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Evaluation of serum interleukin-33 and gene polymorphisms in patients with bronchial asthma

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ABSTRACT

Aims: Asthma is a chronic inflammatory disease of the airways. The aim of the study was to investigate the association between serum interleukin (IL)-33 level and IL-33 gene rs1342326, rs1929992 and rs3939286 polymorphisms in patients with asthma.

Methods: Ninety patients aged 18 years and older, who were diagnosed with asthma, and 90 healthy control subjects were included in the study. Serum IL-33 levels were measured by enzyme-linked immunosorbent assay; and IL-33 gene polymorphisms were studied by real-time polymerase chain reaction.

Results: IL-33 level was higher in patients with asthma (4.16±0.88 pg/mL) compared to the control subjects (1.28±0.37 pg/mL) ($p<0.001$). The frequencies of AA, CC and AC alleles for the rs1342326 polymorphism were 52 (57.8%), 5 (5.6%) and 33 (36.7%) in the patient group, and 53 (58.9%), 7 (7.8%) and 30 (33.3%) in the control group, respectively. The frequencies of CC, TT and CT alleles for the rs1929992 polymorphism were 17 (18.9%), 31 (34.4%), and 42 (46.7%) in the patient group and 13 (14.4%), 41 (45.6%), and 36 (40%) in the control group, respectively. The frequencies of CC, TT and CT alleles for the rs3939286 polymorphism were 46 (51.1%), 7 (7.8%), and 37 (41.1%) in the patient group and 44 (48.9%), 9 (10%), and 37 (41.1%) in the control group, respectively. There was no statistically significant difference between the groups in terms of IL-33 gene polymorphisms (rs1342326, rs1929992 and rs3939286) ($p=0.784$, $p=0.304$, $p=0.863$, respectively).

Conclusions: The current study showed increased serum IL-33 levels in patients with asthma compared to healthy controls but gene polymorphism studies did not show any significant difference.

Introduction

Asthma is a major public health problem that affects approximately 300 million people worldwide. Current evidence suggests asthma is a disease in which genetic origins are important and which has complex inheritance characteristics (1). Interleukin (IL)-33 is a new member of the IL-1 cytokine family (2) and is generally released from epithelial cells (eye, skin, intestine, airway, lymphoid organs, keratinocytes, smooth muscle cells), fibroblasts and endothelial cells that are associated with the external environment (3). IL-33 is known to activate both hereditary and acquired immunity. In hereditary immunity, IL-33 activates group 2 innate lymphoid cells (ILC2s), leading to the release of IL-4, IL-5 and IL-13. In addition to ILC2s, it stimulates macrophages, eosinophils, masts and basophils. IL-33-activated dendritic cells increase IL-5 and IL-13 secretion by stimulating Th2 cells (4). Th2 cells promote B cell proliferation and increases secretion of immunoglobulin (Ig) M, IgG1, IgA and IgE antibodies (4) and they also play a critical role in allergic immune response including airway eosinophilia and mucus hyperplasia (5). IL-33 plays a role in the pathogenesis of many diseases, and studies of the entire genome have implicated IL-33 in the pathogenesis of asthma (6). There is a need for new drugs in patients with asthma. IL-33 can be an important target for potential new treatment studies in asthma treatment and biological treatments that are able to control IL-33 levels in patients with asthma may be a potential new approach (7).

The aim of the study was to investigate the association between serum IL-33 level and IL-33 gene rs1929992 (6.251.588. C/T genotype), rs3939286 (6.210.099. C/T genotype) and rs1342326 (6.190.076. A/C genotype) polymorphisms in patients with asthma.

Methods

The study was designed as a single-center, prospective case-control study. In the study, 90 patients diagnosed with asthma according to the GINA 2015 criteria were recruited in the pulmonary outpatient clinic of our hospital between March 2016 and June 2017. Ninety healthy control subjects were recruited in the study. Exclusion criteria were age under 18 years and presence of conditions other than asthma. Written informed consent was obtained from all participants. The study was approved by the Local Ethical Committee (Gülhane Military Medical Academy Haydarpasa Training and Research Hospital, approval number: 26.02.2016/1491-46-16/1539). The procedures were in line with the Helsinki Declaration.

Serum IL-33 Levels

The Serum IL-33 level was measured by an enzyme-linked immunosorbent assay (ELISA) using a Biotek elx-800 device, Gen5 software and the appropriate commercial kits (Human IL-33 Coated ELISA Kit BMS2048, Bender MedSystems GmbH, Wien, Austria), as per the manufacturer's instructions.

IL-33 Gene Polymorphisms

A 2-mL blood sample was collected in ethylenediaminetetraacetic acid tubes from both the patient and control group subjects. The blood samples were stored in a freezer at -80 °C until the day of analysis, when the blood samples were taken out of freezer and left to thaw at room temperature. VIC and FAM probes were used for polymorphism detection. VIC adenine (A) and FAM cytosine (C) allele was determined for the rs1342326 polymorphism, VIC C, FAM thymine (T) for the rs1929992 polymorphism, and VIC C, FAM T allele for the rs3939286 polymorphism.

Reverse Transcription-polymerase Chain Reaction

Genomic DNA was prepared from peripheral blood samples, using standard protocols. All DNA samples were diluted to a final concentration of 50 ng/µL. DNA samples, 2X master mix (TaqMan Genotyping Master Mix, Applied Biosystems, California, USA) and 20X assays [TaqMan single nucleotide polymorphism (SNP) Genotyping Assays, Applied Biosystems, California, USA] were vortexed and centrifuged for 1 minute. A 96-microwell plate was prepared. Each well was filled with 1.25 µL assay, 12.5 µL of master mix and 11.25 µL of DNA sample (20 ng) to yield a final total reaction volume of 25 µL. No DNA was added to at least two wells for no template control.

Statistical Analysis

The study data were uploaded into IBM SPSS Statistics 23 software. In the analysis of the study data, descriptive statistics were presented as mean and standard deviation for numerical variables, and as a frequency distribution for categorical variables. A chi-square test was used to evaluate the relationship between two independent categorical variables. An independent samples t-test was used to evaluate the significance of differences between the two groups. The Hardy-Weinberg equilibrium for the two groups showed no disequilibrium in genotype distribution

Results

Evaluation of Demographic and Clinical Findings

The study included a total of 180 participants, including 90 patients (53 male/37 female) and 90 control subjects (27 male/63 female). The mean age was 36.6±17.2 years in the patient group and 34.9±8.8 years in the control group. There was no statistically significant difference in the age distribution between the two groups ($p=0.406$), although a significant difference was noted in gender distribution ($p<0.001$). The mean serum IL-33 level was 4.16±0.88 pg/mL in the patient group and was 1.28±0.37 pg/mL in the control group. An independent samples t-test showed a statistically significant difference between the groups in terms of the mean IL-33 levels ($p<0.001$). The mean IL-33 level was significantly higher in the patient group than in the control group. The mean age of asthma onset was 25.54±15.05

years in the patient group. The mean asthma control test (ACT) score was 12.98±4.93 in the patient group. The mean forced expiratory volume in the first second (FEV1) % was 77.53±21.80 and the mean total IgE level was 366.71±817.13 IU/mL in the patient group (Table 1).

The age of asthma onset was <18 years in 24 (26.7%) patients, between 18 and 39 years in 50 (55.5%) patients, between 40 and 59 years in 14 (15.6%) patients, and ≥60 years in two (2.2%) patients. The ACT score was ≥20 points (good asthma control) in 11 (12.2%) patients and <20 points (poor asthma control) in 79 (87.8%) patients. The total IgE level was ≤165 IU/mL in 61 (67.8%) patients and >165 IU/mL in 29 (32.2%) patients (Table 2).

Allele and Genotype Analyses

The rs1342326, rs1929992 and rs3939286 variants of the IL-33 gene polymorphism were studied in 90 patients in the asthma group and in the 90 healthy control subjects. In the patient group, 52 (57.8%) patients were VIC homozygous (AA), five (5.6%) patients were FAM homozygous (CC), and 33 (36.7%) patients were heterozygous (AC) for the rs1342326 polymorphism. The frequencies of AA, CC and AC alleles for the same polymorphism in the control group were 53 (58.9%), 7 (7.8%), and 30 (33.3%), respectively.

In the patient group, 17 (18.9%) patients were VIC homozygous (CC), 31 patients (34.4%) were FAM homozygous (TT), and 42 (46.7%) patients were heterozygous (CT) for the rs1929992 polymorphism. The frequencies of CC, TT, and CT alleles for the same polymorphism in the control group were 13 (14.4%), 41 (45.6%) and 36 (40%), respectively.

In the patient group, 46 (51.1%) patients were VIC homozygous (CC), 7 (7.8%) patients were FAM homozygous (TT), and 37 (41.1%) patients were heterozygous (CT) for the rs3939286 polymorphism. The frequencies of CC, TT and CT alleles of the same polymorphism in the control group were 44 (48.9%), 9 (10%), and 37 (41.1%), respectively. A chi-square test showed no statistically significant difference in the genotype distribution of the three polymorphisms among the three groups (p=0.784, p=0.304, p=0.863, respectively) (Table 3).

In the alleles for the rs1342326 polymorphism in the patient group, 137 (76.1%) A alleles and 43 (23.9%) C alleles were identified. The numbers of A and C alleles for the same polymorphism in the control group were 136 (75.6%) and 44

Table 1. Demographic data, IL-33 levels, asthma control test scores, FEV1% and total IgE levels in the patient and control groups

		Control group (n=90)	Patient group (n=90)	p
Age		34.9±8.8	36.6±17.2	0.406
Gender	**Male**	27 (30%)	53 (58.9%)	<0.001
	Female	63 (70%)	37 (41.1%)	
IL-33 (pg/mL)		1.28±0.37	4.16±0.88	<0.001
Age of asthma onset (years)		-	25.54±15.05	-
ACT score		-	12.98±4.93	-
FEV1 (%)		-	77.53±21.8	-
Total IgE		-	366.71±817.13	-

IL-33: Interleukin-33, IgE: Immunoglobulin E, ACT: Asthma control test, FEV1: Forced expiratory volume in one second

Table 2. Distribution of age at asthma onset and asthma control test scores

		n	%
Age of asthma onset (years)	<18	24	26.7
	18-39	50	55.5
	40-59	14	15.6
	≥60	2	2.2
ACT score	Poor asthma control	79	87.8
	Good asthma control	11	12.2
Total IgE	Negative	61	67.8
	Positive	29	32.2

ACT: Asthma control test, IgE: Immunoglobulin E

Table 3. Evaluation of interleukin-33 gene polymorphisms in the patient and control groups

			Groups		Total	p
			Control group	Patient group		
rs1342326	AA	n	53	52	105	0.784
		%	58.9	57.8	58.3	
	AC	n	30	33	63	
		%	33.3	36.7	35.0	
	CC	n	7	5	12	
		%	7.8	5.6	6.7	
rs1929992	CC	n	13	17	30	0.304
		%	14.4	18.9	16.7	
	CT	n	36	42	78	
		%	40.0	46.7	43.3	
	TT	n	41	31	72	
		%	45.6	34.4	40.0	
rs3939286	CC	n	44	46	90	0.863
		%	48.9	51.1	50.0	
	CT	n	37	37	74	
		%	41.1	41.1	41.1	
	TT	n	9	7	16	
		%	10.0	7.8	8.9	

A: adenine, C: cytosine, T: thymine
rs1342326: AA: VIC homozygous, CC: FAM homozygous, AC: heterozygous
rs1929992: CC: VIC homozygous, TT: FAM homozygous, CT: heterozygous
rs3939286: CC: VIC homozygous, TT: FAM homozygous, CT: heterozygous

(24.4%), respectively, The number of C alleles was 76 (42.2%) and the number of T alleles was 104 (57.8%) in the rs1929992 polymorphism. The numbers of C and T alleles for the same polymorphism in the control group were 62 (34.4%) and 118 (65.6%), respectively. In the rs3939286 polymorphism, the number of C alleles was 129 (71.7%) and the number of T alleles was 51 (28.3%). The numbers of C and T alleles for the same polymorphism in the control group were 125 (69.4%) and 55 (30.6%), respectively. A chi-square test showed no statistically significant difference in the allele distribution of the three polymorphisms among the three groups (p=0.902, p=0.129, p=0.644, respectively) (Table 4).

Table 4. Evaluation of allele distribution in the patient and control groups

			Groups		Total	p
			Control group	Patient group		
rs1342326	A	n	136	137	273	0.902
		%	75.6	76.1	75.8	
	C	n	44	43	87	
		%	24.4	23.9	24.2	
rs1929992	C	n	62	76	138	0.129
		%	34.4	42.2	38.3	
	T	n	118	104	222	
		%	65.6	57.8	61.7	
rs3939286	C	n	125	129	254	0.644
		%	69.4	71.7	70.6	
	T	n	55	51	106	
		%	30.6	28.3	29.4	

A: Adenine, C: Cytosine, T: Thymine

Discussion

The present study investigated serum IL-33 levels and the rs1342326, rs1929992 and rs3939286 variants of the IL-33 gene polymorphisms in patients with asthma and healthy controls. The study revealed that there was a significant difference in serum IL-33 levels between the patient group and the healthy controls, while there was no statistically significant difference between the groups in terms of the studied rs1342326, rs1929992 and rs3939286 polymorphisms.

Koca et al. (8) reported that there was no significant difference in serum IL-33 levels between the patients with Behçet's disease and controls and IL-33 may be related to an increase in Th2 cytokine, while Behçet's disease is known to be related to an increase in Th1 cytokine. They also concluded that there was no significant relationship between the development of Behcet's disease and the rs1929992 and rs7044343 SNPs (8). IL-33 levels were higher in patients with systemic sclerosis than in the control group in the study by Yanaba et al. (9). In a study on patients with systemic lupus erythematosus (SLE), Xu et al. (10) studied the rs1929992 and rs7044343 variants of the IL-33 gene polymorphisms. They showed a significant relationship between the rs1929992 polymorphism and SLE and concluded that this polymorphism could serve as a potential biomarker for SLE (10). Higher IL-33 levels in patients with ankylosing spondylitis than in the control group were reported by Han et al. (11). In a study on patients with rheumatoid arthritis (RA), Li et al. (12) found that the rs7044343 polymorphism reduced IL-33 expression and suggested that this variant was a protective genotype against RA. These studies indicated that IL-33 had both pro-inflammatory and anti-inflammatory effects. The protective effect of IL-33 against myocardial infarction and the development of atherogenesis has been demonstrated (13). High IL-33 levels have been also reported in allergic diseases as asthma and anaphylactic shock (14). The intraperitoneal administration of anti-IL-33 prior to sensitization with ovalbumin administration reduced serum IgE levels, eosinophils and lymphocytes and the IL-4, IL-5 and IL-13 levels in bronchoalveolar lavage fluid, and caused a significant reduction in eosinophilic inflammation and mucus secretion in the lung tissue (15). IL-33 levels were significantly higher in the asthma group than in the control group in the present study. IL-33 blockade may be investigated as a new therapeutic target in patients with asthma.

Sakashita et al. (16) reported a significant correlation between the rs1929992 SNP on the IL-33 gene region and Japanese cedar pollinosis (Odds ratio: 1.82; 95% confidence interval: 1.00-3.31; p=0.048). The rs1929992 gene polymorphism was found to be a risk factor for SLE (17). The present study revealed no correlation between the rs1929992 SNP and asthma.

Charrad et al. (18) reported significantly higher IL-33 levels in the asthma group than in the control group in consistence with the present study. They concluded that the rs1342326 polymorphism reduced the risk of asthma and the rs1342326 C allele reduced the risk of development of atopic asthma (18). Schröder et al. (19) showed a positive correlation between rs1342326 SNP and seasonal allergic rhinitis. There was a significant relationship between rs1342326 SNP in the IL-33 gene region and asthma in a study by Moffatt et al. (20). In contrast, the present study found no correlation between rs1342326 SNP and asthma.

In previous studies, there was no significant relationship between the rs3939286 variant of the IL-33 gene polymorphism and preeclampsia (21) and gout (22). López-Mejías et al. (23) suggested that the rs3939286 gene polymorphism had a protective role against the development of subclinical atherosclerosis. Latiano et al. (24) revealed a statistically significant relationship between achalasia and rs3939286 SNP. There was a significant correlation between nasal polyps and the presence of rs3939286 SNP in the IL-33 gene region in another study (25). The present study found no statistically significant relationship between rs3939286 SNP and asthma.

Predisposing genes for asthma were reported to be transferred to the children from their mothers with atopic asthma (26). There was a significant relationship between the defensin β-1 polymorphism and asthma in female patients, whereas no such relationship was reported in males and this gender difference was considered to be linked to hormone-dependent processes in females (27). The lack of an association between the studied IL-33 gene polymorphisms and asthma may be caused by gender difference, because there were significant gender differences between the patient and control groups in the present study.

The strength of this study was its being a prospective case control study. The patients and controls were unmatched, so chi-square test and unconditional logistic regression analysis were used for statistical analysis. Since Hosmer and Lemeshow test showed that goodness of fit test of the model was not found to be statistically compatible ($p>0.05$) in the study, odds ratio value could not be calculated. The limitation of this study was that the patients with asthma had different asthma phenotypes such as eosinophilic asthma, non-eosinophilic asthma, asthma in elderly, refractory atopic asthma, and asthma with allergic rhinitis. We did not classify and compare them according to phenotypes.

Conclusion

In conclusion, IL-33 levels were significantly higher in the asthma group than in the control group. There was no significant difference between patient and control groups in terms of IL-33 gene polymorphisms (rs1929992, rs1342326 and rs3939286). Our findings demonstrated that asthma patients had a higher level of IL-33, and that IL-33 may be considered as a potential therapeutic target in the future. Further molecular studies and clinical studies addressing therapeutic agents that would reduce IL-33 levels are required.

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Ethics

Ethics Committee Approval: The study was approved by the Local Ethical Committee (Ethical Committee of Gülhane Military Medical Academy Haydarpasa Training Hospital, approval number: 26.02.2016/1491-46-16/1539).

Informed Consent: Written informed consent was obtained from all participants.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Concept: K.C., D.T., T.Ç., Design: K.C., A.F.A.K., Z.K., Data Collection or Processing: K.C., Y.U., Analysis or Interpretation: K.C., O.O., İ.Y., S.Y., Literature Search: K.C., Ö.A., Writing: K.C., D.T.

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References

1. Global Initiative for Asthma (2017). Global strategy for asthma management and prevention. Last Accessed Date: 01.06.2018. Available from: https://ginasthma.org/wp-content/uploads/2017/02/wmsGINA-2017-main-report-final_V2.pdf.
2. Schmitz J, Owyang A, Oldham E, et al. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. Immunity. 2005;23:479-490.
3. Mitchell PD, O'Byrne PM. Epithelial-derived cytokines in asthma. Chest. 2017;151:1338-1344.
4. Rank MA, Kobayashi T, Kozaki H, Bartemes KR, Squillace DL, Kita H. IL-33-activated dendritic cells induce an atypical TH2-type response. J Allergy Clin Immunol. 2009;123:1047-1054.
5. Akdis CA. Therapies for allergic inflammation: refining strategies to induce tolerance. Nat Med. 2012;18:736-749.
6. Bønnelykke K, Sleiman P, Nielsen K, et al. A genome-wide association study identifies CDHR3 as a susceptibility locus for early childhood asthma with severe exacerbations. Nat Genet. 2014;46:51-55.
7. Drake LY, Kita H. IL-33: biological properties, functions, and roles in airway disease. Immunol Rev. 2017;278:173-184.
8. Koca SS, Kara M, Deniz F, et al. Serum IL-33 level and IL-33 gene polymorphisms in Behçet's disease. Rheumatol Int. 2015;35:471-477.
9. Yanaba K, Yoshizaki A, Asano Y, Kadono T, Sato S. Serum IL-33 levels are raised in patients with systemic sclerosis: association with extent of skin sclerosis and severity of pulmonary fibrosis. Clin Rheumatol. 2011;30:825-830.
10. Xu W, Liu Y, Ye D. Association between IL-33 gene polymorphisms (rs1929992, rs7044343) and systemic lupus erythematosus in a chinese han population. Immunol Invest. 2016;45:575-583.
11. Han GW, Zeng LW, Liang CX, et al. Serum levels of IL-33 is increased in patients with ankylosing spondylitis. Clin Rheumatol. 2011;30:1583-1588.
12. Li C, Mu R, Guo J, et al. Genetic variant in IL33 is associated with susceptibility to rheumatoid arthritis. Arthritis Res Ther. 2014;16:R105.

13. Miller AM, Xu D, Asquith DL, et al. IL-33 reduces the development of atherosclerosis. J Exp Med. 2008;205:339-346.

14. Pushparaj PN, Tay HK, H'ng SC, et al. The cytokine interleukin-33 mediates anaphylactic shock. Proc Natl Acad Sci USA. 2009;106:9773-9778.

15. Liu X, Li M, Wu Y, Zhou Y, Zeng L, Huang T. Anti-IL-33 antibody treatment inhibits airway inflammation in a murine model of allergic asthma. Biochem Biophys Res Commun. 2009;386:181-185.

16. Sakashita M, Yoshimoto T, Hirota T, et al. Association of serum interleukin-33 level and the interleukin-33 genetic variant with Japanese cedar pollinosis. Clin Exp Allergy. 2008;38:1875-1881.

17. Zhu X, Xie L, Qin H, et al. Interaction between il-33 gene polymorphisms and current smoking with susceptibility to systemic lupus erythematosus. J Immunol Res. 2019;2019:1547578.

18. Charrad R, Kaabachi W, Berraies A, Hamzaoui K, Hamzaoui A. IL-33 gene variants and protein expression in pediatric Tunisian asthmatic patients. Cytokine. 2018;104:85-91.

19. Schröder PC, Casaca VI, Illi S, et al. IL-33 polymorphisms are associated with increased risk of hay fever and reduced regulatory T cells in a birth cohort. Pediatr Allergy Immunol. 2016;27:687-695.

20. Moffatt MF, Gut IG, Demenais F, et al. A large-scale, consortium-based genomewide association study of asthma. N Engl J Med. 2010;363:1211-1221.

21. Ren X, Guo M, Liu C, et al. A case-control study indicates that no association exists between polymorphisms of il-33 and il-1rl1 and preeclampsia. Cell Physiol Biochem. 2016;38:1406-1414.

22. Liu S, Zhou Z, Wang C, Guo M, Chu N, Li C. Associations between interleukin and interleukin receptor gene polymorphisms and risk of gout. Sci Rep. 2015;5:13887.

23. López-Mejías R, Genre F, Remuzgo-Martínez S, et al. Protective role of the interleukin 33 rs3939286 gene polymorphism in the development of subclinical atherosclerosis in rheumatoid arthritis patients. PLoS One. 2015;10:e0143153.

24. Latiano A, Palmieri O, Bossa F, et al. Impact of genetic polymorphisms on the pathogenesis of idiopathic achalasia: association with IL33 gene variant. Hum Immunol. 2014;75:364-369.

25. Buysschaert ID, Grulois V, Eloy P, et al. Genetic evidence for a role of IL33 in nasal polyposis. Allergy. 2010;65:616-622.

26. Happle R, Schnyder UW. Evidence for the carter effect in atopy. Int Arch Allergy Appl Immunol. 1982;68:90-92.

27. Levy H, Raby BA, Lake S, et al. Association of defensin beta-1 gene polymorphisms with asthma. J Allergy Clin Immunol. 2005;115:252-258.