INTERLEUKINE-33 AND SOLUBLE ST2 AND THEIR CORRELATION WITH ASTHMA SEVERITY AND AS FUTURE THERAPEUTIC TARGETS.
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ABSTRACT
Interleukin-33 is a member of IL-1 family of cytokines and binds to two receptors: ST2 (IL-1R1) and IL-1 receptor accessory protein (IL-1RAP). There are Two isoforms of ST2 proteins: ST2L which is a transmembrane form and a soluble ST2 (sST2) which is a secreted form that can serve as a decoy receptor of IL-33. The IL-33/ST2 signaling pathway activates airway eosinophils that exacerbate airway inflammation. The aim of this study was to analyze the serum levels of IL-33 and its receptor sST2 in patients with bronchial asthma to assess if the serum levels of IL-33 and/or sST2 may be markers of the disease severity and potential therapeutic targets.

Patients and methods: This study was carried out at Microbiology & Immunology and Chest Departments, Faculty of Medicine, Zagazig University Hospitals during the period from December 2012 to September 2013. The study included 30 patients diagnosed as bronchial asthma. Patients were classified to two groups: Group 1: included 15 patients (8 males and 7 females) with a mean age of 36.2 ± 15.8 during exacerbations of bronchial asthma. Group 2: include 15 patients (8 males and 7 females) with a mean age of 37.3 ± 12.8. They were stable asthmatic patients. There were 30 normal healthy persons as a control group. All patients were subjected to full medical history, general and local examinations, Plain chest X-ray PA and lateral views, pulmonary function tests, Liver and kidney functions tests, intradermal skin prick test, measurement of serum levels of IL-33 (WKEA MED), soluble ST2 (sST2) (OmniKine) and total IgE (IMMUNOSPEC) by enzyme linked immunosorbent technique using commercial kits.

Results: There was a high significant increase in the mean serum levels of IL-33 in both groups of patients (p < 0.001) with the highest mean serum level 960 ± 336 ng/L in group 1 followed by 732.2 ± 68.3 ng/L in group 2 while the normal control group mean serum level was 174 ± 41 ng/L. As regards serum levels of sST2, there was a high significant increase in its mean levels in both groups of patients (p < 0.001) with the highest mean serum level 96.8 ± 25 pg/ml in group 1 followed by 83.3 ± 5.3 pg/ml in group 2 while the normal control mean serum level was 33.9 ± 9.6 pg/ml. In acute exacerbated patients there was significant — correlation between FEV1% and serum levels of IL-33 and in stable asthmatic patients there was significant + correlation between PEFR variability and serum levels of sST2.

Conclusion: The serum levels of IL-33 and its receptor sST2 were markedly elevated in patients with bronchial asthma and this supports the concept of sST2 and Interleukin-33 as therapeutic targets in bronchial asthma.

Key words: Interleukin-33, soluble ST2, bronchial asthma.

INTRODUCTION
Bronchial asthma is thought to be T helper 2 (Th2) cell-mediated immune diseases. Th2 cells produce cytokines, such as interleukin (IL)-33 which is also a chemoattractant for human Th2 cells. IL-33 is produced by mast cells after immunoglobulin (Ig) E-mediated activation and is able to trigger mast cells to release proinflammatory cytokines in vitro[1]. IL-33 is a member of the IL-1 family of cytokines and binds to two receptors: ST2 (IL-1R1) and IL-1 receptor accessory protein (IL-1RAP). There are two isoforms of ST2 proteins: ST2L, a transmembrane form, and soluble ST2 (sST2), a secreted form that can serve as a decoy receptor of IL-33. ST2 is highly expressed on mast cells and selectively on Th2 cells [2].

High levels of sST2 have been found in the sera of adults and children with acute asthma [3].

The IL-33/ST2 signaling pathway activates airway eosinophils that exacerbate airway inflammation. This pathway is critical for the progression of IgE-dependent inflammation. Mutations in the gene for IL1RL1 (ST2) have been linked to atopic dermatitis and asthma [4]. Steroids and combination therapies with long-acting β-agonists are the mainstay of asthma treatment and effectively suppress cytokine expression and acute inflammatory symptoms. However,
they do not prevent, reverse or treat the underlying causes of disease. These treatments require constant monitoring and are associated with side-effects and resistance. Therefore, there is an urgent need for new and more effective treatments and cytokines have been extensively investigated as potential therapeutic targets. The aim of this study was to analyze the serum levels of IL-33 and its receptor sST2 in patients with bronchial asthma to assess if the serum levels of IL-33 and/or sST2 may be markers of the disease severity and potential therapeutic targets.

**PATIENTS AND METHODS**

This study was carried out at the Microbiology & Immunology and Chest Departments, Faculty of Medicine, Zagazig University Hospitals during the period from December 2012 to September 2013 after ethics committee / IRB approval. The study included 30 patients diagnosed as bronchial asthma according to GINA 2012\(^5\) as follows:

- Recurrent episodes of wheezing, breathlessness, chest tightness, and coughing, particularly at night or in the early morning.
- Pulmonary function test demonstrating reversible airway obstruction, manifested by postbronchodilator increase in FEV1 > 15%.
- Peak expiratory flow (PEF): Variability by 7-20%.

Patients were classified to two groups:

- **Group I (asthmatic patients during acute exacerbations):**
  
  This group included 15 patients; 8 males and 7 females with a mean age 36.2 ± 1.5 years, during exacerbation of bronchial asthma.
  
  The severity of exacerbations were assessed according to GINA (2012) \(^5\), as mild, moderate, severe and respiratory arrest imminent.

- **Group 2 (stable asthmatic patients):**
  
  This group included 15 patients 8 males and 7 females with a mean age 37.3±12.8 years. They were stable asthmatic patients and the last exacerbation was one month ago. They were classified according GINA 2012 into: controlled, partially controlled and uncontrolled.

- **Control group:**

  There were 30 normal healthy persons as a control group they were 15 males and 15 females with a mean age 34.5 ± 9.

  All patients were subjected to full medical history, general and local examination, Plain chest X-ray PA and lateral views, pulmonary function tests, Liver and kidney function tests. Five ml of blood were taken from patients and control subjects. One ml of blood was collected in EDTA containing tubes for eosinophilic count, sera were separated from the other 4ml and stored at -20 until used for measurement of serum levels of IL-33, sST2 and total IgE.

  Commercial solid phase sandwich enzyme-linked immunosorbent assays were used for measurement of serum levels of IL-33(WKEA MED), sST2 (Omnikine) and total IgE(Immunospec). The assays were performed using the protocols recommended by the manufacturers. The concentration of IL-33, sST2 and total IgE were determined by comparing the optical density of the sample to the standard curve.

**Statistical analysis:**

Statistical analysis was performed with SPSS version19 software package (SPSS, Inc. Chicago). Categorical variables were expressed as proportions, and continuous variables that were or were not normally distributed were expressed as means ±SD or medians (quartiles), respectively. The t-test or Mann–Whitney test was used to compare means or medians between different groups, for variables that were or were not normally distributed, respectively. For all analyses, P value <0.05 was considered significant.
RESULTS

Table (1): Comparison between the means of eosinophil %, total serum IgE, serum IL-33 and serum level of sST2 in the three groups.

<table>
<thead>
<tr>
<th></th>
<th>Control X±SD</th>
<th>Group I (during acute attack) X±SD</th>
<th>Group II (stable Cases in between attacks X±SD)</th>
<th>F</th>
<th>P1</th>
<th>P2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eosinophil %</td>
<td>1±0.9</td>
<td>16.5±2.6</td>
<td>5.13±2.3</td>
<td>354.129</td>
<td>&lt;0.001**</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>IgE (IU/ml)</td>
<td>20.6±15.7</td>
<td>324.7±133.4</td>
<td>68.2±47.3</td>
<td>KW=41.825</td>
<td>&lt;0.001**</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>IL33 (ng/L)</td>
<td>174±41.2</td>
<td>960±336</td>
<td>732.2±68.3</td>
<td>120.433</td>
<td>&lt;0.001**</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>sST2 (pg/ml)</td>
<td>33.9±9.6</td>
<td>96.8±25</td>
<td>83.3±5.3</td>
<td>117.625</td>
<td>&lt;0.001**</td>
<td>0.012*</td>
</tr>
</tbody>
</table>

F = value of ANOVA (analysis of variance) test
P1 means probability of difference among the three groups
P2 means probability of difference between group 1 and group 2.

The mean values of total serum IgE, serum IL-33, sST2 and eosinophilic percentage were higher in acute attacks and in stable asthmatics than control group and the difference is highly significant (p<0.001).

The mean value of serum soluble ST2 in patients in acute attacks was higher than in stable asthmatics and the difference is significant (p=0.012). (Tab and Fig 1)

Fig (1): Comparison between mean values of serum total IgE, IL-33, sST2 and eosinophil percentage in the three groups.

Table (2): Correlation between FEV1% and total IgE, IL-33 & sST2 in group 1 (during acute attack) patients.

<table>
<thead>
<tr>
<th></th>
<th>FEV1%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(r)</td>
</tr>
<tr>
<td>Total IgE</td>
<td>-0.427</td>
</tr>
<tr>
<td>IL-33</td>
<td>-0.776</td>
</tr>
<tr>
<td>sST2</td>
<td>0.256</td>
</tr>
</tbody>
</table>
Table (2) shows that in acute exacerbated patients there is a significant – ve correlation between FEV1% and total IgE as (r) -0.427 and p = 0.113. There is a high significant –ve correlation between FEV1% and IL-33 as (r) -0.776 and P = 0.001, while there is no correlation between FEV1% and sST2.

Table (3): Correlation between PEFR variability and total IgE, IL-33 and sST2 in group 2 patients (stable asthmatic cases).

<table>
<thead>
<tr>
<th>PEFR variability%</th>
<th>(r)</th>
<th>p. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgE</td>
<td>0.366</td>
<td>0.179</td>
</tr>
<tr>
<td>IL-33</td>
<td>0.179</td>
<td>0.522</td>
</tr>
<tr>
<td>sST2</td>
<td>+ 0.524</td>
<td>0.045</td>
</tr>
</tbody>
</table>

Table (3) shows that there is a significant + ve correlation between PEFR variability and serum level of sST2 as (r) + 0.524 and p = 0.045, while there is no correlation between PEFR variability and serum levels of either IgE or IL-33.

Table (4): Correlation between mean serum level of IL-33, esinophil %, total IgE, and sST2 in patients during acute attack.

<table>
<thead>
<tr>
<th>IL-33 in patients during acute attack</th>
<th>(r)</th>
<th>p. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Esinophil%</td>
<td>0.185</td>
<td>0.509</td>
</tr>
<tr>
<td>Total IgE</td>
<td>0.491</td>
<td>0.063</td>
</tr>
<tr>
<td>sST2</td>
<td>-0.381</td>
<td>0.161</td>
</tr>
</tbody>
</table>

There is no correlation between IL-33 and total IgE and eosinophilic % (p>0.05) during acute attacks. There is negative insignificant correlation between IL-33 and soluble ST2 in patients in acute attacks, (P>0.05)

Table (5): Correlation between mean serum IL-33 and total IgE, esinophil % and sST2 in stable asthmatics

<table>
<thead>
<tr>
<th>IL-33 in stable asthmatics</th>
<th>(r)</th>
<th>p. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Esinophil%</td>
<td>0.599</td>
<td>0.018*</td>
</tr>
<tr>
<td>Total IgE</td>
<td>0.6</td>
<td>0.018*</td>
</tr>
<tr>
<td>sST2</td>
<td>-0.099</td>
<td>0.727</td>
</tr>
</tbody>
</table>

There is a significant correlation between mean serum IL-33 and total IgE and eosinophilic % (p<0.05) in between attacks. There is negative insignificant correlation between IL-33 and soluble ST2 in patients in between attacks, (P>0.005).

Table (6): Association between degree of disease severity and level of IL33 in patients during acute attack using ANOVA (analysis of variance) test.

<table>
<thead>
<tr>
<th>IL33(ng/L)</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>X±SD</td>
<td>716.7±28.9</td>
<td>816.7±51.6</td>
<td>1275±335.8</td>
<td>9.229</td>
<td>0.004*</td>
</tr>
<tr>
<td>Range</td>
<td>700-750</td>
<td>750-900</td>
<td>950-1700</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
There is an association between the mean levels of IL-33 in acute asthmatic patients and disease severity and the association is significant (p=0.004).

**Table (7):** Association between degree of disease severity and level of sST2 in patients during acute attack using ANOVA (analysis of variance) test.

<table>
<thead>
<tr>
<th>sST2 (pg/ml)</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>X±SD</td>
<td>78.5±4.5</td>
<td>85.8±1.7</td>
<td>117.3±29.8</td>
<td>5.656</td>
<td>0.019*</td>
</tr>
<tr>
<td>Range</td>
<td>73.4-82</td>
<td>85-89.2</td>
<td>90.8-166.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

There is a significant association between the mean levels of soluble ST2 in acute asthmatic patients and the degree of disease severity, and the association is significant (p=0.019).

**DISCUSSION**

Asthma is a chronic inflammatory disease classically characterized by airway hyperresponsiveness, allergic inflammation, elevated serum IgE levels and increased Th2 cytokine production. Given that IL-33 is a strong inducer of Th2 immune responses its role in asthma has been extensively studied [6]. IL-33, a member of the IL1-cytokine family, is considered to be crucial for the induction of T-helper type 2 cell dominant immune responses such as host defense against nematodes and allergic diseases [7].

IL-33 receptor was first identified as IL-1 receptor-like molecule and termed as ST2. ST2 is an Interleukin-1 receptor family member and exist in both a membrane-bound isoform and a soluble isoform (sST2) [8].

IL-33 is the functional ligand for ST2 and ST2/IL-33 signaling regulate inflammation and immunity [9]. IL-33 and its receptor are part of IL-1 family, and their interactions promote a variety of actions from a number of different cell types. The IL-33/ST2 axis is thought to be intimately involved in the promotion and maintenance of allergic inflammation via a number of cell types that include Th2 cells, mast cells and basophils, and structural cells such as airway epithelium and smooth muscle cells [10]. IL-33/ST2 signaling pathway activates air way eosinophils that exacerbate air way inflammation [11].

In our study, there was a high significant rise in serum levels of both interleukin-33 and its receptor sST2 in both exacerbated and stable asthmatic patients and the rise in exacerbated patients was significantly higher than the rise in stable patients.

Cytokines regulate important biological processes such as the immune response or hematopoiesis and involved in pathogenesis of many diseases. In the physiological state their concentrations in biological fluids and tissues are undetectable or very low. Therefore, any increase in their concentrations suggests activation of pathways involved in an inflammatory response or disease development. That is why cytokines may serve as potential biomarkers of various diseases, and changes of their concentrations may be used in follow-up. Moreover, the cytokine profile in the acute phase of the disease often differs from the chronic phase. Measurements of cytokines concentrations are sufficient to diagnose a disease and their concentrations correlate with the stage of the disease [12].

In our study, in acute exacerbated patients there was insignificant – ve correlation between FEV1% & total IgE and a high significant – ve correlation between FEV1% and IL-33, while there was no correlation between FEV1% and serum level of sST2. In stable asthmatic patients there was significant + ve correlation between PEFR variability and serum level of sST2 while there was no correlation between PEFR variability and serum levels of either IgE or IL-33.

IL-33 is increased in Airway smooth muscle and epithelial cells from asthmatics and this increase positively correlates with asthma severity [13]. Soluble ST2 is decoy receptor that is elevated in the serum of
asthma patients, soluble ST2 association with IL-33, blocks ST2-dependent signaling and the immunological effect of IL-33 \(^{[13]}\). Previous studies reported that serum ST2 protein levels increased in patients with acute exacerbation of atopic asthma which is characteristic of Th2-mediated eosinophilic airway inflammation \(^{[14]}\).

Expression of IL-33 was found in higher levels in endotracheal biopsies from human asthmatic subjects compared to controls. IL-33 expression was particularly evident in those with severe asthma and the expression was mainly located in bronchial epithelial cells \(^{[15]}\).

There are many data suggesting that IL-33 is involved in lung inflammation and support the concept of ST2 as a therapeutic target in asthma. Endobronchial biopsies from adults with mild, moderate, and severe asthma were obtained. Airway smooth muscle cells (ASMC) from asthmatic samples, regardless of severity of disease, expressed increased IL-33 mRNA levels compared with controls. IL-33 protein was predominantly expressed by ASMC and epithelial and endothelial cells in asthmatic lungs but was absent in control samples. Thus, IL-33 is expressed by ASMC in asthmatic lungs and shows promise as a potential inflammatory marker for asthma \(^{[16]}\).

Soluble ST2 binds to IL-33 and functions as a decoy receptor of IL-33. Pretreatment with soluble ST2 suppress IL-33 induced NF-kB activity and IL-4, IL-5 and IL-13 expression \(^{[17]}\). Soluble ST2 has been implicated as an anti-inflammatory mediator in inflammatory responses. Pre-treatment with recombinant sST2 protein attenuates expression of TNF, IL-6 and IL-12 in macrophages \(^{[18]}\). ST2/IL-33 interactions on mast cells may serve not only to promote maturation and activation, but also to maintain their localization within the tissue \(^{[19]}\).

**Conclusion:** The serum levels of IL-33 and its receptor sST2 were markedly elevated in patients with bronchial asthma and this supports the concept of sST2 and Interleukin-33 as therapeutic targets in bronchial asthma.

**REFERENCES**


Interleukine-33 andSoluble St2 and Their Correlation With……

العنوان: إنترليوكيين-33 و في مستويات الذيب وال茼أ مستقبلة للعلاج

المقدمة:
إنترليوكيين-33 هو التهاب مزمن يصيب الشعب الهوائية نتيجة وجود حساسية شديدة للشعب الهوائية تجادل مظاهر وآثار السكتات.

تعتبر الدراسة تأثير التهابات الشعب الهوائية في مرضى إنترليوكيين-33 و مستقبلة الذيب اس تشأ في تأثير التهابات هذه المسارين.

الهدف من البحث:
تهدف هذه الدراسة إلى تقييم الوضع على المستوى المكسي لكل من إنترليوكيين-33 و في مستويات الذيب وماها مستقبلات كلاً.

على التهابات الشعب الهوائية في التهابي الشعبي و دراسة ارتباطها بشدة التهاب هذا التهاب في مرضى استكمالاً كهدف مستقبل للعلاج.

المريضي و طرق البحث:
أجري هذا البحث في قسم الميكروبيولوجيا و البيولوجيا و قسم الصرد بكلية طب الزقازيق وقد اشترط الدراسة على 30 مريضاً باندوك الشعبي (15 مريضاً يعانون من نوبات حادة من الروب الشعبي و 15 مريضاً لا يعانون من نوبات حادة أثناء المتابعة).

تم عمل الأدوات الآثني لكل المريضي الذين شملهم البحث:
- اختي التاريخ المرضي - الفحص الاوليتيكي العام والموضوعي للصرد – عمليّة الاكس على الصدر – اختيارات وظائف
- التنفس والكللي و الكبد.

كما تم ضبط عينات من المريضي و المجموعه الطابع لعمل الاختيارات البيانات:
- اختي الخلايا الأپوزيتي بالدم – قياس مستوى الإنترليوكيين 33 و في مستويات الذيب و مستويات الاجسام المضادة في المصل
- باستخدام الايزي.

نتائج البحث:
- وجود زيادة ذات دلالة إحصائي في المستوى المصلي لكل من إنترليوكيين 33 و في مستويات الذيب و مستويات الاجسام المضادة
- وجود علاقة ذات دلالة إحصائي بين مستوى إنترليوكيين 33 و كل من النسبة المئوية للخلايا الأپويتيي و الاجسام المضادة لكللي.
- وجود علاقة ذات دلالة إحصائي بين مستوي إنترليوكيين 33 و FEV1
- وجود ارتباط ذات دلالة إحصائي بين كل من مستوي إنترليوكيين 33 و في مستويات الذيب و شدة المرض.

المستخلص من البحث:
المريضي المصلي لكل من إنترليوكيين 33 و مستقبلة اس تشأ ليا لهما ارتباط ذو دلاله إحصائيي في استخدامه مستقبلة للعلاج التهابي الشعبي.

اعتبرها هذال مستقبلية لعلاج التهابي الشعبي.