0.2-9.6)	1.5±2.9 (0.4-11.2)	0.664
Albumin (g/L), mean ± SD (min.-max.)	42.4±3.6 (35.4-46.1)	45.4±4.6 (41.1-57.8)	0.137
CAR, mean ± SD (min.-max.)	0.046±0.08 (0.01-0.24)	0.035±0.07 (0.01-0.27)	0.717
CHIT1 (nmol/mL/h), mean ± SD (min.-max.)	9.1±6.8 (0-22.7)	20.9±14.2 (3.9-52.8)	0.014

BPD: Bronchopulmonary dysplasia, CAR: CRP/albumin ratio, CHIT1: Chitotriosidase, CRP: C-reactive protein, F: Female, M: Male, min.-max.: Minimum-maximum, SD: Standard deviation, BMI: Body mass index

James et al. (24) also found that the other factor affecting *CHIT1* activity was age. They have demonstrated a positive correlation with the serum levels of *CHIT1* and age. In another study, *CHIT1* activity was analyzed in adult sarcoidosis patients with a control group (25). The mean ± SD age was 52.2±17.2 years and mean ± SD *CHIT1* activity was 34.2±13.8 nmol/mL/h in the control group in their study. In their adult based study, Bargagli et al. (22) found that mean ± SD *CHIT1* activity was 16.13±13.27 nmol/mL/h in the control group. In the present study, we enrolled children younger than 6 years old and mean (minimum-maximum) serum *CHIT1* activity in the control group was 12.1 (0-37.4) nmol/mL/h. We detected a weak positive relation between age and *CHIT1* levels of BPD patients. The juvenility of the children that were enrolled in the present study might have an important impact on those results. In their study, James et al. (24) did not observe any relationships between *CHIT1* with corticosteroid therapies. They found no effect of steroid treatment on *CHIT1* activity in patients with asthma. The serum *CHIT1* activity was higher in inhaled steroid-free BPD patients compared to inhaled steroid-using ones in the present study, which was not compatible with the aforementioned study.

Not only the serum activity of *CHIT1* but also serum CRP and CAR levels of BPD patients and healthy controls were not statistically different. This finding might be related to the lack of inflammation in BPD patients enrolled in our study.

Study Limitations

There are several limitations of this study. First, the sample size and subgroup size are low. In the comparison of healthy and BPD groups, with an error rate of 5% the power of study is 25% while the power of study is 77% in the comparison of inhaled steroid treated patients and inhaled steroid-free patient groups. We believe that evaluation of *CHIT1* activity in larger groups led us to obtain more significant results. Second, it is well known that 6-10% of population are genetically *CHIT1* activity negative which may effect our results (23). Third, in this study we only evaluated the CRP albumin and CAR level with *CHIT1* activity. However, it would be better if we analyzed other inflammatory markers such as erythrocyte sedimentation rate and procalcitonin. Finally, we did not analysis the relation between severity of BPD and *CHIT1* level due to small number of subgroups.

Conclusion

In this study, serum *CHIT1* activity in BPD patients was not sufficiently statistically different from healthy children to accept it as a potential biomarker for detecting airway inflammation in BPD patients. Although this study provided some evidence that inhaled steroid treatment might decrease serum *CHIT1* activity in BPD, further large population studies are needed to confirm these findings.

Ethics

Ethics Committee Approval: Ethical approval was obtained from Ankara University Clinical Research Ethics Committee (approval no: 21.311.17, date: 25.12.2017).

Informed Consent: Written informed consent was obtained from parents of the children enrolled to the study.

Peer-reviewed: Externally peer-reviewed.

Authorship Contributions

Concept: F.T.E., El.K., N.Ç., Design: F.T.E., Ö.D., Data Collection and Processing: Ö.D., E.K., Z.Ş.H., Analysis or Interpretation: F.T.E., E.K., Literature Search: F.T.E., El.K., E.K., Writing: F.T.E., E.K.

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