The role of Bcl-2 protein, Bax protein and there ratio in peripheral blood lymphocytes of asthmatic patients

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Summary:
Background: Asthma is an inflammatory airway disease; this inflammatory response can be attributed to reduced lymphocyte apoptosis in peripheral blood and in airway tissues. The mechanism behind this could be attributed to decreased Bcl2 protein and increase Bax protein in peripheral blood lymphocytes of asthmatic patients.
Aim: to explore the mechanism behind decreased lymphocyte apoptosis in peripheral blood of asthmatic patients at cellular level.
Method: Ninety four subjects; (44) control and (50) patients were included in this study during the period from (2003) to (2004). The aspirated lymphocytes for each individual were prepared and stained by immunocytochemistry to study the percentage of anti-apoptotic Bcl-2 protein, pro-apoptotic Bax protein and their ratio.
Results: Our results showed that the percentage of Bcl2 protein in peripheral blood lymphocyte of asthmatic patients were significantly higher than control (33.23±11.56, 21.47±4.16)(P=<0.00001).
The percentage of Bax protein in peripheral blood lymphocyte in asthmatic patients was significantly lower than the control (21.43±6.88, 24.89±4.8) (P=0.0323). Bcl2/Bax ratio was positive in asthmatic patients there was significant difference in comparison with the control (1.68±0.57, 0.87 ± 0.17) (P=<0.00001).
Conclusion: This study clarifies the role of these 2 proteins in the process of reduction of apoptosis in peripheral blood lymphocytes of asthmatic patients.
Key words: Lymphocyte apoptosis, asthma, Bcl-2 protein, Bax protein.

Introduction:
Lymphocytes are known to play a pivotal role in asthmatics airway inflammation. Infiltration of lymphocytes into bronchial wall and interaction with inhaler antigen will lead to bronchial epithelial cell damage1 and hence inflammatory process.2 In asthma, where the inflammation is the major problem, reduced lymphocyte apoptosis may play a key role in the pathophysiology that lead to chronic persistent airway inflammation3.

Lymphocyte apoptosis can be induced by tow major pathways:
Receptor-Ligand Interaction: Death inducing receptors of tumor necrosis family receptor (TNFR) includes: FAS, TNFR1, TNFR2, DR4, DR3, TRAIL-R1 (TNF-related apoptosis inducing ligand receptor-1), TRAIL-2, and DR6. These receptors contain both extra cellular domains and intra cellular cytoplasmic death domain (DD)4. Fas induced apoptosis adaptor protein FADD (Fas associated death domain) contains C-terminal DD which promote the interaction with the same DD in trimerized Fas-receptor and N-terminal of caspase -8 5. This complex is called DISC (Death Inducing Signaling Complex) by which pro-caspase-8 was cleaved and activated which in turn will lead to activation and cleavage of caspase-3, a central executioner caspase that will activate...
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Mitochondrial pathway:
The mechanisms by which mitochondria stimulate apoptotic process remain unclear, and could be due to opening of a mitochondria! permeability transition pore and the presence of specific channels for cytochrome c release from mitochondria. Cytochrome c released from mitochondria is under the control of the Bcl-2 family of proteins which include two categories: The first are the apoptotic Inhibitors like (Bcl-2, Bcl-xl) the second apoptotic promoter like (Bax, Bak). After release of cytochrome c from mitochondria it interacts with Apaf-1 (Apoptotic activated factor-1) in the cytoplasm. Apaf-1 activates procaspase-9, to form apoptosom complex that can activate procaspase-3 and pro-caspase-7 And hence execution of apoptotic process.

Inhibition of lymphocyte apoptosis plays an important role in airway inflammation. In this study we try to explain one of the molecular mechanisms for the inhibition of lymphocyte apoptosis in asthmatic patients through studying the anti-apoptotic Bcl-2 protein, pro-apoptotic Bax protein and their ratio.

Patients and Methods
This study included (94) subjects; (44) control and (50) asthmatic patients (24 male and 26 female). They have been diagnosed previously by specialist senior physician, tested by Pulmonary Function Test Unit at Center of Diagnosis and Treatment of Allergy and Asthma in Baghdad province. The aspirated lymphocytes for each individual were prepared to study: the percentage of anti-apoptotic Bcl-2 protein, pro-apoptotic Bax protein and its ratio through immunocytochemistry. This procedure, in brief, a primary antibody reacts with an antigen (Bcl-2 and Bax proteins).

A biotinylated secondary antibody then reacts with the primary antibody. This is followed by the attachment of an enzyme-conjugated streptavidin to the biotins on the secondary antibody. The enzyme converts a substrate to a colored reaction product. High levels of signal amplification are achieved due to the binding of multiple units of secondary antibody to each primary antibody, the binding of multiple enzyme-conjugated streptavidin molecules to each secondary antibody, and the enzymatic conversion of the substrate. Then percentages were obtained the counting the number of positive cells under light microscope (40X and 100X).

Statistical analysis: Student T-test was used to compare between patients and control (unpaired T-test) the difference was considered significant statistically when p < 0.05.

Results
In this study, the results showed that the percentage of Bcl-2 protein in peripheral blood lymphocyte of asthmatic patients were significantly higher than control (33.23±11.56, 21.47±4.16) (p=<0.00001). Table (1) shows immunocytochemical staining of Bcl-2 in lymphocytes of normal and asthmatic patients. While the percentage of Bax protein in peripheral blood lymphocyte in asthmatic patients were significantly lower than control (21.43±6.88, 24.89±4.8) (p=<0.0323).Figurel shows immunocytochemical staining of Bax in lymphocytes of control and asthmatic patients. Bcl-2/Bax ratio was positive in asthmatic patients there was significant different in comparison with control (1.68±0.57, 0.87±0.17) (p=<0.00001).

Table 1: comparison between Bcl-2%, Bax% and its ratio in asthmatic patients and control

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patients</th>
<th>Control</th>
<th>*P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bcl-2%</td>
<td>33.23±11.56</td>
<td>21.47±4.16</td>
<td>0.00001</td>
</tr>
<tr>
<td>Bax%</td>
<td>21.43±6.88</td>
<td>24.89±4.8</td>
<td>0.0323</td>
</tr>
<tr>
<td>Bcl-2/Bax</td>
<td>1.68±0.57</td>
<td>0.87±0.17</td>
<td>0.00001</td>
</tr>
</tbody>
</table>

• P value significant at 0.05
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Discussion:
In this study we found that anti-apoptotic Bcl-2 protein was increased in patients with asthma compared with control. Conversely, the pro-apoptotic Bax protein was decreased. So Bcl-2/Bax ratio in asthmatics was higher than control. This imbalance between Bcl-2/Bax proteins explains one of the molecular mechanisms for the inhibition of lymphocyte apoptosis in asthma. Xue, Xu and Zhange have found a significant increase in percentage of anti-apoptotic Bcl-2 protein in asthmatic patients, and a decrease in the percentage of pro-apoptotic Bax protein in patients compared with control.12

Asthmatic process results from complex interactions between inflammatory cells, mediators and the tissue of airways. Lymphocytes are known to play a major role in asthmatic airway inflammation; the presence of lymphocyte has been correlated to bronchial hyper responsiveness.14 The T112 subset of T lymphocyte produces and release interleukin (IL-4, IL-5, IL-6, IL-10 and IL-13. 15 IL-4 and IL-13 promote B-lymphocyte to switch IgE synthesis and enhance recruitment of eosinophil and mast cells activation and migration to the site of inflammation.16

Figure: Immunocytochemical staining of Bcl-2 of lymphocytes by peroxidase/ DAB stain (brown), counter stained with Meyer's hematoxylin (blue).
A: Peripheral blood lymphocytes of control, black arrow show lymphocyte stained with DAB stains, white arrow show lymphocyte stained with hematoxylin stains. B: Peripheral blood lymphocytes of asthmatic patients black arrow show lymphocyte stained with DAB stains, white arrow show lymphocyte stained with hematoxylin stains. (100X.)

Figure2: Immunocytochemical staining of Bax of lymphocytes by Alkaline phosphatase/chromogen fast A, B stain (red), counter stained with Meyer's hematoxylin (blue). A: Peripheral blood lymphocytes of control, black arrow show lymphocyte stained with chromogen fast A, B stains, white arrow show lymphocyte stained with hematoxylin stains. B: Peripheral blood lymphocytes of asthmatic patients black arrow show lymphocyte stained with chromogen fast A, B stains, white arrow shows lymphocyte stained with hematoxylin stains. (100X)
Bcl-2 protein can inhibit the direct effect of pro-apoptotic (stimulator) Bax proteins by (Bcl-2-Bax interaction) to form heterodimer complex. The mechanism of action of Bax protein on mitochondria membrane is still ambiguous, Bax which form transmembrane channel protein causes changes in membrane potential of mitochondria that lead to release of cytochrome c and other pro-apoptotic protein that causes caspase activation and hence activation of apoptosis. Bax protein will open the mitochondrial permeability transition pore lead to release of mitochondrial apoptotic proteins; cytochrome c, Apaf-1 (apoptotic activator factor-1) and Smac/Diablo (Second mitochondria-derived activator of caspase/Direct IAP-Binding protein with Low isoelectric point). The release of these proteins will complete the activation of apoptotic process in lymphocytes. We conclude that the imbalance between Bcl-2/Bax proteins clarify one of the molecular mechanisms for the inhibition of lymphocyte apoptosis in asthma.

References: