Wissam Ali Al nadawi et al /J. Pharm. Sci. & Res. Vol. 11(6), 2019, 2292-2294

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

TGF-BRIII gene expression and TGF-B1 serum level in Iraqi children with asthma

Wissam Ali Al nadawi, Hazima Mossa Alabassi

Dept of Biology / college of Education for pure Science (Ibn Al-Haitham) / University of Baghdad, Iraq

Abstract

Transforming growth factor-beta signaling pathways involve in many processes that play a key role in pathogenesis of asthma. This study was investigate the serum level of transforming growth factor-beta1 (TGF-BI) in asthmatic patients. And the quantity gene expression, of transforming growth factor receptor III (TGF-BRIII), Elisa technique was used to assess TGF-B1 serum level, while real time PCR used to determine quantity of gene expression. This study is the first study of its type, locally and globally which investigated the quantity gene expression of TGF-BRIII in asthmatic patients. The serum level of TGF-B1 was elevated in patients as compared to control. While the mean of TGF-B1 serum level in patients were (669.2 ± 70.0) as compared to control (361.5 ± 21.5) under p value ($P < 0.001$). The TGF-BRIII gene expression was decrease in the asthmatic patients compared with control group. The (mean ± SE) of gene expression in the patient was (0.18 ± 0.07) as compared to control (1.27 ± 0.2). Down regulate of TGF-BRIII expression, May weakens the inhibitory role of TGF-BRIII on some signaling pathways that TGF-B can mediate it as consequence irreversible structural change in airway vessel may occurred. The elevated serum level of TGF-B in asthma patients may play an important role in reduce TGF-BRIII expression.

INTRODUCTION

Asthma is a chronic bronchial inflammation disease, characterized by frequent attacks of difficult breathing and reversible airflow obstruction, and bronchospasm. [1] Asthmatic Patients suffer from one or more of the symptoms that include wheezing, coughing, chest tightness or shortness of breath.[2] Evidence suggests that, Exposure to inflammation for a long period leads to remodeling of the airways.[3] multiple cellular response can induced by TGF-B including proliferation, differentiation, survival, and apoptosis. In addition, TGF-B involve in the development of several disease including asthma, and cancer.[4] TGF-B considered a fibrotic cytokine, involved in the airway fibrosis, and mediating a key role in airway remodeling.[5][6] Furthermore, TGF-B enhancing airway smooth muscle proliferation in asthmatic patients, that will lead to increasing the airway wall thickness, and involve in structural change.[7] Airway remodeling lead to fixed airflow obstruction, which is regard to be a late and irreversible manifestation of airway remodeling. [8] TGF-B have a dual role, as anti and pro-inflammatory cytokine; participate in initiation, progress, and resolution of inflammatory responses in the airway.[9][10] where TGF-b regulation activation, survival, and chemotaxis of dendritic cells, macrophages, lymphocytes, mast cells, natural killer cells, and granulocytes.[11] Three TGF-B receptors (TGF-BR) were identified Includes, TGF-BRI, TGFBRII, and TGF-BRIII. The latter is the most widespread TGF-BR.[12] the TGF-B signaling initiates either by binding to TGF-BRIII, then TGF-B will present to TGF-BRII by TGF-BRIII or by directly binding to TGF-BRII, When TGF-β binding to TGF-BRII, TGF-βRI is subsequently recruited, and phosphorylated.[13] TGF-BRIII is a TGF- b superfamily co-receptor serve to presenting ligand to TGF-BRII. Furthermore, several studies have been suggested TGF-BRIII play a fundamental role in mediating and regulating TGF-B signaling through TGF-BRII and TGF-BRI.[14] TGF-BRIII has a short cytoplasmic domain lacking to kinase activity. This cytoplasmic domain is not involve in the presentation role, it have essential role to TGF-BRIII enhance TGF-B mediated inhibition of proliferation.[13] In addition, Chu *et al* (2011) [15] demonstrated that TGFBRIII protects agent serve as anti-fibrotic via the inhibition of TGF- B signaling pathway. Also Vilchis *et al* (2001) [16] has been reported that, TGFBRIII inhibits TGF-b signaling by preventing formation TGFBRI–TGFBRII complex and is a potent TGF-b neutralizing agent. Where referred that TGF-B contribute in airway structural remodeling by induce fibrotic tissue within the asthmatic lung.[17] and increases proliferation of airway smooth muscle cells [6] A study performed by Kim, *et al*. (2010)[18] to evaluate the effects of genetic variations in the TGF-β receptor type III on asthma, 19 SNPs for TGFBRIII were found. Nucleotide polymorphisms (SNPs) may result in altered gene expression levels and cause diseases.[19]

MATERIALS AND METHODS

60 children were enrolled in this study, 30 of Iraqi children with asthma, aged 6-18 and 30 Childs apparently healthy Childs as control group, their ages were match with patients. The study has included two parts (serological and molecular). The serological study has been done by using Elisa technique to evaluate the serum level of TGF-B1 and the assay method was carried out by follow the instructions supplied with kit (Human TGFB1 ELISA Version 18). And the supplies company was Diaclone SAS. While, the molecular study was done by using Real time PCR technique to determine the amount of gene expression. First RNA was extracted from peripheral blood of patients and control by using RNA extraction kit from Geneaid Company, made in Taiwan. After RNA extraction, One-step real-time RT-PCR method was performed, that involves, the reverse transcription (cD synthesis) and qPCR steps are both conducted in the same reaction well.by use Go Taq® 1-Step RT-qPCR System, and Go Tag qPCR sybr green Master Mix promega kit. Transferred 1.5µl of each RNA sample into PCR tube, then was added into all tubes, 5µl of Go Tag qPCR Master Mix, 0.25µl of 1-Step RT-qPCR System, 0.25µl of MgCl2,

0.5µl from each forward and reverse primers, sequences of Primers are available in Table1.and finally 2µl from Nuclease Free Water was added. The CT values were normalize to GAPDH. Primer sequences are available Table (1).

Table 1: Primer name and sequences that used in this study.

Primer Name	Sequences
Forward primer TGF-BRIII	5`-TTG GTA GGG TGA GTG TTT CCA-3`
Reverse primer TGF-BRIII	5`-AGA CCG ACA GGA TTT GCC AT-3`
Forward primer GAPDH	5`-AGA AGG CTG GGG CTC ATT TG-3`
Reverse primer GAPDH	5`-AGG GGC CAT CCA CAG TCT TC-3`

Real Time PCR Program, RT. Enzyme Activation Temperature: 37°C, time: 15:00 minutes, Cycle: 1. the Initial denaturation temperature were 95°C, and the time: 5:00 minutes, Cycle: 1. Denaturation temperature: 95°C, Time 20 Second, Cycle 40. Annealing: 60°C, Time: 20 Second, Cycle 40. Extension: 72°C, Time: 20 Second, Cycle 40. Moreover, the Melt on Green, Melt from 72°C to 95°C at 0.3°C/s.

RESULTS

The result was shown in table -2-, which illustrated the TGF-B1 serum level of patients and control. TGFB1 serum level was highly increased in asthmatic patients as compared to control. The (mean ± SE) in patients was (669.2 ± 70) and in control group was (361.5 ± 21.5) the p value ($P < 0.001$).

Table 2: TGF B1 level (mean ± SE) in the studied groups

Control	Patients	Probability
361.5 ± 21.5	669.2 ± 70.0	P < 0.001

TGF-BRIII gene expression was down regulate in patients as compared to control and the (mean ± SE) of the gene expression in patients was (0.18 ± 0.07) as compared to control was (1.27 ± 0.2), a high significantly difference ($P < 0.001$). The result was shown in table-3-.

Table 3: TGF BR3 gene expression level (mean ± SE) in the studied groups

Control	Patients	Probability
1.27 ± 0.2	0.18 ± 0.07	P < 0.001

DISCUSSION

Transforming growth factor-beta1 (TGF-B1), considered a fibrotic cytokine, and implicated in airway remodeling by prompting structural changes.[20] Elevated levels of TGF-beta correlate with subepithelial fibrosis.[4] It promotes differentiation of fibroblasts into myofibroblasts, which are a major producers of ECM. [21] TGF-B1 also implicated in airway microvascular changes, and smooth muscle remodeling. [22] Entire of this event will lead to, thickness, and irreversible remodeling in airway vessel thus, cause a Permanent airflow narrowing. In this study, a high concentration of serum TGF1 was observed in asthmatic patients. This result corresponds to several studies, which estimated a rise in the level of serum TGF-B1. Manuyakorn *et al* (2008) [6] was found, that serum TGF-b1 level highest in patients with asthma compared to healthy group. In addition, a study performed by El-Sayed *et al* (2004) [23] to estimate the concentration of TGF-B1 in children with asthma, they has been reported that the serum TGF-beta1 was significantly elevated in children with asthma compared to controls. In this study elevated level, of TGF-b1 observed in the sera of asthmatic patients. Chu *et al* (2000) [24] was found that peripheral blood neutrophils in patients with asthma released higher levels of TGF-beta than those from normal subjects. also The rise in serum TGFbeta1 could be secondary to its rise in the respiratory tract during acute asthma as proved by Redington *et al* (1997)[25] TGF-BRIII function as co-receptor presentation TGF-b to the TGF-b receptor. [13] in addition, Several studies have reported a fundamental role of TGF-BRIII in regulation the TGF-B signaling pathway. You *et al* (2007) [26] has been establish that TGF-BRIII mediated inhibition of proliferation. Also Several study in epithelial cell have suggested that TGF-BRIII act to preventing TGFBRII and TGFB-R1 complex formation, thus a potent ability to TGF-b signaling inhibition. [16] Furthermore, TGFBRIII appear as anti-fibrotic agent in the lung and heart.[27] TGF-BRIII also, inhibition TGF-b signaling pathway that involved in ECM production [15]. The importance of TGF-BRIII appear on it protective role that play through its ability to inhibition several TGF-B signaling pathways involved in airway remodeling. In this study was found TGF-BRIII gene expression decrease in asthma patients compared with healthy. TGF- b1 decreased TGF-BRIII messenger RNA (mRNA) expression at the transcriptional level in breast cancer and ovarian cell lines. TGF- b1 directly regulates the TGF-BRIII promoter as proved by Hempel *et al* (2008) [28] Where Elevated level of TGF-B1 observed in several cancer type. [29] [30] Same Circumstances in asthma, several study reported increase in the TGF-B1 level in asthma patients including current study, a rise in serum TGF-B1 level, suggest a possible role of TGF-B1 in decrease TGF-BRIII expression in asthma patients.

CONCLUSIONS

Reduce TGF-BRIII gene expression may will lead to attenuate an important preventive role of TGF-BRIII, that mediated through its ability to inhibition proliferation, fibrosis, and increase ECM production that induced by TGF-B1 in airway of asthma patients. Therefore, TGF-BRIII may can used as a therapeutic Target.

REFERENCES

1- National Heart, Lung, and Blood Institute. National Asthma Education and Prevention Program. Expert panel report 3: guidelines for the diagnosis and management of asthma: full report 2007. http://www. nhlbi. nih. gov/guidelines/asthma/asthgdln. pdf. 2007 Aug.

2- Maslan J, Mims JW. What is asthma? Pathophysiology, demographics, and health care costs. Otolaryngologic Clinics of North America. 2014 Feb 1;47(1):13-22.

3- Canonica GW. Treating asthma as an inflammatory disease. Chest. 2006 Jul 1;130(1):21S-8S.

4- Makinde T, Murphy RF, Agrawal DK. The regulatory role of TGF-β in airway remodeling in asthma. Immunology and cell biology. 2007 Jul;85(5):348-56.
5- Mauviel A. Transforming growth factor-β. InFibrosis Research 2005 (pp. 69-80). Humana Press.
6- Manuyakorn W, Kamchaisatian W, Atamasirikul K, Sasisakulporn C, Direkwattanachai C, Benjaponpitak S. Serum TGF-[beta] 1 in Atopic Asthma. Asian Pacific journal of allergy and immunology. 2008 Dec 1;26(4):185.
7- Chen G, Khalil N. TGF-β1 increases proliferation of airway smooth muscle cells by phosphorylation of map kinases. Respiratory research. 2006 Dec;7(1):2.
8- Bergeron C, Tulic MK, Hamid Q. Airway remodelling in asthma: from benchside to clinical practice. Canadian respiratory journal. 2010;17(4):e85-93.
9- Duvernelle C, Freund V, Frossard N. Transforming growth factor-β and its role in asthma. Pulmonary pharmacology & therapeutics. 2003 Aug 1;16(4):181-96.
10- Schmidt-Weber CB, Blaser K. Regulation and role of transforming growth factor-β in immune tolerance induction and inflammation. Current opinion in immunology. 2004 Dec 1;16(6):709-16.
11- Li MO, Wan YY, Sanjabi S, Robertson AK, Flavell RA. Transforming growth factor-β regulation of immune responses. Annu. Rev. Immunol.. 2006 Apr 23;24:99-146.
12- Lopez-Casillas F, Wrana JL, Massagué J. Betaglycan presents ligand to the TGFβ signaling receptor. Cell. 1993 Jul 2;73(7):1435-44.
13- Blobe GC, Liu X, Fang SJ, How T, Lodish HF. A novel mechanism for regulating transforming growth factor β signaling: functional modulation of type III TGF-β receptor expression through interaction with the PDZ domain protein, GIPC. Journal of Biological Chemistry. 2001 Aug 23.
14- Finger EC, Turley RS, Dong M, How T, Fields TA, Blobe GC. TβRIII suppresses non-small cell lung cancer invasiveness and tumorigenicity. Carcinogenesis. 2008 Jan 3;29(3):528-35.
15- Chu W, Li X, Li C, Wan L, Shi H, Song X, Liu X, Chen X, Zhang C, Shan H, Lu Y. TGFBR3, a potential negative regulator of TGF-β signaling, protects cardiac fibroblasts from hypoxia-induced apoptosis. Journal of cellular physiology. 2011 Oct;226(10):2586-94.
16- VILCHIS-LANDEROS MM, MONTIEL JL, MENDOZA V, MENDOZA-HERNÁNDEZ G, LÓPEZ-CASILLAS F. Recombinant soluble betaglycan is a potent and isoform-selective transforming growth factor-β neutralizing agent. Biochemical Journal. 2001 Apr 1;355(1):215-22.
17- Al-Alawi M, Hassan T, Chotirmall SH. Transforming growth factor β and severe asthma: a perfect storm. Respiratory medicine. 2014 Oct 1;108(10):1409-23.
18- Kim HK, Jang TW, Jung MH, Park HW, Lee JE, Shin ES, Cho SH, Min KU, Kim YY. Association between genetic variations of the transforming growth factor β receptor type III and asthma in a Korean population. Experimental & molecular medicine. 2010 Jun;42(6):420.
19- Wang XG, Huang JM, Feng MY, Ju ZH, Wang CF, Yang GW, Yuan JD, Zhong JF. Regulatory mutations in the A 2M gene are involved in the mastitis susceptibility in dairy cows. Animal genetics. 2014 Feb;45(1):28-37.
20- Duvernelle C, Freund V, Frossard N. Transforming growth factor-β and its role in asthma. Pulmonary pharmacology & therapeutics. 2003 Aug 1;16(4):181-96.
21- Tomasek JJ, Gabbiani G, Hinz B, Chaponnier C, Brown RA. Myofibroblasts and mechano-regulation of connective tissue remodelling. Nature reviews Molecular cell biology. 2002 May;3(5):349.
22- Halwani R, Al-Muhsen S, Al-Jahdali H, Hamid Q. Role of transforming growth factor–β in airway remodeling in asthma. American journal of respiratory cell and molecular biology. 2011 Feb;44(2):127-33.
23- El-Sayed ZA, El-Hakim IZ, El-Kerdani TA, Ghanem HM. Serum transforming growth factor-beta1 in asthmatic children. Egyptian Journal of Pediatric Allergy and Immunology (The). 2004;2(1).
24- Chu HW, Trudeau JB, Balzar S, Wenzel SE. Peripheral blood and airway tissue expression of transforming growth factor β by neutrophils in asthmatic subjects and normal control subjects. Journal of allergy and clinical immunology. 2000 Dec 1;106(6):1115-23.
25- Redington AE, Madden J, Frew AJ, Djukanovic R, Roche WR, Holgate ST, Howarth PH. Transforming growth factor-β 1 in asthma: measurement in bronchoalveolar lavage fluid. American Journal of Respiratory and Critical Care Medicine. 1997 Aug 1;156(2):642-7.
26- You HJ, Bruinsma MW, How T, Ostrander JH, Blobe GC. The type III TGF-β receptor signals through both Smad3 and the p38 MAP kinase pathways to contribute to inhibition of cell proliferation. Carcinogenesis. 2007 Sep 3;28(12):2491-500.
27- Hermida N, López B, González A, Dotor J, Lasarte JJ, Sarobe P, Borrás-Cuesta F, Díez J. A synthetic peptide from transforming growth factor-β1 type III receptor prevents myocardial fibrosis in spontaneously hypertensive rats. Cardiovascular research. 2008 Nov 19;81(3):601-9.
28- Hempel N, How T, Cooper SJ, Green TR, Dong M, Copland JA, Wood CG, Blobe GC. Expression of the type III TGF-β receptor is negatively regulated by TGF-β. Carcinogenesis. 2008 Feb 24;29(5):905-12.
29- Tsushima HI, Kawata S, Tamura SH, Ito NO, Shirai YA, Kiso SH, Imai YA, Shimomukai HI, Nomura YA, Matsuda YA, Matsuzawa YU. High levels of transforming growth factor beta 1 in patients with colorectal cancer: association with disease progression. Gastroenterology. 1996 Feb 1;110(2):375-82.
30- Barrett-Lee P, Travers M, Luqmani Y, Coombes RC. Transcripts for transforming growth factors in human breast cancer: clinical correlates. British journal of cancer. 1990 Apr;61(4):612.