

GATA-3 mRNA Expression in Bronchial Asthma Effect of Inhaled Corticosteroid Therapy

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ABSTRACT:

Background/Aim: GATA-3 is a T cell-specific transcription factor and is expressed in TH2 but not TH1 cells. It plays a critical role in TH2 differentiation and allergic airway inflammation. The aim of the work was to study non-invasive tool like induced sputum provide an accurate reflection of the inflammatory milieu in bronchial asthma as regards the expression of GATA-3 mRNA as well as the effect of inhaled corticosteroid therapy on GATA-3 mRNA expression.

Methods: induced sputum samples were collected from 46 subjects (10 healthy controls, 18 non-atopic and 18 atopic asthmatic patients). Atopy is based on positive family history and positive allergic skin test to group of common allergens. For each sample, GATA-3 mRNA expression was detected by RT-PCR and the induced sputum eosinophilic percentage was determined. Sputum induction was repeated for 10 non-atopic and 10 atopic patients after 6 weeks of inhaled steroid therapy (Beclomethasone dipropionate 250 µg twice daily).

Results we demonstrated that 83 % of the studied untreated atopic asthmatic patients had increased expression of GATA-3 mRNA in their induced sputum samples. In contrast, 61 % of the studied untreated non atopic patients had decreased expression of GATA-3 mRNA in their induced sputum samples. However, after long course of corticosteroid therapy both atopic and non atopic treated patients show negative expression of GATA-3 mRNA. Additionally, There were significant association between GATA-3 expression in both asthmatic groups before treatment and FEV1, blood eosinophilic count & induced sputum eosinophilic percentage. So, the severity of asthma could be reflected from the nature of GATA-3 expression in the induced sputum.

Conclusion: Induced sputum provides sufficient sensitivity for examining transcription factors; GATA-3 expression is upregulated in atopic asthma. Corticosteroids block the action of GATA-3 as a part of its effects. Therefore, local delivery of antisense oligonucleotides blocking GATA-3 mRNA in atopic asthmatic patients will be at least as effective as the administration of corticosteroids. In addition, this will escape the numerous side effects of corticosteroids.

Key words: GATA-3 mRNA, Bronchial Asthma, Corticosteroids Therapy.

INTRODUCTION

Asthma is a chronic pulmonary disease characterized by reversible airway obstruction and bronchial hyper-responsiveness. The allergic type is associated with polarization of T

lymphocyte responses and increased secretion of cytokines, especially TH2 cytokines IL-4 and IL-5, involved in the regulation of IgE, mast

cells, basophils, and eosinophils, ultimately leading to inflammation and disease ⁽¹⁾.

IL-4 has been postulated to be critical for the development of TH2 cells in asthmatic airways and the reduced expression of the IL-12 receptor β 2 chain on lung TH1 cells. Furthermore, it is believed to induce IgE isotype switching in B cells in Asthma and promotes goblet cells metaplasia, mucus hypersecretion, and the recruitment of eosinophils by upregulating vascular cell adhesion molecule-1 expression in pulmonary vascular endothelial cells ⁽¹⁾.

IL-5 plays a vital role in the activation of eosinophils and seems to be responsible for the development of a number of eosinophil-associated diseases including bronchial asthma ⁽²⁾. It is essential for terminal differentiation of the committed eosinophil precursor, which also activates and prolongs the survival of the mature cells in the tissues. A large number of T cells expressing IL-5 mRNA exist in the bronchial mucosa of asthmatic patients and, accordingly, bronchoalveolar lavage fluid of atopic and non-atopic asthmatics contains increased concentration of IL-5. Serum IL-5 levels are also elevated in symptomatic asthmatics and decreased after oral prednisolone therapy ⁽³⁾.

The production of the main TH2 cytokines, IL-4 and IL-5, is mediated by the transcription factors GATA-3 and c-MAF. These factors are found at low levels in naïve T cells, and their numbers are increased during TH2 phenotype activation ⁽⁴⁾. GATA-3 is a pleiotropic transcription factor of the C4 zinc finger family expressed in T cells, mast cells, eosinophils, basophils and embryonic brain and kidney. It is one of the GATA family which contains proteins of six members (GATA 1-6) that share a common DNA binding motif (A/T)GATA (A/G) ⁽⁵⁾. GATA-3 has been shown to be essential for the development of the earliest T-cell progenitors. GATA-3 was found to be selectively expressed in TH2 but not in TH1 cells and to play an important role in cytokine gene expression in T-cells. In particular, GATA-3 is important for the

expression of IL-5 in T-cells by transactivation of the IL-5 promoter together with Ets-1 and Ets-2 proteins. Furthermore, GATA-3 weakly transactivates the IL-4 promoter in T cells ⁽¹⁾. Inhibition of GATA-3 expression in TH2 cells has been associated with decreased levels of IL-4, IL-5 and IL-13 ⁽⁴⁾.

In fact, an increased content of this protein was recorded in the lungs of asthma patients ⁽⁶⁾. Recent data indicate a critical role for GATA-3 as a key regulator of both T cell effector function and airway hyperresponsiveness in allergic airway inflammation and suggest that the local delivery of GATA-3 antisense oligonucleotides may be a novel approach for the treatment of airway hyperresponsiveness such as in asthma ⁽¹⁾.

SUBJECTS & METHODS

The present work included: 46 subjects classified into the following groups:

Group I: Included 10 apparently healthy subjects as a control group. They were five males and five females and their ages ranged from 25-57 years.

Group II: Included 18 non-atopic asthmatic patients. They were eight males and 10 females and their ages ranged from 33-65 years

Group III: Included 18 atopic asthmatic patients. They were 11 males and 7 females and their ages ranged from 18-42 years. Atopy is based on positive family history and positive allergic skin test to group of common allergens.

All the studied groups were subjected to the following:

- 1- Detailed history and thorough clinical examination.
- 2- Plain X-ray chest.
- 3- Urine and stool examination
- 4- Pulmonary function tests.
- 5- Allergic skin test.
- 6- Complete blood picture and determination of blood eosinophilic count.

7-Determination of induced sputum eosinophilic percentage

8-Specific laboratory test: investigating the expression of GATA-3 mRNA in the induced sputum by (RT-PCR).

Induced sputum samples were taken from all groups and it was repeated in 10 patients of group 2 and in 10 patients of group 3 after 6 weeks of inhaled steroid therapy (Beclomethasone dipropionate 250 µg twice daily). Pulmonary function tests, complete blood picture, determination of induced sputum eosinophilic percentage and the assessment of the expression of GATA-3 mRNA in the induced sputum by RT-PCR were repeated for those patients after 6 weeks of inhaled steroid therapy.

Sputum induction was performed using a modification of the method of Pin and coworkers⁷. Subjects were administered salbutamol, 200 µg to inhibit possible bronchoconstriction during sputum induction followed by increasing concentrations (3%, 4%, and 5%) of hypertonic saline solution generated by an ultrasonic nebulizer. The procedure was interrupted every 2 min to PEF_R or FEV₁. Subjects were asked to rinse the mouth and blow the nose to minimize contamination with saliva and postnasal drip and also instructed to cough sputum into a sterile container. These procedures were repeated sequentially for 8-min periods at each concentration unless a fall in PEF_R or FEV₁ >10% occurred, in which case the procedure was terminated.

Collection of samples: Induced sputum was collected in autoclaved containers and divided into 2 aliquots, one was used for determination of the sputum eosinophils and the other was used for total RNA extraction and assessment of the expression of GATA-3 by RT-PCR. Two ml of ADTA-anticoagulated venous blood was collected for complete blood count.

Determination of eosinophil in induced sputum: The sputum samples were weighted and mixed with freshly prepared 0.1 µg/µl Dithiothreitol (DTT) in a ratio 1: 4 volume (microliters)/weight (milligrams). The DTT-sputum mixture was completely homogenized

and then mixed with equal volume of Phosphate buffered saline (PBS). The mixture was centrifuged for 5 minutes and the cell pellet was spread on a slide, fixed with methanol and stained with Giemsa stain for an overall differential cell count of 100 nonsquamous cells^(8,9).

Analysis of GATA-3 mRNA expression in the induced sputum using RT-PCR. Total RNA was extracted from sputum using Qiagen RNeasy mini spin column (RNeasy Mini Kit, Qiagen, USA) according to the manufacturer instructions. The concentration of RNA was measured at 260nm (A₂₆₀) absorbance. The A₂₆₀/A₂₈₀ ratio was measured to provide an estimate of the purity of RNA, A₂₆₀/A₂₈₀ ratio greater than 1.6 was accepted⁽¹⁰⁾.

The extracted total RNA was transcribed into cDNA using random hexamers (High Capacity cDNA Kit, Applied Biosystem, USA) and Multiscribe™ reverse transcriptase enzyme. The cDNA was used as a template to amplify GATA-3 using the following primers; GATA-3 F: 5'GACGAGAAAGAGTGCCTCAAG3' and GATA-3 R: 5'TCCAGAGTGTGGTGTGGTG3'. As normalization for sample to sample differences in RNA input, RNA quality, and RT efficiency, the level of β-actin expression was measured in each sample. GATA-3 and β-actin amplification were performed in the same template but in different tubes. β-actin amplification was done using the following primers; F: 5' TTAGCTGTGCTCGC GCTACTCTC 3' and R: 5' GTCGGATTGATG AAACCCAGACACA 3'. The amplification reaction mixtures were performed in a final volume of 50 µl containing 25 µl Taq PCR master mix (25 units Taq DNA polymerase, 1x PCR buffer, and 200 µM of each dNTP) (Taq PCR master kit from Qiagen, Germany), 0.5 µM of each primer, and 5µl cDNA. PCR conditions used an initial denaturation at 94°C for 3 minutes followed by 35 cycles of 30 sec denaturation at 94°C, 30 sec annealing at 60°C, and 1 minutes extension at 72°C, followed by a final extension at 72°C for 10 minutes. The PCR products were resolved on 1.5% agarose gel (Sambrook et al., 1989)⁽¹¹⁾.

RESULTS

Table (1) Patients characteristic

	Control N=10 Mean ± SD	Non - atopic asthmatic N=18	Atopic asthmatic N=18
Sex (M/F)	5/3	8/10	11/7
Age	42.8±9.95	47.06±9.84	28.11±5.88
FEV1% of predicted	108.3±10.98	75.89±6.078	72.72±5.512
Blood eosinophilic count (cells/mm ³)	171±93.51	613.89±145.33	866.67±130.61
Induced sputum eosinophilic %	0.161±0.245	3.00±0.740	4.344±1.033

Table (2): GATA-3 mRNA expression in the induced sputum by Rt-PCR in all studied groups

		GATA-3 mRNA expression by Rt-PCR			
		Decreased expression	Normal expression	Increased expression	Total
Control	N	0	10	0	10
	%	0.00	100.00	0.00	100.00
Non-atopic patients	N	11	7	0	18
	%	61.11	38.89	0.00	100.00
atopic patients	N	0	3	15	18
	%	0.00	16.67	83.33	100.00
Total	N	11	20	15	46
	%	23.91	43.48	32.61	100.00
Chi-square	X ²	50.856			
	P-value	<0.001*			

*Significant P- value < 0.05 - N: number of subjects

- Statistical analysis by Chi-square test

Table (3): GATA-3 mRNA expression in the induced sputum of asthmatic patients (group I, II) by Rt-PCR in relation to some parameters

	Decreased expression	Normal expression	Increased expression	Anova F (p)	Turkey's test (p)
Age (years)	40.64±9.49	39.4±16.07	36.27±5.73	1.97 (0.101)	
FEV1 % of pred	72.64±6.04	79±5.31	70.93±4.01	7.67 (0.002)*	t ₁ <0.034* t ₂ >0.171 t ₃ <0.001*
Blood eosinophilic count	709.01±94.39	525±108.65	906.67±99.76	43.85 (0.001)*	t ₁ <0.001* t ₂ <0.001* t ₃ <0.001*
Induced sputum eosinophilic% count	3.48±0.496	2.33±0.258	4.707±0.665	61.501 (0.001)*	t ₁ <0.001* t ₂ <0.001* t ₃ <0.001*
t ₁ (decreased Vs normal)		t ₂ (decreased Vs increased)		t ₃ (normal Vs increased)	

Table (4): GATA-3 mRNA expression in the induced sputum by Rt-PCR in non atopic asthmatics before and after Corticosteroid treatment compared to the control group

GATA-3 mRNA expression	Non atopic				Control	
	Before ttt		After ttt		N	%
	N	%	N	%		
Negative expression	0	0.00	10	100.00	0	0.00
Decreased expression	11	61.11	0	0.00	0	0.00
normal expression	7	38.89	0	0.00	10	100.00
Increased expression	0	0.00	0	0.00	0	0.00
Chi-square	X ²			51.66		
	P-value			<0.001*		

* Significant, P- value < 0.05 N: number of subjects
 - Statistical analysis by Chi-square test

Table (5): GATA-3 mRNA expression in the induced sputum by Rt-PCR in atopic asthmatics before and after Corticosteroid treatment compared to the control group

GATA-3 mRNA expression	Atopic				Control	
	Before ttt		After ttt		N	%
	N	%	N	%		
Negative expression	0	0.00	10	100.00	0	0.00
Decreased express.	0	0.00	0	0.00	0	0.00
Normal expression	3	16.67	0	0.00	10	100.00
Increased expression	15	83.33	0	0.00	0	0.00
Chi-square	X ²		62.359			
	P-value		< 0.001*			

* Significant, P- value < 0.05 - N: number of subjects

- Statistical analysis by Chi-square test

Table (6): GATA-3 mRNA expression in the induced sputum by Rt-PCR in atopic asthmatics compared to non-atopic asthmatics both before and after Corticosteroid therapy

GATA-3 mRNA expression	Before ttt				After ttt			
	Nonatopic		Atopic		Non atopic		Atopic	
	N	%	N	%	N	%	N	%
Negative expression	0	0.00	0	0.00	10	100.00	10	100.0
Decreased expression	11	61.11	0	0.00	0	0.00	0	0.00
Normal expression	7	38.89	3	16.67	0	0.00	0	0.00
Increased expression	0	0.00	15	83.33	0	0.00	0	0.00
Chi-square	X ²		27.6					
	P-value		< 0.001*					

* Significant, P- value < 0.05 - N: number of subjects

- Statistical analysis by Chi-square test

Table (7): Show Receiver Operating Characteristic (ROC) curve analysis of induced sputum eosinophilic percentage with GATA-3 mRNA expression by RT-PCR in all studied subjects

ROC curve of Induced sputum eosinophils (%) with GATA-3 mRNA					
Cut off	Sens.	Spec.	PPV	NPV	Accuracy
> 28	96.0	100.0	100.0	95.2	99.8

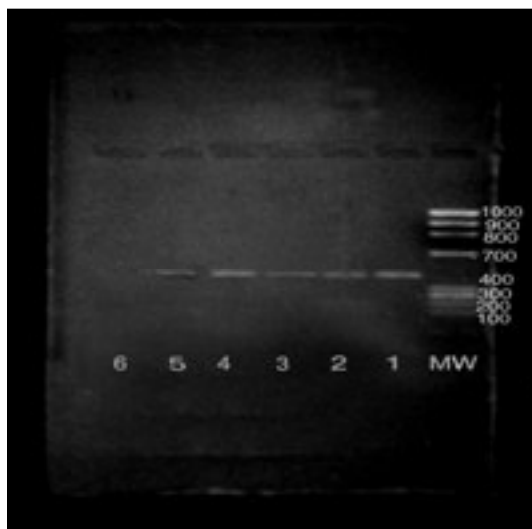


Figure (1): RT-PCR analysis for GATA-3 mRNA expression (444 bp) by agarose gel electrophoresis and ethidium bromide staining shows:

Lane MW: Molecular weight ladder standard (1000-100 bp)
 Lane1: Induced sputum from normal healthy control subject (normal expression)
 Lane2: Induced sputum from non-atopic asthmatic patient before corticosteroid therapy (normal expression).
 Lane3: Induced sputum from non-atopic asthmatic patient before corticosteroid therapy (decreased expression).
 Lane4: Induced sputum from non-atopic asthmatic patient before corticosteroid therapy (normal expression).
 Lane5: Induced sputum from non-atopic asthmatic patient before corticosteroid therapy (decreased expression).
 Lane6: Induced sputum from non-atopic asthmatic patient after 6 weeks of corticosteroid therapy (negative expression).

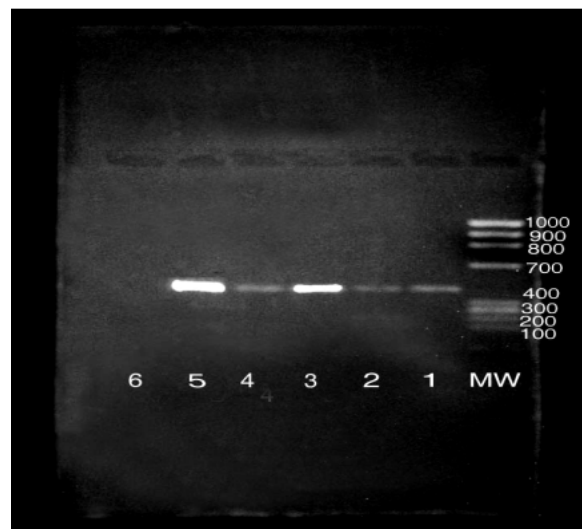


Figure (2): RT-PCR analysis for GATA-3 mRNA expression (444 bp) by agarose gel electrophoresis and ethidium bromide staining shows:

Lane MW: Molecular weight ladder standard (1000-100 bp)
 Lane 1: Induced sputum from normal healthy control subject (normal expression)
 Lane 2: Induced sputum from atopic asthmatic patient before corticosteroid therapy (normal expression)
 Lane 3: Induced sputum from atopic asthmatic patient before corticosteroid therapy (increased expression)
 Lane 4: Induced sputum from atopic asthmatic patient before corticosteroid therapy (normal expression)
 Lane 5: Induced sputum from atopic asthmatic patient before corticosteroid therapy (increased expression)
 Lane 6: Induced sputum from atopic asthmatic patient after 6 weeks of corticosteroid therapy (negative expression)

DISCUSSION

Asthma is a chronic disease characterized by reversible airway obstruction, bronchial hyperresponsiveness, and airway inflammation and remodeling. In most cases, the asthmatic inflammatory process results from inappropriate immune responses to common environmental antigens in a genetically susceptible individual⁽¹²⁾.

The current study demonstrated that 83 % of the studied untreated atopic asthmatic patients had increased expression of GATA-3 mRNA in their induced sputum samples. In contrast, 61 % of the studied untreated non-atopic patients had decreased expression of GATA-3 mRNA in their induced sputum samples. In addition, 100% of the control group had normal expression; the difference was statistically significant between the non-atopic and the atopic groups as compared to the control group and as compared to each other.

The reciprocal nature of the GATA-3 expression in atopics and non-atopics appeared to correspond to patterns of in vitro studies of T cell differentiation systems, where cells committed to the TH2 pathway show an increase in the levels of GATA-3 while TH1 conditions lead to a decrease of GATA-3 levels^(13,14).

Recent published data showing that increased GATA-3 expression in bronchial biopsies and bronchoalveolar lavage cells from atopic asthmatics^(15,16). Additionally allergen-induced up-regulation of GATA-3 mRNA expression in peripheral blood mononuclear cells (PBMC) in 88 % of atopic asthmatics tested which occurred concomitantly with production of the cytokines IL-4, IL-5 and IL-13. Moreover, they demonstrated down-regulation of GATA-3 mRNA expression in 75 % of the non-atopics tested. This appeared consistent with underlying TH1-like responsiveness. The puzzle of why this TH1-like responsiveness in non-atopics is not associated with in vivo manifestations of delayed type hypersensitivity (DTH) remains to be solved. It is likely that

some form of negative feedback within the overall response of the non-atopics, such as concomitant IL-10 production, may be involved, but no direct evidence on this issue is currently available. It is also of interest to speculate on the mechanism(s) underlying GATA-3 down-regulation in the non-atopic subjects. A direct role of IL-12 in GATA-3 repression has been proposed. However, a recent study failed to observe a decrease in GATA-3 expression following culture with IL-12. It may be possible that interaction with other TFs, such as the recently described repressor of GATA (ROG) and T-bet play an active role in GATA-3 repression⁽¹⁴⁾.

Taha *et al.*,⁽⁴⁾ demonstrated that higher expression of IL-4R α , IL-5R α , GATA-3, c-MAF and STAT6 in induced sputum samples obtained from atopic asthmatics in relation to those of healthy control subjects. These findings provide clear evidence that, in addition to the expression of the soluble mediators that have been reported previously, cell surface receptors and intracellular TFs can also be detected within samples of induced sputum. Furthermore, it illustrated the close relationship that exists between the expression of TH2 cytokine receptors and the related TFs. It is clear that GATA-3 is necessary for the expression of IL-5, and the inhibition of its expression in TH2 cells has been associated with decreased levels of IL-5, IL-4 and IL-13.

But the study of Christodoulopoulos *et al.*,⁽¹⁷⁾ the first scientists to characterize and compare the expression of the TH2 cytokine-associated transcription factors in bronchial tissue specimens in atopic and non-atopic asthmatics, found that GATA-3 and c-MAF are up-regulated in asthma, regardless of the atopic status.

In the present study, there was no association between GATA-3 mRNA expression in the induced sputum and the age of the asthmatic patients as well as no association could be found between its expression and certain sex of the included patients. However, there was

significant association between GATA-3 mRNA expression in the induced sputum and FEV1. So, the severity of asthma could be reflected from the nature of GATA-3 expression in the induced sputum. This result is in agreement with the study of Nakamura *et al.*,⁽¹⁶⁾ which reported that the number of cells expressing GATA-3 transcripts correlates significantly with reduced airway caliber and airways hyperresponsiveness in asthmatic subjects.

The present study also demonstrated a significant association between GATA-3 mRNA expression in the induced sputum and blood eosinophilic count as well as induced sputum eosinophilic percentage. There was no previous study had demonstrated this association however, the study of Taha *et al.*, (2003)⁽⁴⁾ which illustrated the close relationship that exists between the expression of TH2 cytokine receptors and the related TFs and that GATA-3 is necessary for the expression of IL-5, and the inhibition of its expression in TH2 cells has been associated with decreased levels of IL-5, IL-4 and IL-13.

Glucocorticoids are used to treat chronic inflammatory diseases such as asthma. Glucocorticoid binding to its receptor (GR) can have a dual effect on gene transcription. Activated GR can activate transcription (transactivation), or by interacting with other transcription factors such as NF-kappaB suppress transcription (transrepression) of target genes⁽¹⁸⁾. Recently, the major anti-inflammatory effects of glucocorticoids appear to be due largely to interaction between the activated glucocorticoid receptor and transcription factors, notably NF-kappaB and activator protein-1⁽¹⁹⁾.

In the present study after 6 weeks of inhaled corticosteroid therapy, all the treated patients either in the non-atopic or the atopic groups had negative expression of GATA-3 mRNA. The difference was statistically significant between GATA-3 mRNA expressions in the induced sputum by Rt-PCR before steroid therapy in atopic asthmatics in comparison to non-atopic asthmatics. However, there is no difference in GATA-3 mRNA expression in the induced

sputum by Rt-PCR after steroid therapy between the two groups.

It is evident that corticosteroids potently suppress TH2 cytokine gene expression as an important part of its therapeutic role in atopic asthma⁽²⁰⁾. So, intrapulmonary blockade of GATA-3 expression could cause an abrogation of signs of lung inflammation including infiltration of eosinophils and TH2 cytokines production and this antisense-induced blockade of GATA-3 will be at least as effective to suppress lung inflammation as the administration of corticosteroids⁽¹⁾.

Finally, this and similar studies open up new avenues for future research in this and related areas of disease immunopathogenesis. In relation to allergy, it is becoming clear that the disease involves aberrant expression of a range of TH2-associated cytokines, and it appears that therapies targeted at single cytokine will not have broad efficacy. The possibility of targeting upstream events in the disease process, as exemplified by the potential role of GATA-3 in regulating a range of TH2 cytokine genes, provides alternatives for future drug development. While specific pharmacological tools to test this possibility in humans are yet to be developed, it is important to know that an experimental model of atopic asthma, in which expression of a dominant-negative mutant of GATA-3 in mice in a T cell specific fashion led to concomitant down-regulation of IL-4, IL-5 and IL-13, and parallel attenuation of asthma-like manifestations and IgE production. This provides important proof-of-principle for the approach⁽¹⁴⁾.

CONCLUSION

Induced sputum provides sufficient sensitivity for examining transcription factors; GATA-3 expression is upregulated in atopic asthma. Corticosteroids block the action of GATA-3 as a part of its effects. Therefore, local delivery of antisense oligonucleotides blocking GATA-3 mRNA in atopic asthmatic patients will be at least as effective as the administration of

corticosteroids. In addition, this will escape the numerous side effects of corticosteroids.

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الملخص العربي

جاتا-٣ أم.ر.ن.أ في الربو الشعبي تأثير العلاج الأستنشاقى بالكورتيزون

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قسم الباثولوجيا الأكلينيكية، قسم الصدر* جامعه طنطا

الربو الشعبي مرض صدري مزمن يتسبب في زيادة استجابة الشعب الهوائية وانسداد جزئي بالممر الهوائي ويرتبط النوع المناعي منة بزيادة عدد الخلايا اللمفاوية المساعدة من النوع TH2 وزيادة افرازها للأنترلوكينز، ٤ وكذلك ١٣ ويقوم عامل النسخ جاتا -٣ بدور كبير في هذة العملية حيث انه يزيد من عدد وفعالة TH2 مما يزيد من حدة التهاب الانسجة التنفسية.

الغرض من البحث : يهدف هذا البحث الي دراسة مدي ظهور العامل -GATA 3MRNA في البلغم المستصث لمرضي الربو الشعبي المناعي والفيرمناعي باعتبارها طريقة امنة وبسيطة لدراسة الطبيعة الالتهابية للشعب الهوائية في هذا المرض مما يسهل التشخيص والعلاج.

المرضي وطرق البحث : اشتملت هذة الدراسة علي ٤٦ شخصا في ثلاث مجموعات وقد تم دراسة ظهور العامل GATA 3MRNA في البلغم السمتصث بواسطة ؛} وكذلك تحديد نسبة خلايا الايزينوفيل في العينات ودراسة علاقتها . وقد تم اعادة هذة الاختبارات ل٢٠ شخصا بعد العلاج بالمورتيزون لمدة طويلة.

نتائج البحث : هناك اختلاف في طريقة ظهور جاتا-٣ ام.ر.ن.أ في البلغم المستصث لمرضي الربو الشعبي المناعي عند مرضي الربو الشعبي الغير مناعي وكذلك عن المجموعة الضابطة وكذلك زيادة نسبة خلايا الايزنوفيل في البلغم المستصث في كلا من الربو المتاعي والغير مناعي عند المقارنة مع مجموعة الاصحاء وانه يوجد علاقة ذات دلالة احصائية بين ظهور جاتا-٣ ام.ر.ن.أ ونسبة خلايا الايزينوفيل في الربو الشعبي بنوعية.

الخلاصة : ان احباط جاتا-٣ في خلايا الشعب الهوائية قد يلعب دورا هاما في تخفيف حدة الربو الشعبي المناعي .