Evaluation of the Role of Interleukein-2 and Interleukein-4 in the Immunopathogenesis of Steroid Therapy Resistance in Iraqi Asthmatic Patients

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Abstract

Interleukins (IL-2 and IL-4) are increased in asthmatics and were reported to induce resistance to steroid therapy in some patients who fail to get benefit from glucocorticoids when used in full dose and for long period of time. In this context, the present study was conducted on Iraqi patients to provide additional laboratory mean, beside the clinical diagnosis, for the decision whether the asthma is steroid sensitive or resistant by monitoring the level of immunoglobulins, complement proteins and interleukins among asthmatic patients (steroid sensitive or resistant) and the possible contribution of other factors like age, sex and environments in the development of steroid resistance. A total number of 55 asthmatics and 28 normal subjects were enrolled in the study. Patients were diagnosed clinically as steroid sensitive (SSA) and steroid resistant (SRA) and blood samples were taken from all subjects included in the study for the measurement of immunoglobulins (IgA, IgG, IgM and IgE), complement proteins (C3 and C4), interleukins (Il-2 and Il-4), and total and differential WBC counts.The results showed no age, sex and residence dependency of acquired steroid resistance, while smoking habit (and may be the atopic allergy) constitute marked predisposing factors. The level of IgA and IgE were high in both SRA and SSA, while IgG level was low in SRA. Complement proteins (C3 and C4) were not differ in asthmatic patients in comparison with control group. The interesting results were those concerning interleukins. The levels of IL-2 and IL-4 were very high in SRA than in SSA. These are parallel with high lymphocyte and neutrophil counts in blood samples of those patients.In conclusion, beside clinical diagnostic features concerning the dose and duration of therapy with glucocorticoids, monitoring the levels of IL-2 and IL-4 could provide additional laboratory diagnostic measures for the convincing decision that asthma is steroid resistant.

Key words: steroid resistant asthma, steroid sensitive asthma, IL-2, IL-4.

الخلاصة

يزداد مستوى بعض الوسائط الالتهابية والمناعية عند مرضى الربو وقد يكون ذلك سببا لظهور بعض العلامات المرافقة أو المهيجة للمرض .وقد لوحظ أن مستوى الانترليوكينات ومن بينها interleukin-2 and interleukin-4 يزداد عند مرضى الربو وقد يُنتج مقاومة ضد العلاج بالستيرويدات عند بعض المرضى الذين اظهروا فشل الاستجابة للستيرويدات عند استخدامها بجرعها القصوى ولفترة طويلة .وفي هذا السياق فقد أجريت الدراسة الحالية لإضافة وسيلة مختبرية بالإضافة إلى التشخيص السريري للتمكن من التقرير فيما إذا كانت حالة الربو مستجيبة أو مقاومة للعلاج بالستيرويدات وذلك من خلال مراقبة مستوى الغلوبينات المناعية (immunoglobulins) ، البروتين التكميلية complement proteins) و الانترليوكينات) interleukins) بين مرضى الربو) مستجيبون أو مقاومون للستيرويدات(بالإضافة لدراسة المساهمة المحتملة للعوامل الأخرى مثل العمر والجنس والعوامل البيئية في تطوير المقاومة للستيرويدات. أجريت الدراسة على 55 مريضاً بالربو و28 من الأصحاء في مستشفى الناصرية العام وللفترة من شباط 2005 إلى تشرين أول 2005 .تم اخذ بيانات كاملة عن المرضى والذين كانوا قد شخصوا سريرياً كمقاومين أو مستجيبين للعلاج بالستيرويدات .وتم اخذ عينات الدم من كل الأشخاص المشاركين بالدراسة وذلك لقياس مستوى الغلوبينات المناعية (IgA, IgG, IgM, IgE)، والبروتينات التكميلية (C3 و C4)، والانترليوكينات (IL-2 و IL-4(بالإضافة لقياس عدد كريات الدم البيضاء وإحصائها التفاضلي (Differential count) أظهرت نتائج هذه الدراسة أن مقاومة العلاج بالستيرويدات غير معتمدة على العمر أو الجنس أو منطقة السكن (إن كانت حضرية أم قروية(، بينما كان للتدخين)وربما للحساسية المفرطة(تأثيرا واضحا كعوامل مهيئة لحدوث المقاومة. إن مستوى IgA و IgE كان عالياً عند كلا الصنفين من مرضى الربو) المقاومين والمستجيبين للستيرويدات(، بينما كان مستوى IgG منخفضاً عند المجموعة المقاومة للستيرويدات. وقد وجد أيضاً بان مستوى البروتينات التكميلية (C3 و C4) غير مختلف عند كلا الصنفين من المرضى بالمقارنة مع مجموعة الأشخاص الأصحاء .من النتائج المثيرة في هذه الدراسة تلك المتعلقة بالانترليوكينات (IL-2 و IL-4) والتي كانت مستوياتها عالية جداً عند المجموعة المقاومة للستيرويدات .وهذه النتائج جاءت متوازية مع العدد المرتفع لكريات الدم البيضاء اللمفاوية والمعتدلة عند أولئك المرضى. يمكن الاستنتاج من هذه الدراسة انه بالإضافة إلى التشخيص السريري فيما يتعلق بالجرعة ومدة العلاج بالستيرويدات لمرضى الربو فان مراقبة مستوى تركيز الانترليوكينات IL-2 و IL-4(يمكن أن يضيف وسيلة مختبرية للمساعدة في تقرير فيما إذا كانت حالة الربو من النوع الذي يستجيب أو يقاوم العلاج بالستيرويدات.

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Introduction

Increased responsiveness to a variety of stimuli is a common feature involved in the one of the interesting lung diseases, asthma [1]. Whether hypersensitivity to inhaled allergens is present or not, asthma is categorized broadly into extrinsic and intrinsic types, respectively[2,3]. The incidence of extrinsic asthma occurs most frequently between the age of 3 and 45 years, although its onset may be at any age [4]. Serum immunoglobulin E (IgE) antibody level is raised in most of those patients [5]. Intrinsic asthma, on the other hand, has a primary onset before the age of 3 years, or after 35 to 45 with, however, some hypersensitivity to drugs, particularly aspirin and some other manifestations including nasal polyposis and urticaria [6]. Other category of asthma are causally related to different inducing factors including occupational, exercise-induced and aspirin-induced asthma[7,8]. The role of immunity and related inflammatory cytokines in the pathogenesis of asthma has been extensively studied. Interleukin-2 (IL-2) is a known T-cell growth factor which induces clonal expansion of T-lymphocytes [9]. Activated T-cells, B-cells and mast cells have the ability to synthesise IL-2 [10,11]. The second important T-cell growth factor is interleukin-4 (IL-4), which is synthesized by some types of T-lymphocytes, basophils, eosinophils and mast cells to function as mitogenic and lymphocyte differentiation factor[11]. These cytokines have, beside these functions, anti-tumor effects on some types of tumors[12,13]. In addition to activation of immune system during the pathogenesis of asthma, neuropeptides (substance P and neurokinine A) and nitric oxide have shown to play major roles in the initiation of cascade of responses including vasodilation, mucous secretion, plasma protein extravasation, leukocyte adhesion and activation, and bronchoconstriction that comprise the classical signs and symptoms of asthma [14]. These pathogenic mechanisms are controlled to some extent by the use of glucocorticoids (GC) [15]. However, some resistance to this mode of therapy is continuously increased and is manifested by failure to improve baseline morning (AM) pre-bronchodilator forced expiratory volume during the first second (FEV1) by greater than 15% following 7-14 days of 20mg twice daily oral prednisolone [16]. Some patients do not have an absolute resistance, but rather GC insensitivity and some might respond to higher doses of prednisolone or require longer period of therapy [17]. Patients with steroid resistant asthma (SRA) have higher level of immune activation (raised levels of T-cells and eosinophils with high level of IL-2 and IL4)[18]. This accompany by persistent respiratory symptoms, nocturnal exacerbations and chronic airway obstruction together with poor clinical and physiologic responses to oral glucocorticoids (GCs) therapy [17]. In clinical practice, setting any patient not responding to 40-60mg/day prednisolone after 3 weeks of therapy should be suspected to be SRA [16]. The clinical efficacy of GCs therapy is the result of the combinations of inhibitory effects on the process of inflammation including decreased trafficking of inflammatory cells and inhibition of inflammatory cytokines production [19]. This efficacy has shown to be altered in SRA [20]. The correlation between increased inflammatory cytokines (IL-2 and IL-4) and the development of steroidal resistance have been studied. Cytokines were reported to induce activation of transcriptional factors that interfere with GC binding to their nuclear recognition sites [21,22]. IL-2 and IL-4 were shown to promote the synthesis of altered GC binding protein (GCRβ), which reported to be a dominant negative inhibitor of the classic ligand binding protein for GC (GCRα) [23,24,25]. Factors contributing to GC insensitivity via immune activation may include allergen exposure which is reported to decrease GC receptor binding affinity and steroid responsiveness in atopic asthmatics [26] possibly by IL-2 and IL-4 dependent mechanisms[17]. On the other hand, superantigen secretion by bacterial or viral agents my contributed to poorly controlled asthma and reduce GC sensitivity. In this context, staphylococcal enterotoxin B is a potent inducer of GCRβ isoform in T-cells [27]. According to previously mentioned immunopathogenic features of steroid resistant asthma, this study was conducted to monitor the role of IL-2 and IL-4 in the immunopathogenesis of steroidal resistance asthma among Iraqi patients; and to monitor any role for immunoglobulins, complement proteins, age, sex, residence and smoking habit on the incidence of SRA.

Patients, Materials and Methods

This study was conducted on 55 asthmatic patients (25 females and 30 males) and another 28 healthy persons (18 females and 20 males) in a single-blind technique. The study was carried out in AL-Nasseriya General Hospital from February 2005 till October 2005. The age range of healthy subjects and patients was 16-73 years with average age ± SD (39.4 ± 14.67). Steroid sensitive asthmatics (SSA) were 28 (10 females and 18 males) and

SRA were 27 (12 female and 15 males). Patients' selection was based on special criteria including (a) presence of no acute infection at the time of study, (b) presence of no any other chronic infection, (c) steroidal therapy must be discontinued at least for 2 weeks and (d) presence of no systemic disease that my be associated with steroid resistance. Patients were diagnosed for steroidal resistance depending on the history of steroidal therapy and the clinical decision. Other patient's information were collected in a specially prepared sheet including: age, sex, chief complain, type and dose of steroid used, predisposing factors, associated symptoms, medical history, family history of steroid resistance, smoking habit, residence (civilian or rural), and presence of atopic allergy.

Materials:

IL-2 and IL-4 Elisa Kit (Immunotech, Marseille, France), Single Radial Immuno-diffusion Test Kit for immunoglobulins (BINDARIDTM The Binding Site Ltd., Birmingham, UK), Single Radial Immunodiffusion Test Kit for Complements (BINDARIDTM The Binding Site Ltd., Birmingham, UK), IgE Elisa Kit (Immunotech, Marseille, France).

Methods:

Blood samples were drawn, left for clotting and then centrifuged for 5-10 minutes at 2000 rpm (using Centrifuge, K24, Coold With Rotor Number 905, WIR 12x10 ML, Janetzki, Germany) for separation of serum, which was kept frozen unless analyzed immediately. Serum levels of IL-2 and IL-4 were determined using ELISA kits (Immunotech, Marseille, France), based on the interaction between monoclonal antibody bound to the wells of a microtiter plate to the IL-2 and IL-4 found in the serum. The antigen-antibody complex was detected by the addition of a chromogenic substrate, and the intensity of color was recorded colometrically (using Spectrophotometer SP6-500, Pye-Unicam, England); accordingly serum levels of IL-2 and IL-4 were calculated utilizing a standard curve prepared for this purpose [(28)]. Serum level of the Immunoglobulins (IgG, IgA, and IgM) and the complement proteins (C3 and C4) were determined using SRID kits (BINDARIDTM The Binding Site Ltd., Birmingham, UK). Equal volumes of reference sera and test samples were added to wells in an agarose gel containing a monospecific antiserum. The samples diffused radially through this gel and the tested compound (antigen) being assayed by forming a precipitin ring with the monospecific antiserum; rings diameters were measured (using Microwell System Reader 2305, Organon Teknika, Austria), and concentrations were determined using standard curve prepared for this purpose[(29)]. IgE was determined using IgE ELISA kit (Immunotech, Marseille, France)

Statistical Analysis

All results were presented as a mean ± SEM. Comparisons were made using Chi-square, Student's *t*-test and ANOVA. P values less than 0.05 were considered significant.

Results

In this study, age distribution of the volunteers enrolled in the study revealed that the incidence of SRA was varied but generally is great in the middle and older ages (Table 1).

Table (1): Distribution of patients according to their age among steroid resistant and sensitive asthmatic patients.

Age (years)	SRA (%) (n=27) (32.5%) of total (83) patients	SSA (%) (n=28) (33.73%) of total (83) patients
10-20	---	3.57
21-30	11.11	17.85
31-40	33.33	28.57
41-50	11.11	21.42
51-60	11.11	14.28
> 61	33.33	14.28

SRA = Steroid resistant asthma.
SSA = Steroid sensitive asthma

However, no significant difference (P>0.1) was noticed in the incidence of SRA among female (55.56%) and male (44.44%) with male/female ratio of 0.33 (Table 2). On the other hand, the incidence of SRA was shown to be high in those with negative family history (77.77%) in comparison to those with positive family history (22.23%) (P<0.01) as shown in table (2). In addition, steroid resistance was more pronounced in civilian (66.67%) than in rural areas (33.33%); but however the difference was not significant (P>0.1) (Table 2). It is clearly shown that smoking may comprise significant predisposing factor for steroid resistance (55.56%) when smoking SRA patients were compared to non-smoking

SRA patients (44.44%) (Table 3). Atopic allergy, on the other was more pronounced in patients with SRA (66.66%) in comparison to SRA patients with no symptoms of atopic allergy (34.34%); however, the difference was not significant, P>0.5 (Table 3).

Table (2): Sex distribution, family history and residence among SRA and SSA patients included in the study.

Asthm-atic groups	Sex		Family history		Residence	
	Male (%)	Female (%)	Positive (%)	Negative (%)	Civilian (%)	Rural (%)
SSA (n=28)	64.28	35.72	71.42	28.57	82.14	17.85
SRA (n=27)	44.45	55.55	22.22	77.78	66.67	33.33
P-values	>0.1		<0.01		>0.1	

Table (3): Smoking habit and atopic allergy among steroid resistant and steroid sensitive asthmatic patients.

Asthmatic groups	Smoking		Atopic allergy	
	Non-smokers (%)	Smokers (%)	Present (%)	Absent (%)
SSA (n=28)	82.15	17.85	71.43	28.57
SRA (n=27)	44.45	55.55	66.66	33.34
P-values	<0.05		>0.5	

SRA group of patients exhibited varying patterns of immunoglobulin levels as shown in figure (1). There is significant elevation in IgA level (3105.55 ± 225.12) and IgE level (295.17 ± 61.5) in comparison to control group (2346.11 ± 142.7 and 39.05 ± 4.5, respectively) (P<0.05). However, the levels of these immunoglobulins also were significantly high in SSA group (3815.71 ± 302.61 and 387.85 ± 52.5, respectively) (P<0.05). Moreover, only IgA levels were shown to be significantly different between SRA and SSA. The level of IgG level did not differ significantly in SRA (13615.56 ± 993.23) over that in control group (12798.33 ± 746.27) (P>0.05); but it was significantly high in SSA (15890.36 ± 892.08) (P<0.05). Lastly, the level of IgM did not differ significantly among control (1626.89 ± 139.6), SRA (1407.11 ± 187.9) and SSA (1593.82 ± 136.38) groups (P>0.05) as shown in figure (1).

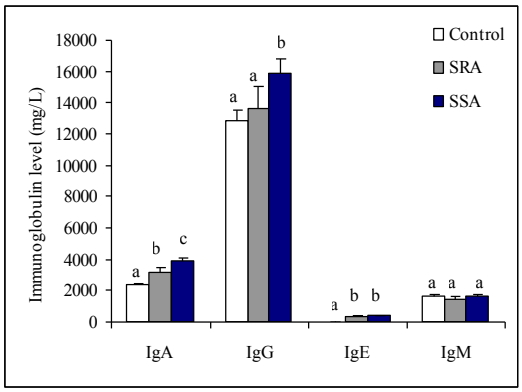

Fig. (1): Immunoglobulins level among SSA (n=28) and SRA (n=27) patients. Values are (mean ± SEM). Non-identical superscripts (a, b, c) considered significant, P<0.05 analyzed by ANOVA.

In this study also, the levels of complement protein (C3) did not differ significantly in SRA (1834.44 ± 46.48) and SSA (1666.78 ± 58.21) in comparison with control group (1699.44 ± 46.48), P>0.05 (Fig. 2). The same profile was seen in the second complement protein (C4) who its level show comparable values in SRA (398.44 ± 20.3) and SSA (389.82 ± 25.5) to that in control group (375.72 ± 23.5), P>0.05 as shown in figure (2).

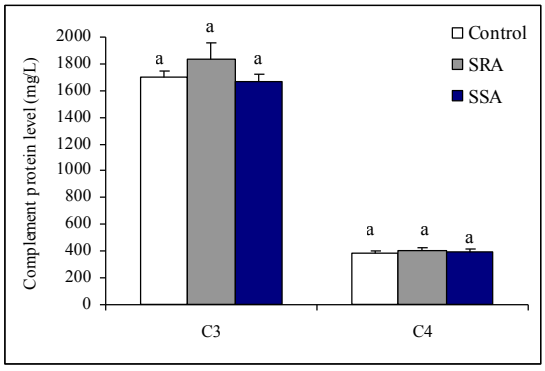

Fig. (2): Complement proteins level among SSA (n=28) and SRA (n=27) patients. Values are (mean ± SEM). No significant difference among groups, P>0.05 analyzed by ANOVA.

The interesting results in this study were those related to cytokines (IL-2 and IL-4) levels. Both cytokines were significantly high in SRA (46.11 ± 2.31) and SSA (18.28 ± 0.398) in comparison to control group (8.38 ± 0.63), P<0.05; however, its level among SRA was significantly higher than that in SSA group (P<0.05) as shown in figure (3).

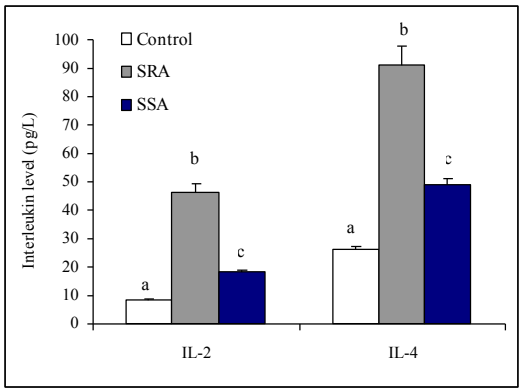

Fig. (3): Interleukins level among SSA (n=28) and SRA (n=27) patients. Values are (mean ± SEM). Non-identical superscripts (a, b, c) considered significant, P<0.05 analyzed by ANOVA.

On the other hand, the same profile was seen for IL-4 who its level was significantly high in SRA (91.33 ± 4.42) and SSA (49.03 ± 2.13) in comparison to control group (26.05 ± 11), P>0.5; with significant difference among the two groups of asthmatics, P<0.05 as shown in figure (3). Total WBC counts were significantly high in SRA and SSA in comparison to control group, P<0.05 (P<0.05) as shown in figure (4).

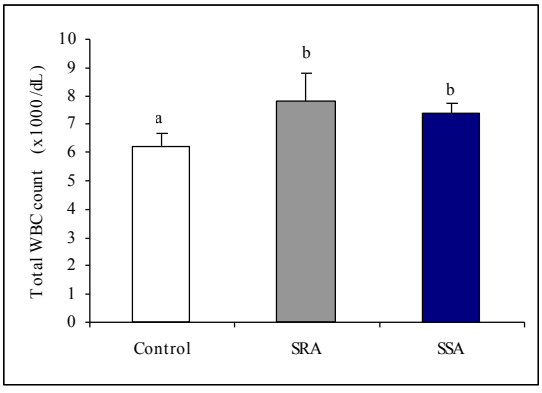

Fig. (4): Total WBC count among SSA (n=28) and SRA (n=27) patients. Values are (mean ± SEM). Non-identical superscripts (a, b) considered significant, P<0.05 analyzed by ANOVA.

Further, the count did not differ significantly among SRA and SSA groups (P>0.05). Neutrophils were significantly high in SRA and SSA groups in comparison to control group (P<0.05). However, their level did not differ significantly among SRA and SSA as shown in figure (5), P>0.05. Lymphocyte levels were low in both groups of asthmatics using steroidal therapy in companion to control group; however it was slightly lower in SSA than in SRA, but the difference was not significant (P>0.05). Differential count of other WBCs did not show any significant difference in SRA and SSA in comparison to control group (P>0.05) as shown in figure (5).

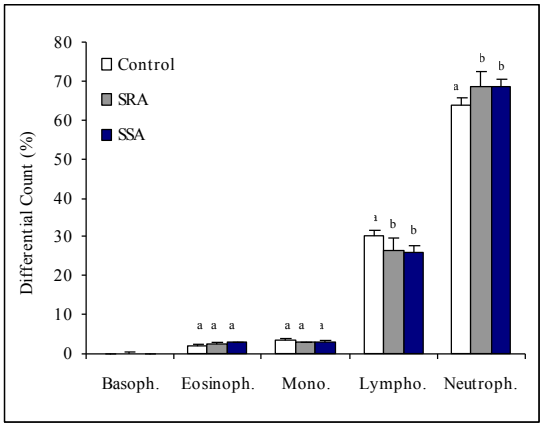

Fig. (5): Differential WBC count among SSA (n=28) and SRA (n=27) patients. Values are (mean ± SEM). Non-identical superscripts (a, b) considered significant, P<0.05 analyzed by ANOVA.

Discussion

The present study showed that the incidence of steroid resistance is increased among all patients' age rang. This is come in agreement with that reported by Ishioka and his co-workers[30]. However, resistance to steroid did not significantly affected by sex variation, but may be linked to other factors such as smoking habit and presence of atopic allergy, a finding supported by what reported by others [31,32]. Atopic allergy and immune activation is clearly suggested in this study to underlay steroid resistance and this is supported further by finding in this study that the levels of IgA and IgE were high in those patients [33]. Furthermore, the low or un increase level of IgG in SRA in comparison to SSA group suggest that the decrease in this immunoglobulin to be one factor contributing to steroid insensitivity. This has been solidified by the question; why Nathan and Erwin introduced IgG as intravenous immunoglobulin in the treatment of steroid resistance; this in turn based on the ability of IgG to decrease the levels of IL-2 and IL-4 *in vitro*, an effect thought to be involved in the potentiation of inhibitory effect of glucocorticoids on cell proliferation and cytokine secretion[34].The link between the high concentrations of IL-2 and IL-4 and the development of SRA relay on the increased resistant of lymphocytes to the action of GCs in a theory suggest altered splicing of the GCR

pre-mRNA genes induced by these cytokines [35]. The results is the generation of a second GCR, termed GCRβ, which does not bind GC but antagonizes the transactivating activity of the classic GCR [25]. Thus, increased expression of GCRβ could account for glucocorticoid insensitivity among asthmatic patients [36,37]. For this reason, the use of high dose of glucocorticoids might make down regulation to the classical glucocoricoid receptors (GCRα), leaving the inhibitory isoform (GCRβ) to be predominate, and that is why resistance occurs to GCs [23]. The levels of C3 and C4 did not differ significantly in this study among SRA and SSA in comparison with baseline. For this reason we suppose monitoring the level of these complements is without benefit to decide wither the patient has steroid sensitivity of resistance. This speculation was come in agreement with that reported by Liao and his associates [38].

The elevated levels of IL-2 and IL-4 seen in this study are correlated well with the acquired resistance to steroid therapy. Positive correlation was existed between the increased levels of IL-2 and IL-4 among SRA patients although the correlation failed to reach the level of statistical significance (data not shown). These results came in agreements with those reported by Kam and his co-workers (1993) in that the combination of IL-2 and IL-4 induced T cell resistance to GCs and increase GCRβ expression in the T cells of normal subjects [39]. In mice IL-2 alone can induce T cell resistance to GC [40]. The mechanism of such resistance involves a defect in nuclear translocation of the GC receptors. This in turn depends upon the phosphorylation of GC receptors [41]. Thus, the results obtained in this donate a possible usefulness of IL-2 and IL-4 as predictive immune markers for the development of steroid resistance and to be a possible underling cause for such resistance [23, 42]. The levels of total WBC were increased in both groups of asthmatic patients. Further, the percentage of lymphocytes and neutrophils were high in SRA group in comparison to SSA group, although the change was not significant. The high level of these leukocytes in SRA based on the theory that glucocorticoids intake could inhibit cell proliferation because of the high dose of steroid used in asthmatic patients as what happen in SSA patients [43].In conclusion, beside clinical diagnostic features concerning the dose and duration of therapy with glucocorticoids, monitoring the levels of IL-2 and IL-4 could provide additional laboratory diagnostic measures for the convincing decision that asthma is a steroid resistant.

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