Anti-VEGF treatment suppresses remodeling factors and restores epithelial barrier function through the E-cadherin/β-catenin signaling axis in experimental asthma models

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Abstract. Besides maintaining a physical barrier with adherens junctional (AJ) and tight junctional proteins, airway epithelial cells have important roles in modulating the inflammatory processes of allergic asthma. E-cadherin and β-catenin are the key AJ proteins that are involved in airway remodeling. Various mediators such as transforming growth factor-β (TGF-β), epidermal growth factor (EGF), fibroblast growth factor (FGF), platelet derived growth factor (PDGF), insulin-like growth factor (IGF), tumor necrosis factor-α (TNF-α) and angiogenic factors, such as vascular endothelial growth factor (VEGF), are released by the airway epithelium in allergic asthma. The signaling pathways activated by these growth factors trigger epithelial-mesenchymal transition (EMT), which contributes to fibrosis and subsequent downregulation of E-cadherin. The present study used a mouse asthma model to investigate the effects of anti-VEGF, anti-TNF and corticosteroid therapies on growth factor and E-cadherin/β-catenin expression. The study used 38 male BALB/c mice, divided into 5 groups. A chronic mouse asthma model was created by treating 4 of the groups with inhaled and intraperitoneal ovalbumin (n= 8 per group). Saline, anti-TNF-α (etanercept), anti-VEGF (bevacizumab) or a corticosteroid (dexamethasone) were applied to each group by intraperitoneal injection. No medication was administered to the control group (n=6). Immunohistochemistry for E-cadherin, β-catenin and growth factors was performed on lung tissues and protein expression levels assessed using H-scores. Statistically significant differences were observed in E-cadherin, β-catenin, EGF, FG, and PFGF ($P<0.001$ for all) as well as the IGF H-scores between the five groups ($P<0.005$). Only anti-VEGF treatment caused E-cadherin and β-catenin levels to increase to the level of non-asthmatic control groups ($P>0.005$). All treatment groups had reduced TGF-β, PDGF and FGF H-scores in comparison with the untreated asthma group ($P=0.001$). The EGF and IGF levels were not significantly different between the untreated asthmatic and non-asthmatic controls. The results suggested that anti-VEGF and TNF-α inhibition treatments are effective in decreasing growth factors, in a similar manner to conventional corticosteroid treatments. Anti-VEGF and TNF inhibition therapy may be an effective treatment for remodeling in asthma while offering an alternative therapeutic option to steroid protective agents. The data suggested that anti-VEGF treatment offered greater restoration of the epithelial barrier than both anti-TNF-α and corticosteroid treatment.

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Introduction

A strong link between allergen exposure, sensitization and asthma development has been reported (1). Epithelial permeability is indicated to be markedly increased in allergic asthma, allowing allergens and irritants to penetrate into the subepithelial area, thus stimulating type 2 immune responses and constituting an important process in the immunopathology of allergy (2). Various mediators, such as transforming growth factor β (TGF-β), epidermal growth factor (EGF), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), insulin-like growth factor (IGF), tumor necrosis factor-α (TNF-α) and angiogenic factors are released by airway epithelium, inflammatory cells and structural cells (3-6). The signaling pathways activated by these growth factors trigger epithelial-mesenchymal transition (EMT), which contributes to fibrosis and subsequent downregulation of E-cadherin (6). Published studies suggest that EMT commonly occurs in epithelial cells in asthma and may contribute to airway remodeling (7,8). Remodeling comprises structural alterations to the airway wall, including epithelial

cell loss, goblet cell hyperplasia, hypertrophy of the airway smooth muscle, basement membrane thickening and increased angiogenesis (9).

Protection of the integrity of the barrier function of the airway epithelium depends on adherens junctional (AJ) and tight junctional (TJ) proteins that link cells together (10,11). AJ proteins are part of two main complexes, the E-cadherin-β-catenin complex and the nectin-afadin complex, that have important roles in arranging cell-cell adhesion and intercellular motility (12). It has been reported that the expression of E-cadherin, β-catenin and zonula occludens-1 are reduced and that their functions are impaired in asthmatic epithelial cells in comparison with healthy cells (13-17).

Inhaled or oral corticosteroids are the most common drugs used for asthma therapy in the majority of patients with asthma (18). Despite providing clinical and symptomatic improvement, existing therapies are insufficient to prevent the onset of airway remodeling (9). The influence of various therapeutic agents on epithelial barrier dysfunction, inhibition of airway remodeling and controlling adaptive immunity guide the development of further therapeutic strategies, targets and rational treatment (19).

Vascular endothelial growth factor (VEGF) is a signaling protein and the main regulator of endothelial cell growth. It is released by many cells that stimulate the formation of blood vessels and is a classic profibrotic growth factor (20). The overexpression of VEGF in the lung can lead to significant airway remodeling (21). Therefore, antagonizing VEGF is important in the prevention of remodeling and in preserving epithelial barrier function (3,22).

An example of a proinflammatory cytokine that plays a role in the pathogenesis of asthma is TNF-α (4,23). TNF-α is usually secreted by macrophages, lymphocytes and mast cells in the lungs (24,25). However, lung epithelial cells may also release TNF-α (25,26). It has been reported that patients with severe asthma have a high amount of TNF-α in the airways in comparison to healthy individuals (24,25). Tissue damage occurs via a TNF-α driven effect in the airways and remodeling may develop as a result (24). Therefore, anti-TNF therapy may be useful to treat severe asthma. Co-prevention of remodeling and tissue injury may be possible by antagonism of TNF-α.

The present study aimed to use an experimental asthma mouse model, to investigate the effects of anti-VEGF, anti-TNF and corticosteroid therapies on growth factors and E-cadherin-β-catenin expression.

Materials and methods

Study animals. Male BALB/c mice (n=38; age, 6-8 weeks; weight, 18-20 g) were used to simulate chronic asthma. The mice were kept in a pathogen-free animal facility at a controlled temperature of 20-24˚C with 60±10% relative humidity and had free access to food and water on a 12-h light-dark cycle. The study was performed in accordance with the suggestions specified in the Guide for the Care and Use of Experimental Animals (27) and ethics approval was obtained from the Ethics Committee of Dokuz Eylül University.

Sensitization protocol. Mice were divided into five groups: i) Control group (n=6); ii) untreated asthma group (n=8); iii) TNF-α receptor blocker group (n=8); iv) anti-VEGF group (n=8); and v) corticosteroid group (n=8). Mice in the control group were not administered medication. The chronic asthma model was applied according to a previously described protocol in all groups except for the control group (28). The duration of the experiment was 10 weeks (2 weeks of ovalbumin injection and 8 weeks of nebulization). Mice were sensitized by injecting 10 μg (0.1 ml) ovalbumin (grade V; Sigma-Aldrich; Merck KGaA) intraperitoneally on the 1st and 14th day of the experiment. After the 3rd week of the experiment, mice were nebulized with 2.5% OVA aerosol in sterile saline for 30 min, 3 days per week for 8 weeks. Nebulized saline inhalation was applied to the mice in the control group. The mice were sacrificed after ketamine hydrochloride (50 mg/ml and 200 mg/kg) anesthesia was applied via the IP route on the first day after nebulized treatment was completed.

Study design. Chronic asthma developed in 32 mice. The treatments given to the study group were as follows: i) Control group, medication was not applied; ii) untreated asthma group, IP saline was administered once per week for 2 weeks; iii) TNF-α receptor blocker group, IP etanercept (Wyeth Europa Ltd.) was administered at a dose of 0.01 mg/dose (0.5 mg/kg) twice per week for two weeks; iv) anti-VEGF group, IP bevacizumab (Avastin®; Roche Diagnostics) was administered at a dose of 0.15 mg/dose 5 mg/kg once per week for 2 weeks and v) corticosteroid group, IP dexamethasone (Dekort®; Deva Holding A.S.) was administered at a dose of 1 mg/kg for 7 days. The treatments commenced at the same stage as the nebulization treatment, but in the case of the corticosteroid group only, the treatments commenced in the second week of nebulization treatment.

Histopathological and immunohistochemical evaluations. Murine lung tissue was fixed at room temperature with 10 % buffered formalin for three days, and a 5 mm thick horizontal slice was obtained from the middle zone of the left lung. Formalin-fixed lung tissue was embedded in paraffin for use in histopathological evaluation. Tissue blocks were serially cut into 5 μm sections. The sections were stained with hematoxylin and eosin (H&E) for histological evaluation (29). Immunostaining for E-cadherin (cat. no. sc-8426; mouse monoclonal IgG1; Santa Cruz Biotechnology, Inc.), β-catenin (cat. no. E-5: sc-7963; mouse monoclonal IgG1; Santa Cruz Biotechnology, Inc.), TGF-β1 (cat. no. bs-0086R; rabbit polyclonal IgG1; BIOSS), FGF-1 (cat. no. C-19: sc-1884; goat polyclonal IgG; Santa Cruz Biotechnology, Inc.), PDGF-A (cat. no. N-30: sc-128; rabbit polyclonal IgG; Santa Cruz Biotechnology, Inc.), EGF (cat. no. EGF-10: sc-57088; mouse monoclonal IgG1; Santa Cruz Biotechnology, Inc.) and IGF-1 (cat. no. H-70: sc-9013; rabbit polyclonal IgG; Santa Cruz Biotechnology, Inc.) was performed according to manufacturer's instructions (30). Sections were washed three times (5 min each) with PBS, followed by incubation at 4˚C for 1 h with biotinylated IgG and then with streptavidin-peroxidase conjugate. For anti-mouse and anti-rabbit primary antibodies, the Histostain Plus IHC secondary antibody system was used (cat. no. 85-9643; broad spectrum; Invitrogen; Thermo Fisher Scientific, Inc.).

Table I. Growth factors H-scores in the study groups.

	Study groups					
Protein	Non-asthmatic control	Untreated asthma	Etanercept	Bevacizumab	Dexamethasone	P-value[a]
TGF-β	202.5 (180-240)	270 (254-280)[b]	151 (130-172.5)[c]	157.5 (150-187.5)[c]	217.5 (177.5-225)[c]	<0.001
PDGF	150 (138-163)	216.5 (207-226.5)[b]	150 (135-169)[c]	145 (133-169)[c]	138 (130-152.5)[c]	<0.001
FGF	237 (225-259)	351 (329-360)[b]	263 (252-280)[c]	217 (187-236)[c]	266 (245-280)[c]	<0.001
EGF	232.5 (210-240)	290 (260-310)	232.5 (182.5-240)	210 (140-225)[d]	260 (210-300)	0.005
IGF	117.5 (90-130)	97.5 (89-100)	95 (90-100)	80 (72.5-80)	147 (130-165)	<0.001

[a]Kruskal-Wallis statistical analysis (expressed as median-interquartile range). The Bonferroni correction was applied for the post-hoc analysis results, where P<0.005 was regarded as a statistically significant difference for the two group comparisons; [b]Mann-Whitney U-test, P<0.005 vs. non-asthmatic control; [c]Mann-Whitney U-test, P<0.001 vs. untreated asthma; [d]Mann-Whitney U-test, P=0.002 vs. untreated asthma.

For anti-goat primary antibodies, ImmunoCruz goat ABC Staining System was used (cat. no. sc-2023; Santa Cruz Biotechnology, Inc.). The staining process was performed according to the manufacturer's instructions. After washing with PBS three times for 5 min, these sections were colored with 3,3'-diaminobenzidine for signal detection and then counterstained with Mayer's hematoxylin at room temperature for 2 min. All antibodies were used at 1:100 dilution. The sections were mounted using Entellan® mounting medium (HX265767; Merck KGaA) and evaluated using a light microscope (model BX40; Olympus Corporation). Control samples were processed similarly except for the use of IgG instead of a primary antibody (30).

The samples were evaluated by two experienced pathologists blinded to the clinical and serological characteristics of the rodents. Based on the nuclear and intracytoplasmic density of immunostaining of the airway epithelium, an H-SCORE [∑ Pi (I+1)] was calculated for all growth factors, E-cadherin and β-catenin. Both the ratio of positive immunostained cells to all cells in the selected fields and the respective H-scores were calculated. I is the intensity score (0, 1, 2 or 3 corresponding to the presence of negative, weak, intermediate or strong staining, respectively). Pi is the percentage of epithelial cells stained with each intensity between 0 and 100% (30). There were 25 slides per animal. From each slide 10 different areas (x400 magnification) were evaluated under a microscope and the percentage of cells with different staining densities was determined.

Statistical analysis. Statistical analysis was performed using SPSS 15.0 (SPSS, Inc.). The statistical significance between the 5 groups was assessed by Kruskal-Wallis test followed by the Dunn-Bonferroni post-hoc test. H-SCORES were compared between pairs of groups (two by two) using the Mann-Whitney U test. P<0.05 was accepted as statistically significant.

Results

TGF-β1 H-scores. TGF-β1 H-scores were compared between the five groups, and there was a statistically significant difference (P<0.001). The highest TGF-β1 H-scores were observed in the untreated asthma group and the lowest scores were observed in the TNF-α blocker group (202.5 vs. 151). There were no statistically significant differences among the TGF-β1 H-scores of the etanercept, bevacizumab and dexamethasone treatment groups and the non-asthmatic group (P>0.001, for all). The differences among the TGF-β1 H-scores of the etanercept, bevacizumab and dexamethasone treatment groups and the untreated asthma group were statistically significant (P<0.001 for all; Table I; Figs. 1 and 2).

PDGF-A H-scores. PDGF-A H-scores were compared between the five groups, and there was a statistically significant difference (P<0.001). The highest PDGF-A H-scores were observed in the untreated asthma group and the lowest scores were seen in the corticosteroid group (216.5 vs. 138). No statistically significant difference was found among the PDGF-A H-scores of the etanercept, bevacizumab and dexamethasone treatment groups in comparison with the non-asthmatic group (P>0.005, for all). The comparison of PDGF-A H-scores among the etanercept, bevacizumab, dexamethasone treatment groups and the untreated asthma group were statistically significantly (P<0.001 for all; Table I; Figs. 1 and 2).

FGF-1 H-scores. When FGF-1 H-scores were compared between the five groups, there was a statistically significant difference (P<0.001). The highest FGF-1 H-score was observed in the untreated asthma group and the lowest score in the anti VEGF group (351 vs. 217). When the FGF-1 H-scores of the etanercept, bevacizumab and dexamethasone treatment groups were compared with the non-asthmatic group no statistically

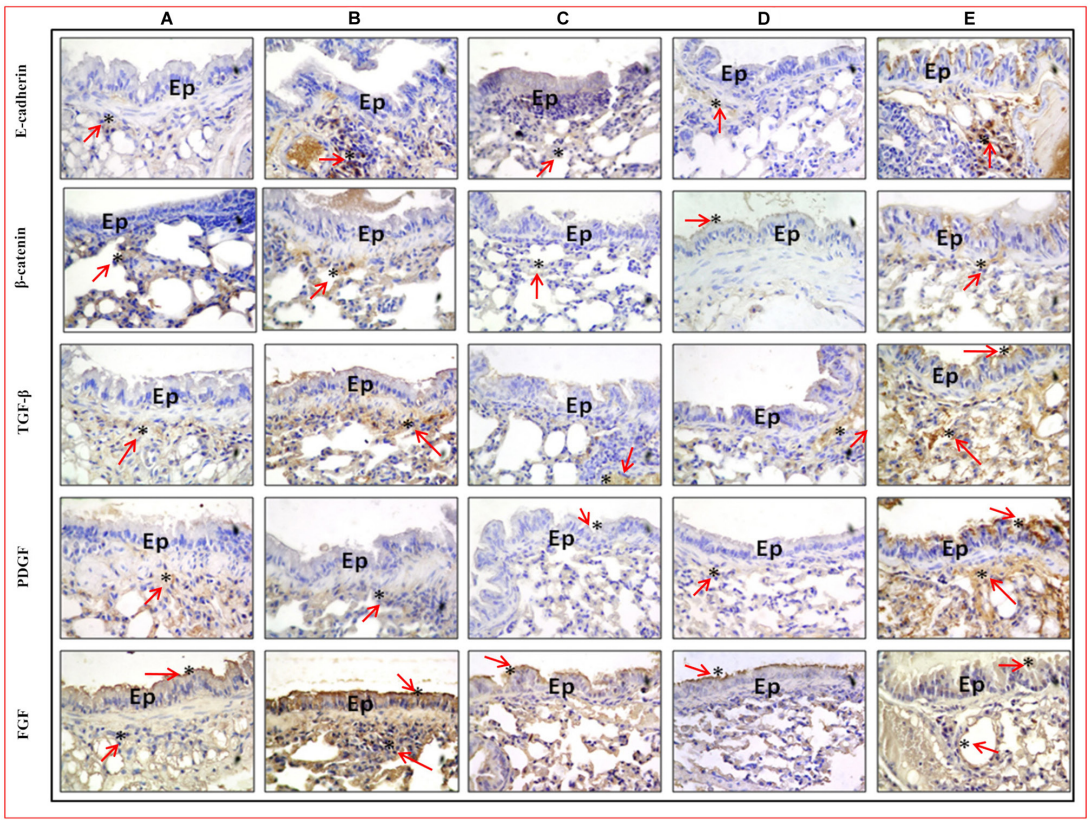

Figure 1. Immunohistochemistry of lung tissue sections from asthma model and control mice. E-cadherin, β-catenin, TGF-β, PDGF, FGF staining of the five groups (original magnification, x100; insert, x400). (A) Non-asthmatic control, (B) untreated asthma, (C) TNF-α blocker, (D) corticosteroid and (E) anti-VEGF groups. *Immunoreactivity. Ep, epithelium. TNF-α, tumor necrosis factor-α; VEGF, vascular endothelial growth factor; TGF-β, transforming growth factor-β; PDGF, platelet derived growth factor; FGF, fibroblast growth factor.

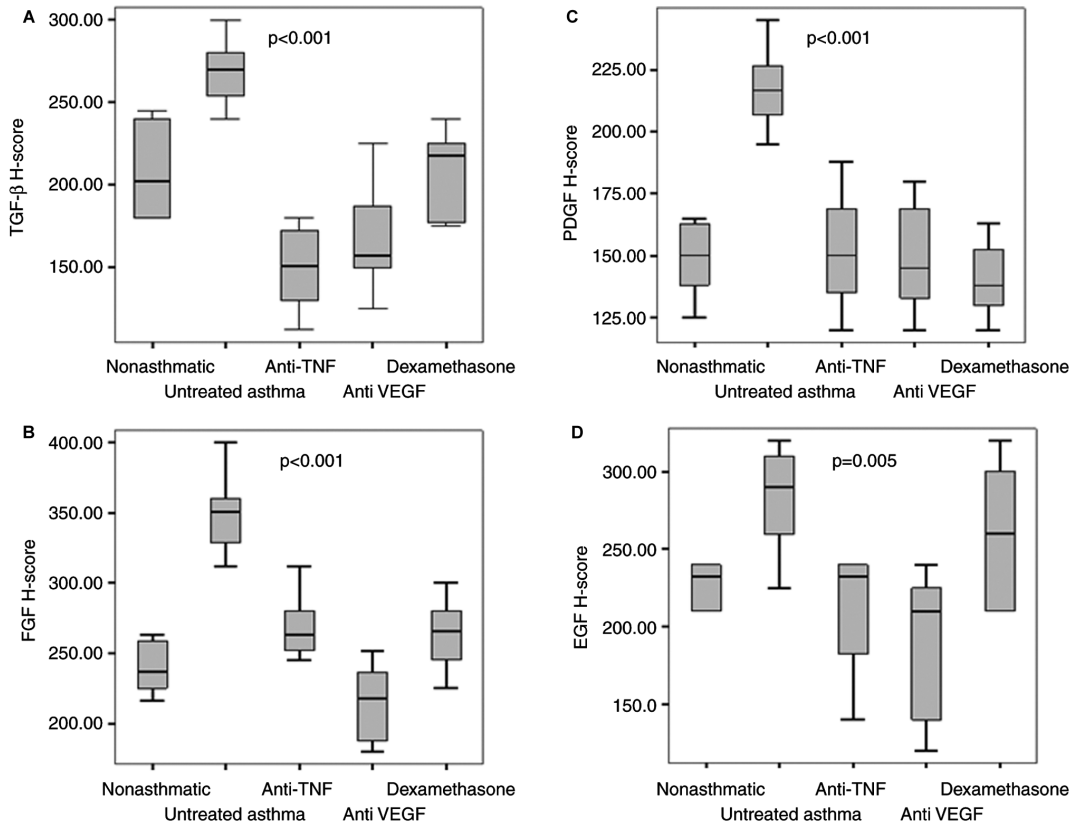

Figure 2. Boxplot of growth factors among the five groups. (A) TGF-β, (B) FGF, (C) PDGF and (D) EGF. TGF-β, transforming growth factor-β; PDGF, platelet derived growth factor; FGF, fibroblast growth factor; EGF, epidermal growth factor; TNF-α, tumor necrosis factor-α; VEGF, vascular endothelial growth factor.

Table II. E-cadherin and β-catenin H-scores in the study groups.

	Study groups					
Protein	Non-asthmatic control	Untreated asthma	Etanercept	Bevacizumab	Dexamethasone	P-value[a]
E-cadherin	225 (200-234)[b]	125 (118-40)	158.5 (138-172.5)[c]	193.5 (181-195)[d]	138 (129-161)[c]	<0.001
β-catenin	191.5 (188-200)[b]	120 (115-127.5)	138 (125-154)[c]	175 (150-182.5)[d]	135 (130-140)[c]	<0.001

[a]Kruskal-Wallis statistical analysis (expressed as median-interquartile range). The Bonferroni correction was applied for the post-hoc analysis results, where P<0.005 was regarded as a statistically significant difference for the two group comparisons; [b]Mann-Whitney U-test, P=0.002 vs. untreated asthma; [c]Mann-Whitney U-test, P<0.005 vs. Non-asthmatic control; [d]Mann-Whitney U-test, P>0.05 vs. Non-asthmatic control.

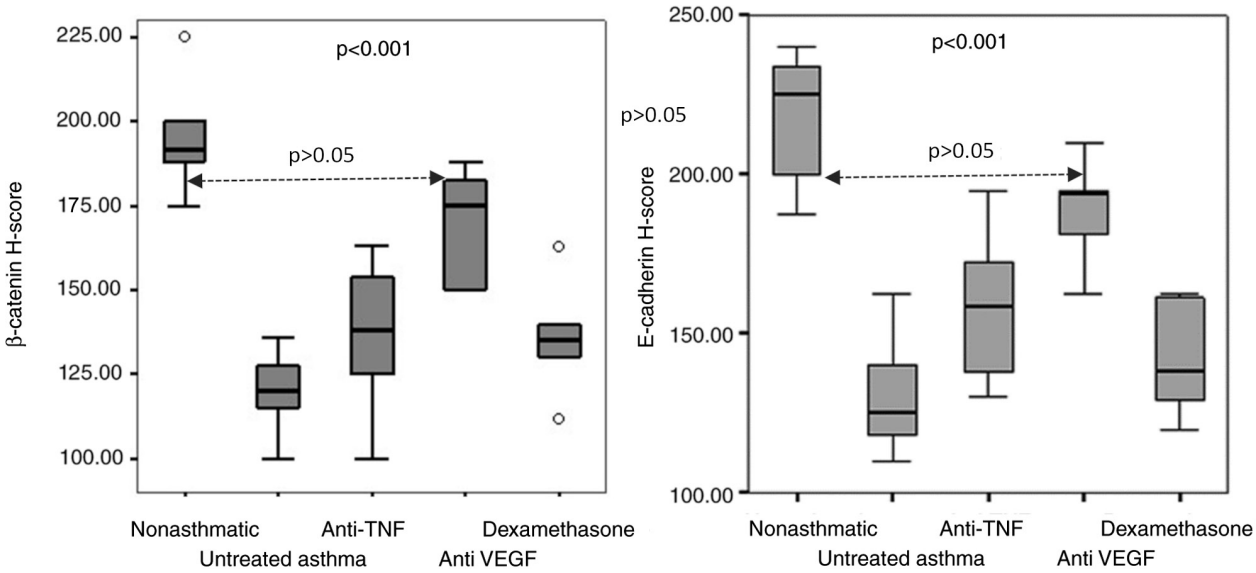

Figure 3. Boxplot of β-catenin and E-cadherin among the five groups. TNF-α, tumor necrosis factor-α; VEGF, vascular endothelial growth factor.

significant difference was found (P>0.005, for all). The differences among the FGF-1 H-scores of the etanercept, bevacizumab, dexamethasone treatment groups and the untreated asthma group were statistically significant (P<0.001 for all; Table I; Figs. 1 and 2).

EGF H-scores. The EGF H-scores between the five groups were significant (P=0.001). The highest EGF-H-score was observed in the untreated asthma group and the lowest score in the anti-VEGF group (290 vs. 210; P=0.002). The comparison of EGF H-scores between the bevacizumab treatment group and the untreated asthma group indicated a statistically significant difference (P=0.002). When the EGF H-scores of the etanercept and dexamethasone treatment groups were compared with the non-asthmatic group, no statistically significant difference was observed (P>0.005; Table I; Figs. 1 and 2).

IGF H-scores. There was a statistically significant difference when the IGF H-scores were compared between the five groups (P<0.001). The highest IGF H-score was found in the corticosteroid group, and the lowest score was in the anti-VEGF group (147 vs. 80; P=0.001). The IGF H-scores of untreated asthma, etanercept, bevacizumab and dexamethasone treatment groups were not significantly different from those in healthy controls (P>0.005, for all). The differences between the IGF H-score in the corticosteroid group in comparison to the untreated asthma, etanercept and anti-VEGF groups was statistically significant (P=0.001 for all; Table I; Fig. 1).

E-cadherin H-scores. The H-scores of E-cadherin between the five groups were significantly different (P<0.001). The highest E-cadherin H-score was observed in the non-asthmatic control group and the lowest score was in the untreated asthma group (225 vs. 125; P=0.002). The E-cadherin H-scores of the anti-VEGF group were closest to the non-asthmatic control groups (193.5 vs. 225; P=0.023; Table II). The H-scores of the TNF-α receptor blocker and corticosteroid treatment groups were significantly lower than the control group (P=0.003 and P=0.002, respectively). Although the H-scores of the TNF-α receptor blocker and corticosteroid treatment groups were higher than the untreated asthma group, the differences were not significant (158.5 vs. 125 and 138 vs. 125; P=0.013 and P=0.138, respectively; Table II; Figs. 1 and 3).

β-catenin H-scores. There was a statistically significant difference in β-catenin H-scores (P<0.001) between the five groups. The highest β-catenin H-score was found in the non-asthmatic control group and lowest score was in the untreated asthma group (191.5 vs. 120; P=0.002). Among the etanercept, bevacizumab and dexamethasone treatment groups, the β-catenin H-scores of the bevacizumab group were closest to the non-asthmatic control groups (175 vs. 191.5; P=0.002). The TNF-α receptor blocker and corticosteroid treatment groups had lower β-catenin H-scores than the control group (P=0.002 and P=0.002, respectively). Although the β-catenin H-scores of the etanercept and the corticosteroid treatment groups were higher than those in the untreated asthma group, the differences were not significant (138 vs. 120 and 135 vs. 120; P=0.056 and P=0.023, respectively; Table II; Figs. 1 and 3).

Discussion

The results suggested that the levels of TGF-β, PDGF and FGF were increased in the untreated asthma group compared with non-asthmatic controls. All treatment groups had reduced TGF-β, PDGF and FGF H-scores in comparison with the untreated asthma group. It was determined that E-cadherin and β-catenin levels were reduced in the untreated asthma group compared to the non-asthmatic control and that their levels increased in non-asthmatic controls following treatment with anti-VEGF treatment.

In asthma, growth factors can trigger inflammation and structural changes in the airways (e.g., epithelial barrier dysfunction and remodeling). This is a process in which EMT causes epithelial cells to dedifferentiate into more mobile mesenchymal cells, such as fibroblasts (6,31,32). EMT can be stimulated by various signals and molecules, including tyrosine kinase receptors, including FGF, IGF, EGF, PDGF and VEGF, and the TGF-β and Wnt/β-catenin pathways (5,6). The studies conducted to date have indicated that the asthmatic airway epithelium is an important source of profibrogenic growth factors, including FGF, IGF, EGF, TGF-β, PDGF and VEGF (6,33-35). Similar to these studies, the present study determined that TGF-β, PDGF and FGF levels were higher in asthmatic mice than control mice; however, IGF and EGF levels were not different from the control group in the present study.

The airway epithelium creates a structural and immunological barrier against environmental stimuli, such as aeroallergens, microorganisms and particulate matter (36). Changes in the asthmatic epithelium include epithelial shedding and the destruction of ciliary cells (37). E-cadherin is important in maintenance of the cytoskeleton and TJ functional integrity (38). Reduced E-cadherin activity causes TJ barrier integrity to deteriorate (38,39). In addition, E-cadherin plays a role in the repression of intracellular signaling pathways, the arrangement of the epithelium and cell proliferation and differentiation (37).

β-catenin is a membrane-bound protein and forms a key AJ component, interacting with E-cadherins and connecting them to the cytoskeleton. Disruption of this epithelial barrier function allows easier access of inhalant allergens to the antigen-presenting cells in the submucosa, causing stimulation of the Th2 type mediated immune response and EMT (13,14,38). A gradual loss of E-cadherin is considered a hallmark of EMT (5,6). Membrane levels of E-cadherin-β- catenin have been found to be reduced in the asthmatic epithelial cell in comparison with healthy cells (13-17). When the E-cadherin-β-catenin structure is disrupted, β-catenin is released into the cytosol and translocates to the nucleus, where it activates the Wnt/β-catenin signaling pathway and contributes to the EMT; this process is involved in tissue remodeling (7,14,40-42).

Studies on mice have shown that repetitive antigen load causes extensive E-cadherin loss and that there is a correlation between the number of allergen exposures and E-cadherin levels (39,43). In biopsies of the bronchial mucosa of patients with atopic asthma, E-cadherin levels in the respiratory epithelia were shown to be lower than non-atopic and non-asthmatic cases (17,44,45). Similarly, a study published by our group showed that patients with asthma had lower levels of E-cadherin in their airways (46). Similar to previous studies, the present study suggested that E-cadherin and β-catenin levels were lower in asthmatic model mice.

VEGF has significant roles in inflammation, angiogenesis and the vascular permeability of airways and in subepithelial collagen deposition, airway smooth muscle hyperplasia and the development of physiological abnormalities of the airway (33). Bevacizumab is a humanized monoclonal antibody that inhibits the activation of VEGF-R and tumor growth (20). TGF-β, IGF, FGF and PDGF were shown to stimulate the secretion of VEGF (33,47). Similar to these studies, the correlation of increased TGF-β levels and the decrease in E-cadherin and β-catenin levels was shown in the present study as well (11,14,32,40,45,48-50). TGF-β has also caused a significant increase in transcriptional activation of β-catenin (14,40). The available knowledge suggests that VEGF potentially affects the signal pathway, and thus triggers myofibroblast transformation (51). In the present study of asthmatic model mice, TGF-β, PDGF and FGF levels decreased with treatment to show no difference from non-asthmatic controls. These results suggest that EMT can be controlled by treatment approaches via VEGF.

Although the role of VEGF in the pathogenesis in asthma and remodeling is known, the effect of antagonizing VEGF on epithelial barrier integrity and AJ structure is not fully understood (33). Most knowledge on the effects of VEGF on epithelial barrier integrity was obtained from studies of the retinal endothelium (30,52). VEGF exposure causes decreased transepithelial resistance and increased vascular permeability. Permeability-enhancing agents also change AJ (53). After exposure to VEGF, the expression of VE-cadherin, β-catenin, occludin, claudin and ZO-1 proteins in the endothelium decreased *in vitro* (54-57). Furthermore, in diabetic retinopathy, VEGF-R1 antagonism has been shown to prevent vascular leakage and retinal leukostasis, degeneration and disorganization of ZO-1 and VE-cadherin (55,57). Similarly, in the present study, E-cadherin and β-catenin levels of asthmatic mice treated with anti-VEGF increased to levels of control mice without asthma. Overall, in the experimental asthma mouse model, anti-VEGF therapy appeared to cause an elevation in epithelial barrier proteins, E-cadherin and β-catenin levels. Therefore, the use of anti-VEGF therapy in asthma may be a therapeutic option for the restoration of epithelial barrier.

TNF-α is released from the airways in asthma and may play a role in the pathogenesis of allergic inflammation

through the activation of transcription factors (4). TNF-α was shown to upregulate TGF-β expression in mice lung fibroblasts (58). TNF-α stimulates TGF-β expression in severe asthma, causing fibroblast growth and maturation into myofibroblasts (59). TNF-α enhances the effect of TGF-β1 on EMT induction in human bronchial epithelial cells (HBECs) (60). The effectiveness of a TNF-α receptor antagonist in severe asthma therapy has recently been studied (25). In the present study of asthmatic mice models, etanercept was shown to downregulate the expression of PDGF and FGF as well as TGF-β. Despite the apparent efficacy in treatment, the mechanism of action of anti-TNF-α is not fully understood, which is limitation of the present study. Further cellular and molecular mechanistic studies are needed to determine how these changes occur in the future. TNF-α causes epithelial barrier dysfunction in other tissues, and previous studies have shown that TNF-α in combination with IFN-γ disrupted the TJ in HBECs (61-63). Therefore, the inhibition of TNF-α can be considered as an option in maintaining epithelial integrity.

In another study, TNF-α stimulation in bronchial epithelial cell culture has been shown to reduce E-cadherin and β-catenin expression; however, improvements were observed with TNF-α inhibition and corticosteroid therapy (48). In contrast to these studies, E-cadherin and β-catenin expression partially increased with corticosteroid treatment and TNF-α inhibition in the present study, but did reach the levels of healthy controls. This may have been caused by the difference between inflammatory processes of Th2 type inflammation caused by the OVA-induced asthma model and Th1 type inflammation caused by TNF-α.

In experimental asthma model studies, corticosteroids were shown to decrease the release of TGF-β while dexamethasone was shown to decline the release of TGF-β, IGF, and FGF. ICS was shown to inhibit IGF expression (23,64,65). All glucocorticosteroids have been shown to inhibit PDGF and collagen I-induced proliferation and hypocontractility in airway smooth muscle cells (66). The anti-angiogenic effects of dexamethasone on VEGF release in FGF-treated human airway smooth muscle cells via the p38MAPK pathway were also demonstrated *in vitro* (47). Similar to these studies, the present study suggested that dexamethasone decreased TGF-β, PDGF and FGF levels.

By inducing tight junction formation and increasing transepithelial resistance, corticosteroids play a crucial role in the function and maintenance of cell-cell contact. However, the potential effects of returning to the original state of AJs (E-cadherin, β-catenin) and healing the epithelial barrier are not fully clear (18,48,67). Glucocorticoid administration in Doerner and Zuraw (68) did not substantially change E-cadherin mRNA downregulation mediated by TGF-β1. Song *et al* (67) showed that E-cadherin distribution could be partially salvaged following pretreatment with dexamethasone in experimental asthma models. While the downregulation of hypoxia-induced loss of ZO-1 expression was inhibited by dexamethasone in the human corneal epithelia, it did not affect the E-cadherin vs. β-catenin (69). In the present study, E-cadherin and β-catenin expression did not reach the level of healthy controls with dexamethasone treatment similar to the aforementioned studies.

The limitations of our study are the inability to look at the molecular level of β-catenin in terms of quantity, only the adherens proteins, and the inability to show a barrier function such as transepithelial resistance as physical data. Further cellular and molecular mechanistic studies are needed to determine how these changes occur in the future.

In the present study, the effects of conventional corticosteroid treatment, VEGF inhibition treatments and TNF-α antagonistic treatment on growth factor and E-cadherin-β-catenin expression were compared. These proteins play roles in the pathogenesis of asthma (3-6). Growth factors TGF-β, PDGF and FGF expression levels were high in asthmatic mice models and E-cadherin and β-catenin expressions were low. Anti-VEGF and TNF-α inhibition treatments are effective in decreasing growth factors similar to conventional corticosteroid treatments. However, corticosteroid and TNF-α inhibition treatment were not effective in increasing E-cadherin and β-catenin levels. This leads to epithelial barrier function disturbance. Anti-VEGF treatment increased the expression of AJ proteins in bronchial epithelium, and VEGF and TNF inhibition may provide an alternative therapeutic option to steroid-sparing agents. They are also a more effective treatment of epithelial barrier restoration and remodeling in asthma. However, before making firm recommendations, further clinical studies are needed to investigate the *in vivo* efficacy and safety profile of anti-VEGF treatment in asthma.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

HY and OY were involved in the conception, hypotheses delineation and design of the study. AT wrote and HY revised the manuscript. FF, AT and ETK took part in the acquisition of the data. SI and MK participated in the analysis and interpretation of the data. HY and OY confirmed the authenticity of the raw data. All authors confirmed that they have read and approved the final manuscript.

Ethics approval and consent to participate

Ethics approval was obtained from the Ethics Committee of Dokuz Eylül University and the Animal Experiment Unit for the implementation of all experimental procedures.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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