INTERLEUKINE-33 AND SOLUBLE ST2 AND THEIR CORRELATION WITH ASTHMA SEVERITY AND AS FUTURE THERAPEUTIC TARGETS.

**Ensaf A. Azazi , Ashraf Elsayed El-shora*, Enas A. Tantawy
and Marwa A. Elsayd .**

Microbiology and Immunology Department, Faculty of Medicine. Zagazig university
*Chest Department, Faculty of Medicine.Zagazig university

ABSTRACT

Interleukin-33 is a member of IL-1 family of cytokines and binds to two receptors: ST2 (IL-1-R1) 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 I**: included 15 patients (8 males and 7 females) with a mean age of 36.2 $\pm$ 15.8 during exacerbations of bronchial asthma. **Group 2**: include 15 patients (8 males and 7 females) with a mean age of 37.3 $\pm$ 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 $\pm$ 336 ng/L in group 1 followed by 732.2 $\pm$ 68.3 ng/L in group 2 while the normal control group mean serum level was 174 $\pm$ 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 $\pm$ 25 pg∕ml in group 1 followed by 83.3 $\pm$ 5.3 pg∕ml in group 2 while the normal control mean serum level was 33.9 $\pm$ 9.6 pg∕ml. In acute exacerbated patients there was significant – ve correlation between FEV1% and serum levels of IL-33 and in stable asthmatic patients there was significant + ve 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 sST$_2$ 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 5.8 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.

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

F =value of ANOVA (analysis of variance) test

P_1: means probability of difference among the three groups

P_2: means probability of difference between group1 and group2**.**

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 values of total serum IgE ,serum IL-33 and eosinophilic percentage were higher in acute attacks than patients in stable asthmatics 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)**
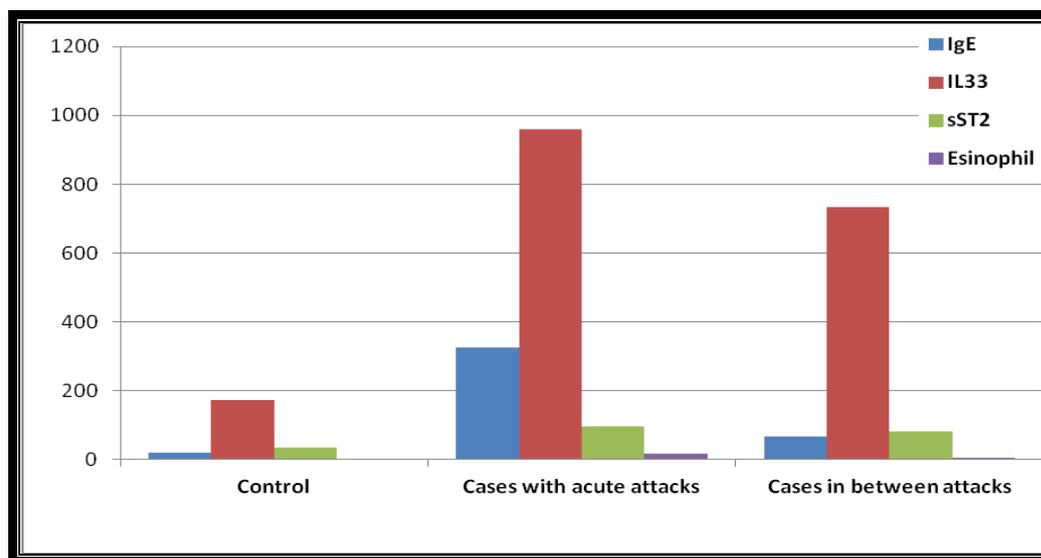
Fig (1): Comparison between mean values of serum total IgE, IL-33, sST2 and eosinophil percentage in the three groups.

Table (2): Correlation between FEV1% and total IgE, IL-33 & sST2 in group1(during acute attack) patients**.**

	FEV1%	
	(r)	p. value
Total IgE	- 0.427	0.113
IL-33	- 0.776	0.001
sST2	0.256	0.356

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).

	PEFR variability%	
	(r)	p. value
IgE	0.366	0.179
IL-33	0.179	0.522
sST2	+ 0.524	0.045

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 .

	IL-33 in patients during acute attack	
	(r)	p. value
Esinophil%	0.185	0.509
Total IgE	0.491	0.063
sST2	-0.381	0.161

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>o.o5)

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

	IL-33 in stable asthmatics	
	(r)	p. value
Esinophil%	0.599	0.018*
Total IgE	0.6	0.018*
sST2	-0.099	0.727

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.

IL33(ng/L)	Mild	Moderate	Severe	F	P
X±SD	716.7±28.9	816.7±51.6	1275±335.8	9.229	0.004*
Range	700-750	750-900	950-1700		

There is an association between the mean levels of IL-33 in acute athmatic 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.

sST2 (pg/ml)	Mild	Moderate	Severe	F	P
X±SD	78.5±4.5	85.8±1.7	117.3±29.8	5.656	0.019*
Range	73.4-82	85-89.2	90.8-166.9		

There is a significant association betweeen 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 Th_2 cytokine production. Given that IL-33 is a strong inducer of Th_2 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 ST_2 . 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/ST_2 axis is thought to be intimately involved in the promotion and maintenance of allergic inflammation via a number of cell types that include Th_2 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 interlukin-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 ST_2 is decoy receptor that is elevated in the serum of

asthma patients, soluble ST_2 association with IL_{33}, blocks ST_2L-dependent signaling and the immunological effect of IL_{33} [13]. Previous studies reported that serum ST_2 protein levels increased in patients with acute exacerbation of atopic asthma which is characteristic of Th_2-mediated eosinophilic airway inflammation[14].

Expression of IL-33 was found in higher levels in endotrachial 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 ST_2 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 ST_2 binds to IL-33 and functions as a decoy receptor of IL-33 . Pretreatment with soluble ST_2 suppress IL-33 induced NF-kB activity and IL-4, IL-5 and IL-13 expression [17]. Soluble ST_2 has been implicated as an anti-inflammatory mediator in inflammatory responses. Pre-treatment with recombinant sST_2 protein attenuates expression of TNF, IL-6 and IL-12 in macrophages[18]. ST_2/IL-33 interactions on mast cells may serve not only to promote maturation and activation, but also to maintain their localizsation 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 sST_2 and Interleukin-33 as therapeutic targets in bronchial asthma.

REFERENCES

1- M. Ali, G. Zhang and W.R. Thomas (2009): Investigations into the role of ST2 in acute asthma in children. Tissue Antigens; 73: 206–12.

2- M. Lohning, A. Stroehmann and A.J. Coyle (2010): T1/ST2 is preferentially expressed on murine Th2 cells, independent of interleukin 4, interleukin 5, and interleukin 10, and important for Th2 effector function. Proc Natl Acad Sci USA.; 95 : 6930–5.

3- K. Oshikawa, K. Kuroiwa and K. Tago (2001): Elevated soluble ST2 protein levels in sera of patients with asthma with an acute exacerbation. Am J Respir Crit Care Med.; 164:277–81.

4- M. Shimizu, A. Matsuda and K. Yanagisawa (2011): Functional SNPs in the distal promoter of the ST2 gene are associated with atopic dermatitis. Hum Mol Genet.; 14:2919–27.

5- Global Strategy for Asthma Management and Prevention, Global Initiative for Asthma (GINA) (2012): Available from: http://www.ginasthma.org/.

6- M. Iikura, H. Suto, N. Kajiwara, K. Oboki, T. Ohno, Y. Okayama, H. Saito, S.J. Galli and S. Nakae (2007): IL-33 can promote survival, adhesion and cytokine production in human mast cells. Lab Invest, 87:971-978.

7- K. Oboki, T. Ohno, N Kajiwara, H. Saito and S. Nakae (2010): IL-33 and IL-33 receptors in host defense and diseases. Allergol Int; 59:143-60.

8- G. Kaiko, J. Horvat, K. Beagley and P. Hansbro (2008): Immunological decision-making: how does the immune system decide to mount a helper T-cell response? Immunology. 123:326–338.

9- S. Lécart, N. Lecointe, A. Subramaniam, S. Alkan, R. Chen, V. Boulay, J. Pène, K. Kuroiwa, S. Tominaga and H. Yssel (2012): Activated, but not resting human Th2 cells, in contrast to Th1 and T regulatory cells, produce soluble ST2 and express low levels of ST2L at the cell surface. Eur J Immunol;32:2979-87.

10- J. Corren, W. Busse, E.O. Meltzer, L. Mansfield, G. Bensch and J. Fahrenholz (2010): A randomized, controlled, phase 2 study of AMG 317, an IL-4Ralpha antagonist, in patients with asthma. Am J Respir Crit Care Med. 2010;181:788–796.

11- O.E. Savenije, M. Kerkhof and NE. Reijmerink (2011): Interleukin-1 receptor-like 1 polymorphisms are associated with serum IL1RL1-a, eosinophils, and asthma in childhood. J Allergy Clin Immunol.; 127:750–6.

12- R.G. Hamilton and P.B. Williams (2010): Specific IgE Testing Task Force of the American Academy of Allergy, Asthma &

Immunology; American College of Allergy, Asthma and Immunology. Human IgE antibody serology: a primer for the practicing North American allergist/ immunologist. J Allergy Clin. Immunol; 126 (1):33–38.

13- S. Sanada, (2007): J. Clin Invest . 117:1538.

14- D.R. Robinson, Q. Hamid, S.Ying, A. Barkans, J. Bentley, A.M. Corrigan and A.B. Kay, (1992) : Predominant TH2-like broncho-alveolar T-lymphocyte pop-ulation in atopic asthma, N. Engl. J. Med. 326 : 298–304.

15- D. Préfontaine, J. Nadigel, F. Chouiali, S. Audusseau, A. Semlali, J. Chakir, JG. Martin And Q. Hamid ,(2010): Increased IL-33 expression by epithelial cells in bronchial asthma. J Allergy Clin Immunol; 125:752-4.

16- H.D. Brightbill, S. Jeet, Z Lin, D. Yan, M. Zhou, M. Tan, A. Nguyen, S. Yeh, D. Delarosa, S.R. Leong, T. Wong, Y. Chen, M. Ultsch, E. Luis, S.R. Ramani, J. Jackman, L. Gonzalez, M.S. Dennis, A. Chuntharapai and M.Balazs, (2010): "Antibodies specific for a segment of human membrane IgE deplete IgE-producing B cells in humanized mice". J Clin Invest. 120 (6): 2218–29.

17- H. Hayakawa, M. Hayakawa, A. Kume and S.I.Tominaga, (2007): Soluble ST2 blocks IL-33signaling in allergic airway inflammation. J Biol Chem;282:26369–80.

18- K. Hikawa, K. Kuroiwa, H. Tago, K. Iwahana, S.Yanagisawa, S.I. Ohno, Y. Tominaga and M. Sugiyama (2011): Elevated soluble ST2protein levels in sera of patients with asthma with an acute exacerbation, Am. J. Respir. Crit. Care Med. 164: 277–281.

19- F.Y. Liew, N.I. Pitman and I.B. McInnes. (2010): Disease-associated functions of IL-33: the new kid in the IL-1 family. Nat Rev Immunol, 10:103-11

<div dir="rtl">

انترليوكين-33 و إس تى 2 الذائب
وارتباطهما بشدة ازمة الربو و كهدف مستقبلى للعلاج

المقدمة:

الربو الشعبى هو التهاب مزمن يصيب الشعب الهوائية نتيجة وجود حساسية شديدة للشعب الهوائية تجاه مهيجات و مثيرات معينة. و تلعب السيتوكينات دورا اساسيا فى حدوث التهابات المسارات الهوائية. كما يقوم انترليوكين-33 و مستقبلة الذائب اس تى 2 بدور هام فى تنشيط الخلايا الايوزينية فى المسارات الهوائية مما يودى الى زيادة التهاب هذة المسارات.

الهدف من البحث:

تهدف هذه الدراسه الى القاء الضوء على المستوى المصلى لكل من انترليوكين 33 و اس تى 2 الذائب واهميتهما كدلالات على التهاب المسارات الهوائية فى الربو الشعبى و دراسة ارتباطهما بشدة المرض لامكانية استخدامهما كهدف مستقبلى للعلاج.

المرضى و طرق البحث:

اجرى هذا البحث فى قسم الميكروبيولوجيا و المناعة و قسم الصدر بكلية طب الزقازيق و قد اشتملت الدراسه على 30 مريضا بالربو الشعبى (15 مريضا يعانون من نوبات حادة من الربو الشعبى و 15 مريضا لا يعانون من نوبات حاده اثناء المتابعه الدوريه بوحدة الحساسيه) بالاضافة الى 30 شخصا طبيعيا كمجموعه ظابطه.

تم عمل الاجراءات الاتيه لكل المرضى الذين شملهم البحث:

-اخذ التاريخ المرضى – الفحص الاكلينيكى العام و الموضعى للصدر – عمل اشعة اكس على الصدر – اختبارات وظائف التنفس و الكلى و الكبد.

كما تم سحب عينات دم من المرضى و المجموعه الظابطه لعمل الاختبارات الاتيه:

-عد الخلايا الايوزينيه بالدم – قياس مستوى انترليوكين 33 و اس تى 2 المذاب و مستوى الاجسام المضاده هفى المصل باستخدام الاليزا.

نتائج البحث:

- وجود زياده ذات دلاله احصائيه فى المستوى المصلى لكل من انترليوكين 33 و اس تى 2 المذاب و مستوى الاجسام المضاده الكلية(ﻫ) فى مرضى الربو الشعبى مقارنة بالمجموعه الظابطه.

- وجود علاقه ايجابيه ذات دلاله احصائيه بين مستوى انترليوكين 33 و كل من النسبه المئويه للخلايا الايوزينيه و الاجسام المضاده الكليه ﻫ.

- وجود علاقه سالبه ذات دلاله احصائيه بين FEV1 و مستوى انترليوكين 33 فى مرضى الربو الشعبى اثناء النوبات الحاده.

- وجود ارتباط ذو دلاله احصائيه بين كل من مستوى انترليوكين 33 و اس تى 2 الذائب و شدة المرض.

المستخلص من البحث:

المستوى المصلى لكل من انترليوكين 33 و مستقبله اس تى 2 لهما ارتباط ذو دلاله احصائيه بشدة المرض و هذا يطرح امكانية اعتبارهما هدفا مستقبليا لعلاج الربو الشعبى.

</div>