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

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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-B1) 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 ± 5E) 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 asthmatic 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, TGF-BRII, 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-BRII or by directly binding to TGF-BRII. When TGF-B binding to TGF-BRII, TGF-BRII 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-BRII. [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 TGFFRIII 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 TGFBR1–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 TGFBI 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 Taq 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.

<table>
<thead>
<tr>
<th>Primer Name</th>
<th>Sequences</th>
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<tbody>
<tr>
<td>Forward primer TGF-BRIII</td>
<td>5<code>-TGG GTA GGG TGA GTG TTT CCA-3</code></td>
</tr>
<tr>
<td>Reverse primer TGF-BRIII</td>
<td>5<code>-AGA CCG ACA GGA TTT GCC AT-3</code></td>
</tr>
<tr>
<td>Forward primer GAPDH</td>
<td>5<code>-AGA AGG CTG GGG CTC ATT TG-3</code></td>
</tr>
<tr>
<td>Reverse primer GAPDH</td>
<td>5<code>-AGG GCC CAT CCA CAG TCT TC-3</code></td>
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Real Time PCR Program, RT. Enzyme Activation Temperature: 37°C, time: 15:00 minutes, Cycle: 1. the Initial denaturation temperature was 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. TGF-B1 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

<table>
<thead>
<tr>
<th>Control</th>
<th>Patients</th>
<th>Probability</th>
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<tr>
<td>361.5 ± 21.5</td>
<td>669.2 ± 70.0</td>
<td>P &lt; 0.001</td>
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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

<table>
<thead>
<tr>
<th>Control</th>
<th>Patients</th>
<th>Probability</th>
</tr>
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<tbody>
<tr>
<td>1.27 ± 0.2</td>
<td>0.18 ± 0.07</td>
<td>P &lt; 0.001</td>
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</table>

DISCUSSION
Transforming growth factor-beta1 (TGF-B1), considered a fibrotic cytokine, and implicated in airway remodeling by prompting structural changes. Elevated levels of TGF-beta correlate with subepithelial fibrosis. It promotes differentiation of fibroblasts into myofibroblasts, which are a major producers of ECM. TGF-B1 also implicated in airway microvascular changes, and smooth muscle remodeling. 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) 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) 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) 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) TGF-BRIII function as co-receptor presentation TGF-b to the TGF-b receptor. In addition, Several studies have reported a fundamental role of TGF-BRIII in regulation the TGF-B signaling pathway. You et al (2007) has been establish that TGF-BRIII mediated inhibition of proliferation. Also Several study in epithelial cell have suggested that TGF-BRIII act to preventing TGFBR1 and TGFBR1 complex formation, thus a potent ability to TGF-b signaling inhibition. Furthermore, TGFBR1II appear as anti-fibrotic agent in the lung and heart. TGF-BRIII also, inhibition TGF- b signaling pathway that involved in ECM production . 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) Where Elevated level of TGF-B1 observed in several cancer type. 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.

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