Plasma Transforming Growth Factor β1 level in Asthmatic Children

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ABSTRACT

Airway remodeling is a characteristic feature of asthma. The mechanism of this remodeling is thought to involve Transforming growth factor - β1 (TGF-β1). We compare plasma level of TGF-β1 among asthmatic patients and healthy children and to evaluate these levels with atopic status. 50 asthmatic patients divided into: 30 asthmatics in between attack and 20 during attack . As regard to atopy they were subdivided into: atopic during and in between attack and non-atopic during and in between attack. Group II (Control group): this group included 20 healthy volunteers. All subjects were subjected to history taking, chest X-ray, CBC, intra-dermal skin tests, serum level of total IgE and determination of plasma TGF-β1. There was statistical significant difference between asthmatics and control group, also between asthmatics inbetween attack and control group as regard the mean total leucocytic count. Regarding the mean eosinophilic count, there was extremely statistical significant difference between asthmatics (during and in between attack) with control group, there was statistical significant difference between asthmatic patients inbetween attack and during attack. Regarding the mean neutrophil count there was statistical significant difference in asthmatics compared to the control group, there was no statistical significant difference between asthmatics inbetween attack and asthmatics during attack. Regarding to serum level of total IgE, there was statistical significant difference between both asthmatic group and the control group. But no statistical significant difference between asthmatic patients inbetween attack and during attack. Regarding plasma level of TGF β1, there was significant difference between asthmatic group and the control group, also between asthmatics during and inbetween attack. The most risky offending allergen was mixed pollens (71.4%), followed by hay dust (61.9%), smoke (57.1%), house dust (47.6%), mixed fungi (38%), cotton (28.5%), wool (19%) and lastly, human hair (9.5%). There was statistical significant difference as regard the mean total leucocytic and neutrophil count in non- atopic patients compared to atopic patient. Statistical significant difference as regard the mean serum level of total IgE in atopic patients in between attack compared to the other groups. There was also statistical significant difference between both groups of atopic patient, also both groups of non-atopic as regard to serum total IgE. As regard the mean level of plasma TGF β1, there was statistical significant difference in non-atopic asthmatic patients inbetween attack compared to other groups. There was also statistical significant difference between both groups of atopic and both group of non-atopic patients. This study concludes that patients with non atopic asthma have elevated plasma TGFβ1 levels that may reflect a post-infective phenomenon that serves to down-regulate the host immune response

INTRODUCTION

Asthma is a chronic disease of the airways that affects people all over the world. Prevalence has increased over recent years, especially in children and young people, in associations^ with atopy rather than an increased recognition of the disease[1]. Approximately 10% of the population are affected, though there is variation between countries, and it is estimated that 1-2% of the total health care expenditure for developed countries is asthma related [2].

Asthma is characterized by persistent inflammation and remodeling of the airways with associated bronchial hyperactivity in response to one or more allergens, resulting in episodic obstruction of airflow [3]. Asthmatic airway tissues have increased immune cells including basophiles, mast cells, eosinophiles, and T lymphocytes that are able to produce proinflammatory cytokines and chemokines. The selective accumulation of immune cells and associated cytokines is considered a central event in the pathogenesis of allergic asthma[4]. Since production of Th2 cytokines is highly associated with allergic asthma, it is rational to deduce that nonallergic individuals may be associated either with a failure to recognize the allergen or the suppression of Th2 responses [5].

High level of IgE are associated with asthma in both adults and children [6]. IgE has been associated with allergic diseases and asthma . The studies of the effects of anti-IgE therapy have confirmed that it plays a role in these diseases[7]. Transforming growth factor- β1 (TGF-β1) is a multifunctional cytokine that has been implicated in the pathogenesis of asthma. TGF-β1 is important in growth, development, transformation, tissue repair, fibrosis

and modulation of inflammatory immune responses and has a critical role in the remodeling process[8]. TGF-β1 is expressed by airway epithelial cells, eosinophils, helper T type2 lymphocytes, macrophage, and fibroblast and may be bound and stored in the subepithelial extracellular matrix of the airways [9].

The aim of this work was to compare plasma level of TGF-β1 among asthmatic patients and healthy children and to evaluate these levels with atopic status.

PATIENTS, MATERIALS & METHODS

This study was carried out at the Allergy and Immunology Unit of Microbiology and Immunology Department, and Pediatric Pulmonology Unit; Faculty of Medicine, Zagazig University during the period from September 2008 to March 2010. This study included 70 subjects that were divided into two groups: Group I (Patient group): 50 asthmatic patients divided into: 30 asthmatics in between attack (17 males and 13 females with the mean age 6.68 Y ± 2.66) and 20 during attack (12 males and 8 females with the mean age 7.45 Y ± 2.26). As regard to atopy they were subdivided into: atopic during and in between attack and non-atopic during and in between attack. Their ages ranged from 2 to 12 years. Group II (Control group): this group included 20 healthy volunteers. The controls were 11males and 9 females of the same age. All subjects included in this study were subjected to the following: history taking, chest X-ray, CBC, intra-dermal skin tests, serum level of total IgE and determination of plasma TGF-β1.

The Skin Intradermal Test was done using house dust, human hair, smoke, cotton, wool, mixed fungus (Aspergellus niger, Aspergellus Flavus, Aspergellus fumigatus), mixed pollens and hay dust [10].

Determination of Serum Total IgE Level by ELISA: (Monobind Inc. Lack forest, CA 92630, USA). According to manufacturer instructions it was read at 450 nm. The concentration of total serum IgE was calculated corresponding to the absorbance from standard curve.

Determination of Plasma level of TGF-β1 by using DRG TGF-β1 ELISA (DRG instruments GmbH, Frauenbergstr.18, D35039 Marburg, Germany). Venous blood sample were taken under complete aseptic conditions. To minimize platelet degranulation and subsequent false increase in the level of the estimated plasma TGF-β_1, a tourniquet was applied only when necessary and was removed after insertion of the 21-gauge needle for venipuncture [11]. Then blood sample were collected in sterile EDTA containing tube , were centrifuged for separation of the plasma for 20 minutes at 4000 xg's (≈ 6500 rpm) at 4°C using cold centrifuge and kept frozen at -70°C.

The Assay was performed after acidification and neutralization of plasma according to the manufacturer's instructions. The absorbance at 450 nm is measured with a microtiter plat reader, the intensity of color development is proportional to the TGF-β_1 concentration in the sample. Standard curve was constructed and Plasma TGF-β1 was estimated. Sensitivity of the kit was 1.9 pg/mL

RESULTS

As shown in table (1) there was statistical significant difference between asthmatics and control group, also between asthmatics inbetween attack and control group as regard the mean total leucocytic count. Regarding the mean eosinophilic count, there was extremely statistical significant difference between asthmatics (during and inbetween attack) with control group, there was statistical significant difference between asthmatic patients inbetween attack and during attack. Regarding the mean neutrophil count there was statistical significant difference in asthmatics compared to the control group, there was no statistical significant difference between asthmatics inbetween attack and asthmatics during attack.

Regarding to serum level of total IgE, as shown in table (2) there was statistical significant difference between both asthmatic group and the control. But no statistical significant difference between asthmatic patients inbetween attack and during attack. Regarding plasma level of TGF β1, there was significant difference between asthmatic group and the control group, also between asthmatics during and inbetween attack. The most risky offending allergen was mixed pollens (71.4%), followed by hay dust (61.9%), smoke (57.1%), house dust (47.6%), mixed fungi (38%), cotton (28.5%), wool (19%) and lastly, human hair (9.5%).

There was statistical significant difference as regard the mean total leucocytic and neutrophil count in non- atopic patients compared to atopic patient. Statistical significant difference as regard the mean serum level of total IgE in atopic patients in between attack compared to the other groups . There was statistical significant difference between both groups of atopic patient, also both groups of non-atopic (Table 3,4). As regard the mean level

of plasma TGF β1,there was statistical significant difference in non-atopic asthmatic patients inbetween attack compared to other groups. There was also statistical significant difference between both groups of atopic and both groups of non-atopic (Table 5).

Table (1): Mean Total leucocytic count, absolute eosinophil count , absolute neutrophil count and multiple comparisons showing least significant different among groups.

Asthmatic group	Total leucocytic count			Absolute eosinophil count (10^9/L)			Absolute neutrophil count /cmm		
	Mean ± SD	Control group	Asthmatics during attack	Mean ± SD	Control group	Asthmatics during attack	Mean ± SD	Control group	Asthmatics during attack
During attack (n=20)	7.56 ± 1.47	0.093		43.8 ± 5.58	0.000***		3822.0 ± 754.14	0.017*	
In between attack (n=30)	7.82 ± 1.61	0.015*	0.52	40.9 ± 4.53	0.000***	0.039*	3686.4 ± 504.99	0.061	0.435
Control group (n=20)	6.8 ± 0.93			11.7± 3.97			3357.9 ± 551.86		
F	3.21			299.2			3.23		
P	0.047*			0.000***			0.046*		

Table (2): Mean Serum level of total IgE , TGF β1 and multiple comparisons showing least significant different among groups.

Asthmatic group	Serum level of total IgE (kU/L)			Plasma level of TGF β1 (pg/mL)		
	Mean ± SD	Control group	Asthmatics during attack	Mean ± SD	Control group	Asthmatics during attack
During attack (n=20)	290.11 ± 96.41	0.000***		3.90 ± 0.85	0.046*	
In between attack (n=30)	272.61 ± 93.24	0.000***	0.738	6.85 ± 3.42	0.000***	0.000***
Control group (n=20)	25.82 ± 9.93			2.40 ± 0.73		
F	14.23			23.7		
P	0.000***			0.000***		

Table (3): Mean TLC, absolute eosinophil , absolute neutrophil counts and mean total IgE , TGF β1 among different groups according to atopy.

	Mean ± SD of			Mean ± SD of	
	Total leucocytic count	Absolute eosinophil count (10^9/l)	Absolute neutrophil count /cmm	Total IgE (kU/L)	TGF β1 (pg/mL)
Atopic*					
During attack(8)	6.01 ± 0.99	45.0 ± 5.71	3052.0 ± 290.28	139.72 ± 42.58	4.81 ± 0.25
In between attack(13)	6.17 ± 0.85	40.1 ± 4.09	3320.46 ± 554.41	508.83 ± 17.16	3.96 ± 0.66
Non-atopic					
During attack(12)	8.59 ± 0.48	43.0 ± 5.59	4337.9 ± 458.32	390.36 ± 133.12	3.29 ± 0.45
In between attack(17)	9.08 ± 0.5	41.5 ± 4.87	3966.23 ± 199.22	91.96 ± 25.51	9.05 ± 2.98
Control group(20)	6.80 ± 0.93	11.7 ± 3.97	3357.9 ± 551.86	25.82 ± 9.93	2.40 ± 0.73
F	44.58	148.81	16.6	68.61	46.49
P	0.000***	0.000***	0.000***	0.000***	0.000***

Table (4): Multiple comparisons showing least significant different among different groups regarding total IgE.

	Control	Atopic		Non-atopic	
		During attack(8)	In between attack(13)	During attack(12)	In between attack(17)
Atopic*					
During attack(8)	0.006**		0.000***	0.000***	0.248
In between attack(13)	0.000***	0.000***		0.003**	0.000***
Non-atopic					
During attack(12)	0.000***	0.000***	0.003**		0.000***
In between attack(17)	0.040*	0.248	0.000***	0.000***	

Table (5): Multiple comparisons showing least significant different among different groups regarding plasma TGF β1 level.

	Control	Atopic		Non-atopic	
		During attack (8)	In between attack(13)	During attack(12)	In between attack(17)
Atopic*					
During attack(8)	0.000***		0.233	0.038*	0.000***
In between attack(13)	0.007**	0.233		0.291	0.000***
Non-atopic					
During attack(12)	0.125	0.038*	0.291		0.000***
In between attack(17)	0.000***	0.000***	0.000***	0.000***	

DISCUSSION

Bronchial asthma is unlikely to be a single disease. It is more likely to be a series of complex, overlapping or phenotypes each defined by its unique interaction between genetic and environmental factors. Asthma is characterized by chronic lung inflammation and airway remodeling associated with wheezing, shortness of breath, chest tightness, coughing and acute bronchial hyperresponsiveness to a variety of stimuli [12].

Airway remodeling is the general description for the thickening and restructuring of the airways seen in asthmatic patients. The characteristics of airway remodeling include subepithelial fibrosis, myofibroblast hyperplasia, myocyte hyperplasia and hypertrophy, together with epithelial damage, goblet cell metaplasia, edema and increased vascularity [13]. The transforming growth factor-beta1 is a central mediator of tissue fibrosis and structural remodeling [14].

TGF- β1 is produced by nearly all cell types of the immune system. The role of TGF- β1 is more complex and involves a dual role as both an anti-inflammatory and a pro-inflammatory cytokine [15]. However, the secretion of TGF- β1 immediately after an allergic disorder contributes to fibrosis and the irreversible changes associated with airway remodeling in chronic asthma, thus pointing toward a pro-inflammatory role for this cytokine [16].

In our study there was increased total leucocytic count (TLC) in asthmatics during and in between attack compared to the control group with their mean 7.56 ± 1.47, 7.82 ± 1.61 and 6.8±0.93 respectively and there were statistical significant difference between asthmatics (during and in between attack) and control group (P < 0.047).

Our results were in agreement with **Hansen et al [17],** they found increased TLC on their studies as they are pivotal in airway inflammation and their infiltration into bronchial wall; interaction with inhaler antigen leads to bronchial cell damage and airway inflammation.

In our study there was increased absolute eosinophilic count in asthmatic during and in between attack more than control group with their mean count 43.8 ± 5.58, 40.9 ± 4.53 and 11.7±3.97 respectively and there was extremely statistical significant difference (P<0.000).

Our findings were in line with **Nomura et al[18],** who found that TGF-beta1 were significantly correlated with eosinophil counts, also, the spontaneously released amount of TGF- beta1 was correlated positively with both neutrophils and eosinohils[19] and the eosinophils are the major source of TGF-beta 1[20].

In our study, increased absolute neutrophil count in asthmatics during attack more than in between attack and the control group with their mean 3822 ± 754.14, 3686.4 ±504.99 and 3357.9 ± 551.86 respectively and there was statistical significant difference, P value <0.046. Our results were in agreement with **Xiao-yan et al[19],** who reported that the percentages of eosinophils and neutrophils were higher in asthmatics than those in normal controls.

In our study there was increased serum level of total IgE in asthmatics during and in between attack more than the control group with their mean level 290.11 ± 96.41, 272.61 ± 93.24 and 25.82 ± 9.93 respectively and there was extremely statistical significant difference between both asthmatic patients during and in between attack with control group, but no statistical significant difference between asthmatic patients in between attack and during attack.

Our results were in line with those of **Bettiol et al [21],** who found that atopic asthma displays raised total serum IgE**.** Also, current attacks of asthma showed an association with total IgE adjusted for specific IgE, sex and age which did not vary by bronchial responsiveness. Individuals with current wheezing and bronchial responsiveness without attacks of asthma also showed an adjusted association with total IgE, which remained for persons without specific IgE. These finding reinforce the evidence that asthma is associated with increased levels of total IgE even in subjects negative for specific IgE to common aeroallergens [22].

In our study there was increased plasma level of TGF β1 in asthmatics in between attack more than during attack and the control group with their mean level 6.85 ± 3.42 , 3.90 ± 0.85 and 2.40±0.73 respectively and there was extremely significant difference between asthmatics (in between attack) and each of during attack and the control group (P < 0.000). There was statistical significant difference between asthmatics during attack and the control group (P<0.046).

Our results are in line with **Vignola et al [23],** who found that the plasma level of TGF β1 was higher in stable asthmatic patients without treatment compared to those seen in healthy control subjects or patients with severe asthma. Peripheral blood neutrophils also expressed TGF beta-1 protein and mRNA, and blood neutrophils from asthmatics spontaneously released significantly higher levels of TGF beta-1 than those of normal controls [24].

In our study, the most risky offending allergen was mixed pollens (71.4%), followed by hay dust (61.9%), smoke (57.1%), house dust (47.6%), mixed fungi (38%), cotton (28.5%), wool (19%) and lastly, human hair (9.5%). In addition, 80% of patients showed positive skin test for more than one allergen. Our results are in line with **Etewa[25],** who found that the commonest allergens were mixed pollens, followed by hay dust, then smoke and house dust mite. This is explained by the global climate change hypothesis where there is now a considerable evidence of impacts of climate change on aeroallergens, particularly pollen. It appears that plants produce a greater quantity of pollen with a stronger allergenicity under increased CO_2 concentrations and temperatures[26,27].

In our study, increased total leucocytic count in non atopic patients either during or in between attack more than atopic patients either during or in between attack and control group with their mean counts 8.59 ± 0.48, 9.08 ± 0.5, 6.01±0.99, 6.17±0.85and 6.80±0.93 respectively and there was extremely statistical significant difference (P<0.000) .

Regarding absolute eosinophil count, there was increase absolute eosinophilic count in asthmatic patients either atopic or non atopic during or in between attack more than control group with their mean counts 45.0 ± 5.71 , 40.1 ± 4.09, 43.0 ± 5.59 , 41.5 ± 4.87and 11.7 ± 3.97 respectively and there was extremely statistical significant difference (P < 0.000) .

Regarding absolute neutrophilic count, increased in non atopic asthmatics either during or in between attack more than atopic either during or in between attack or control group with their mean counts 4337.9 ± 458.32 , 3966.23 ± 199.22 , 3052.0± 290.28 , 3320.46 ± 554.41 and 3357.9 ± 551.86 respectively and there was extremely statistical significant difference (P < 0.000).

Our results were in line with **Joseph et al[28],** who found that the total white blood cell count in the non atopic asthmatic group was significantly higher compared with the atopic and healthy control groups. The mean absolute neutrophil count was also significantly higher compared with atopic patients and healthy control group. The percentages of eosinophils were higher in asthmatics than those in normal controls**,** as the eosinophils play an important role in the pathogenesis of air way remodeling in asthma through the production of various fibrogenic cytokines such as TGF beta-1 [19 ,29].

In our study there was increase serum level of total IgE in atopic patients in between attack compared with atopic during attack, non atopic (during and in between attack) and control

group with their mean 508.83 ± 17.16 , 139.72 ± 42.58, 390.36±133.12, 91.96±25.51 and 25.82±9.93 respectively with extremely statistical significant difference (P<0.000). This is concordant with **Blaiss [30],** who reported that serum IgE levels were higher in asthmatic patients than in non asthmatics and the median total serum IgE level was significantly higher in atopic asthmatic patients compared with the non atopic asthma and control groups[28]. But **Srivastava et al[31]**, who found that, raised serum IgE was detected in all groups of asthmatic patient compared to the control group, they observed lower IgE levels in late onset asthma compared to early onset asthma patients.

In our study there was increased level of plasma TGF β1 in non atopic asthmatic patients in between attack compared with non atopic during attack ,atopic (during and in between attack) and control group with their mean 9.05 ± 2.98, 3.29 ± 0.45, 4.81 ± 0.25, 3.96 ± 0.66 and 2.40±0.73 respectively with extremely statistical significant difference, (P<0.000).

Our results were in line with **Joseph et al[28],** who found that the median value of plasma TGF beta-1 was significantly higher in stable non atopic asthmatic group compared with controls and atopic asthmatic patients **,** also , the TGF beta-1 suppresses airway inflammation [32]. Accordingly, our observation of increased TGF β1 levels in the plasma of nonatopic asthmatic patients might reflect a protective mechanism to suppress ongoing inflammation in the airways[33]**.**

Our results found agreement with **Minshall et al[34],** who reported that many studies performed in humans showing that eosinophils are a major cellular source of TGFβ1 in asthmatic lungs by demonstrating a liner relationship between the degree of eosinophils and the expression of TGFβ1 in different lung tissues. Also**,** positive correlation between eosinophil counts and the number of cells staining positive for TGFβ1 in plasma samples of asthmatics [18].

Our results were in line with **Bossé and Rold [35],** who found that blood neutrophils in normal and asthmatic individuals were shown to express TGFβ1. Neutrophilis could contribute significantly to the increased expression of TGFβ1 particularly prominent in non atopic asthma and in more sever forms of the disease. The median absolute neutrophil count in the non atopic asthmatic patients was significantly higher compared with a topic asthmatic patients and healthy controls [36].

Regarding plasma TGF β 1 level, there was extremely statistical significant difference between non atopic patients in between attack and each of control group, atopic patients (during and inbetween attack) and non-atopic during attack, P<0.000. There was highly statistical significant difference between atopic patients inbetween attack and control group, P =0.007. There was statistical significant difference between atopic patients during attack and non atopic during attack. **Joseph et al [37],** found that the median value of plasma TGFβ1 was significantly higher in non atopic asthmatic patients compared with controls and atopic asthmatic patients. The plasma TGFβ1 was highest in stable asthmatic groups when compared to the moderate asthma and asthma in remission. These data support the role of TGFβ1 in airway remodeling in asthma [38].

This study concludes that patients with non atopic asthma have elevated plasma TGFβ1 levels that may reflect a post-infective phenomenon that serves to down-regulate the host immune response. We recommended that novel therapies for asthma targeted to manipulate TGF- beta1 could change the outcome of many children suffering from this disease.

REFERENCES

1. **Koster E, Wijga A, Koppelman, G, Postma, Brunekreef B, et al (2011):** Uncontrolled asthma at age 8: The importance of parental perception towards medication. *Pediatric Allergy and Immunology, no. doi: 10.1111/j.1399-3038.01150.x.*
2. **Moore WC and Pascual R (2010):** "Update in asthma ". *American journal of respiratory and critical care medicine* 181 (11): 1181–1187.
3. **Fanta CH (2009):** "Asthma". *N Engl J Med* 360 (10): 1002–14.
4. **Walker C, Virchow JC, Bruijnzeel PB and Blaser K (2009):** T cell subset and their soluble products regulate eosinophilia in allergic and nonallergic asthma. *J Immunol; 146:1829-1835.*
5. **Woodruff P, Modrek B, Choy D, Jia G, Abbas A, et al (2009):** T-helper type 2-driven inflammation defines major subphenotypes of asthma. *Am J Respir Crit Care Med; 180:388-395.*
6. **Sunyer J,Anto J, Castellsague J, Soriano J and Roca J (1996) :** Total serum IgE is associated with asthma independently of specific IgE levels. The Spanish Group of the European study of asthma. *Eur Respir J.*, 9: 1880 – 1884.

7. **Ong YE , Menzies-Gow A , Barkans J . Benyahia F , Ou TT and Ying S (2005) :** Anti-IgE (Omalizumab) inhibits late - phase reaction and inflammatory cells after repeat skin allergen challenge. J Allergy Clin Immunol., 116 (3) : 558-564.
8. **Ueda T, Niimi A, Matsumoto H, et al (2008):** TGFB1 promoter polymorphism C-509T and pathophysiology of asthma. *J Allergy Clin Immunol; 121: 659*-664.
9. **Kay B, Phipps S, and Robinson D.S (2008):** A role for eosinophils in airway remodelling in asthma. Trends in Immunology; *Volume 25, Issue 9 Pages 477-482.*
10. **IndrajanaT, Spieksma F. and Voorhost R (1971):** Comparative study of the intracutaneous, scratch and prick tests in allergy. *Ann Allergy; 29: 639-650. Cited from***:** LI, J.T.(2002): Allergy Testing. *American Family Physician*; 66: 4.
11. **Kropf, J.; Schurek, J.O. and Wollner, A. (1997):** Irnmunologieal measurement of Transforming Growth Factor-beta 1 (TGF-beta 1) in blood, *assay development and comparison; 43: 1965-1974.*
12. **Borish L, Culp J (2010):** Asthma: a syndrome composed of heterogenous diseases. *Ann Allergy Asthma Immunol; 101:8-11.*
13. **Busse W, Banks S and Noel P (2010):** Future research directions in asthma: An NHLBI Working Group Report. *Am J Respir Crit Care Med.* 170: 683-690.
14. **Howell J and McAnulty R (2006):** TGF-beta: Its role in asthma and therapeutic potential. *Curr Drug Targets;* 7 (5): 547-565.
15. **Xie S, Sukkar M and Issa R (2005):** Regulation of TGF-β1induced connective tissue growth factor expression in airway smooth muscle cells. *Am. J. Physiol. Lung Cell Mol. Physiol.* 288: L68-L76.
16. **Schmidt-Weber C and Blaser K (2006):** The role of TGF-beta in allergic inflammation. *Immunol Allergy Clin North Am.* 26 (2): 233-244.
17. **Hansen G, Berry G, DeKrayf R, Umetsu DT(2007):** Allergen specific Th1 cells fail to counterbalance Th2 cells induced airway hyper activity but because sever airway inflammation. *J Clin Invest; 103:175-183.*
18. **Nomura A, Uchida Y, Sakamoto T, Ishii Y, Masuyama K, et al (2009):** increase in collagen type 1 synthesis in asthma: the role of eosinophils and transforming growth factor –beta. *Clin. Exp. Allergy, 32:860-865.*
19. **Xiao-Yan GAI, Young- chang SUN and Wen-li CAO (2006):**Effect of dexamethasone on the release of transforming growth factor β1, interleukin-8, interleukin-10 and RANTES release by sputum cells in severe asthma. *Chinese Medical Journal; 119 (18) 1567-1571.*
20. **Cho J, Miller M, Baek K, Han J W, Lee S, et al(2004):** Inhibition of airway remodeling in IL-5- deficient mice, *J Clin. Inves.113, pp.551-560.*
21. **Bettiol J , Bartsch P , Louis R, De Groote D, Gevaerts Y,et al (2000):** Cytokine production from peripheral whole blood in atopic and nonatopic asthmatics:relationship with blood and sputum eosinophilia and serum IgE levels. *Allergy, volume 55 ,Number 12, pp. 1134-1141(8) 2000.*
22. **Sunyer J, Anto J, Castellsague J, Soriano J and Roca J (2008):** Total serum IgE is associated with asthma independently of specific IgE levels. The Spanish Group of the Europian Study of Asthma. *Eur Respir J;9: 1880-1884.*
23. **Vignola A, Chiappara G, Chanez P,et al (2005):** Transforming Growth Factor-β expression in mucosal biopsies in asthma and chronic bronchitis. *Am J Respir Crit Care Med; 156:591-599.*
24. **Chu H, Trudeau J, Balzar S and Wenzel S (2008):** peripheral blood and airway tissue expression of transforming growth factor β by neutrophils in asthmatic subject and normal control subject.*J Allergy Clin Immunol; 106:1115-1123.*
25. **Etewa, R. (2005):** Some Biochemical Markers in Atopic Patients and PCR-Based Assay for Detection of R576 Interleukin-4 Receptor α Allele Gene *MD., Thesis Medical Biochemistry Department, Faculty of Medicine, Zagazig University*.
26. **Beggs, P.J. (2004):** Impacts of climate change on aeroallergens: past and future. *Clin Exp Allergy;* 34: 1507-1513.
27. **Beggs, P.J. and Bambrick, H.J. (2005):** Is the Global Rise of Asthma an Early Impact of Anthropogenic Climate Change? *Environ. Health Perspect. 113(8): 915-919.*
28. **Joseph j, Sheela B, Padmanabhan B, Safa W, Maries J, et al (2003):** *AnnAllergy Immunol;91:472-476.*
29. **Kato Y, Fujisawa T, Nishimori H, Katsumata H, Atsuta J,et al (2009):** Leucotriene D4 induces production of transforming growth factor- beta1 by eosinophils. Int.Arch. *Allergy Immunol., 137 (Supple. 1): 17-20.*

30. **Blaiss MS (2005):** Epidemiology and pathophisiology of immunoglobulin E-mediated asthma. *Allergy Asthma Proc., 26(6):423-427.*
31. **Srivastava N, Srivastava L and Gupta S (2002):** Studies on serum complement and IgE in bronchial asthma Clinical and Experimental Allergy *;12:560-569.*
32. **Heneda k, Sano k, Tamura G, Sato T, Habu S,et al (2009):** TGF- β induced by oral tolerance ameliorates experimental tracheal eosinophilia. *J Immunol.;159:4484-4490.*
33. **Nakao A, Miike S, Hatano M, et al (2008):** Blockade of transforming groeth factor βSmad signaling in T cells by overexpression of Smad7 enhances antigen-indused airway inflammation and airway reactivity. *J Exp Med;192:151-158.*
34. **Minshall E, Leung D, Martin R, et al (2010)**: Eosinophil-associated TGF-β1 mRNA expression and airway fibrosis in bronchial asthma. *Am J Respir Cell Mol Biol;17:326-333.*
35. **Bosse and Rold-Pleszcznski (2010):** Controversy surrounding the increased expression of TGF β1 in Asthma, *respire Res; 8 (1):66.*
36. **Abdulkhalik S, Josef J, Benedict S, Badrinath P, Josef M ,et al(2010) :** Elevation of plasma Transforming Growth Factor beta 1 level in stable nonatopic asthma. *Ann Allergy Athma. Immunol; 91 (5):472-476.*
37. **Joseph M, Wassef S, Josef J, Benedict S, Badrinath P ,et al (2010) :** Elevation of plasma Transforming Growth Factor beta 1 level in stable nonatopic asthma. *Ann Allergy Athma. Immunol; 91 (5):472-476.*
38. **Manuyakorn W, Kamchaisatian W, Atomosirikui K, Sasisakulporn C, Direkwattanuh ,et al (2008):** Plasma TGF-β1 in atopic asthma. *Asian Pack.Allergy.Immunol; 26 (4) 185-189.*

مستوى معامل النمو التحولى بيتا ١ فى بلازما أطفال الربو الشعبى

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يعد الربو الشعبى فى الأطفال من أكثر الأمراض الصدرية المزمنة شيوعا ويتصف بأعراض مختلفة منها الكحة وضيق فى الشعب والشعيبات الهوائية مع التهابات وأزيز ، وتختلف حدة المرض من مريض إلى آخر .التحور فى الشعب الهوائية قد يلعب دورا هاما فى الفسيولوجيا المرضية للربو الشعبى . ويعد معامل النمو التحولى بيتا -١ مادة محركة للخلية ذات عدة وظائف حيث يعمل كمنشط ومضاد للالتهابات .

تستهدف هذه الدراسة قياس مستوى معامل النمو التحولى بيتا -١ فى بلازما الأطفال المصريين المصابين بالربو الشعبى أثناء وبين نوبات المرض مقارنة بالأطفال الأصحاء .

أجريت هذه الدراسة في وحدة الحساسية والمناعة وبقسم الصدر بمستشفي الأطفال الجامعي بكلية الطب جامعة الزقازيق خلال الفترة من سبتمبر ٢٠٠٨ إلي مارس ٢٠١٠. شملت الدراسة ٧٠ طفل وقد تم تقسيمهم إلي مجموعتين:-

المجموعة الأولي (مجموعة المرضي): تشمل ٥٠ مريضا بالربو الشعبي وتتراوح أعمارهم من ٢ وحتى ١٢ سنة وقد تم تقسيمهم إلي:-

أ. ٣٠ مريض بين نوبات المرض (١٧ من الذكور و ١٣ من الإناث).

ب. ٢٠ مريض أثناء نوبات المرض (١٢ من الذكور و ٨ من الإناث).

فيما يتعلق باختبار الحساسية عن طريق الجلد تم تقسيمهم إلي:-

- إيجابا أثناء وبين نوبات المرض علي التوالي (حساس).
- سلبا أثناء وبين نوبات المرض علي التوالي (غير حساس).

المجموعة الثانية (المجموعة الضابطة): شملت هذه المجموعة ٢٠ متطوعا من الأصحاء ١١ من الذكور و ٩ من الإناث من نفس الفئة العمرية.

ولقد تم إجراء الآتي على المرضى و أفراد المجموعة الضابطة:

التحليل الكامل للتاريخ المرضى. الفحص الأكلينيكى العام والخاص للصدر. الفحوصات: أشعة سينية على الصدر خلفي أمامي وجانبي.تحليل بول وبراز لاستبعاد الإصابة بالطفيليات.اختبار بطريقة الحقن بالجلد للتعرف على المواد الغريبة المسببة للحساسية .

اختبارات الدم: عد خلايا الدم البيضاء ومشتقاتها من الأيزينوفيل والنيتروفيل. قياس مستوى الجلوبيولين المناعي الكلى (هـ) فى مصل الدم بالاليزا. قياس مستوى معامل النمو التحولي بيتا -١ فى بلازما الدم بالاليزا.

***نتائج البحث :**

و قد أسفرت الدراسة عن النتائج التالية:

زيادة نسبة كلا من خلايا الدم البيضاء والأيزينوفيل والنيتروفيل والجلوبيولين المناعي الكلي (هـ) في مرضي الربو الشعبي مقارنة بالمجموعة الضابطة. زيادة عدد الأيزينوفيل في المرضي أثناء نوبات المرض مقارنة بين المرضي بين نوبات المرض والمجموعة الضابطة مع وجود فرق ذو دلاله إحصائية.

فيما يتعلق بخلايا الدم البيضاء: لا يوجد فرق ذو دلالة إحصائية بين المرضي بين نوبات المرض وكلا من المجموعة الضابطة والمرضي أثناء نوبات المرض.

فيما يتعلق بالجلوبيولين المناعي الكلي (هـ): لا يوجد فرق ذو دلالة إحصائية بين المرضي أثناء وبين نوبات المرض.

زيادة معامل النمو التحولي ب ١ في المرضي بين وأثناء نوبات المرض مقارنة مع المجموعة الضابطة. وكان هناك فرق ذو دلالة إحصائية بين المرضي بين نوبات المرض وكلا من المرضي أثناء نوبات المرض والمجموعة الضابطة. أيضا وجود فرق ذو دلالة إحصائية بين المرضي أثناء نوبة المرض والمجموعة الضابطة.

أن المواد الأكثر شيوعا كسبب لحساسية الصدر هي حبوب اللقاح المختلفة ثم يليها قش الأرز، الدخان، تراب المنزل، الفطريات المختلفة، القطن، الصوف و شعر الإنسان و أن هناك أكثر من ٨٠% من المرضى يعانون من حساسية لأكثر من مادة.

زيادة خلايا الدم البيضاء والنيتروفيل في المرضي الغير الحساسة مقارنة مع المرضي الحساسة أو المجموعة الضابطة مع وجود فرق ذو دلاله إحصائية.

زيادة عدد الأيزينوفيل في مرضي الربو سواء الحساسة أو غير الحساسة مقارنة بالمجموعة الضابطة مع وجود فرق ذو دلاله إحصائية.

زيادة نسبة الجلوبيولين المناعي (هـ) في المرضي الحساسة بين نوبات المرض مقارنة مع المجموعات الأخرى والمجموعة الضابطة مع وجود فرق ذو دلاله إحصائية.

زيادة مستوي معامل النمو التحولي ب ١ في المرضي غير الحساسة بين نوبات المرض مقارنة بالمجموعة الضابطة والمجموعات الأخرى مع وجود فرق ذو دلاله إحصائية.

فيما يتعلق بخلايا الدم البيضاء: وجود فرق ذو دلاله إحصائية بين المرضي الغير حساسة سواء أثناء أو بين نوبات المرض وكلا من المرضي الحسا سه والمجموعة الضابطة. ولكن لا يوجد فرق ذو دلاله إحصائية بين المرضي أثناء أو بين نوبات المرض (سواء حساسة أم لا).

فيما يتعلق بعدد الأيزينوفيل: وجود فرق ذو دلاله إحصائية بين المرضي الحساسة وغير الحساسة-أثناء وبين نوبات المرض- مقارنة مع المجموعة الضابطة. أيضا وجود فرق ذو دلالة إحصائية بين المرضي الحساسة أثناء وبين نوبات المرض.

فيما يتعلق بعدد النيتروفيل: وجود فرق ذو دلالة إحصائية بين المرضي غير الحساسة -أثناء وبين نوبات المرض- مقارنة مع كلا من المجموعة الضابطة والمرضي الحساسة-أثناء وبين نوبات المرض- ,أيضا وجود فرق ذو دلالة إحصائية بين المرضي غير الحساسة أثناء وبين نوبات المرض. ولكن لا يوجد فرق ذو دلالة إحصائية بين المرضي الحساسة -أثناء وبين نوبات المرض – مقارنة مع المجموعة الضابطة.

فيما يتعلق بالجلوبيولين المناعي الكلي (هـ): وجود فرق ذو دلالة إحصائية بين المرضي غير الحساسة أثناء المرض مقارنة بكلا من المجموعة الضابطة والمرضي الحساسة أثناء المرض وغير الحساسة بين نوبات المرض. أيضا وجود فرق ذو دلالة إحصائية بين المرضي الحساسة أثناء المرض مقارنة بالمجموعة الضابطة. لكن لا يوجد فرق ذو دلالة إحصائية بين المرضي الحساسة أثناء المرض وغير الحساسة بين نوبات المرض.

فيما يتعلق بمعامل النمو التحولي ب ١: وجود فرق ذو دلالة إحصائية بين المرضي غير الحساسة بين نوبات المرض مقارنة بكلاً من المجموعة الضابطة والمرضي الحساسة -أثناء وبين نوبات المرض- والمرضي غير الحساسة بين نوبات المرض. لا يوجد فرق ذو دلالة إحصائية بين المرضي غير الحساسة أثناء المرض مقارنة بكلا من المجموعة الضابطة والمرضي الحساسة بين نوبات المرض. وجود فرق ذو دلالة إحصائية بين المرضي الحساسة بين نوبات المرض والمجموعة الضابطة. وجود فرق ذو دلالة إحصائية بين المرضي أثناء المرض الحساسة وغير الحساسة. لا يوجد فرق ذو دلالة إحصائية بين المرضي الحساسة أثناء وبين نوبات المرض.

التوجيهات :

علاجات جديدة للربو الشعبي تستهدف استخدام معامل النمو التحولي ب-١ يمكن أن يغير في نتائج العديد من الأطفال الذين يعانون من هذا المرض.