A Study of transforming growth factor Beta (TGF β) in bronchial asthma and its relation with disease severity

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Abstract: Introduction: Allergic asthma is classified as a type 1 hypersensitivity reaction. In which IgE plays the main role. The airway hypersensitivity is normally suppressed by regulatory T cells. CD4+ T reg cells secrete latent TGF-β efficiently suppress allergen-specific airway inflammation and hyperresponsiveness. **Aim:** The aim of the work is to study the level of serum TGFβ1 in patients with bronchial asthma of variable severity. **Subjects and Methods**: It included three groups; 20 patients with mild asthma, 20 patients with severe asthma and 10 apparent healthy subjects were taken as controls. The diagnosis of bronchial asthma based on Global Initiative for asthma (GINA 2014). All included subjects were subjected to full history taking, clinical examination, Pulmonary Function Tests (spirometry), Complete Blood Count (CBC), total IgE and serum TGFβ1 assessment by ELISA. **Results**: The included subjects were 21 males and 29 female their mean age ware 33± 9.3 there ware no significant difference between groups regarding age and sex (p = 0.626) there was no significant difference (P = 0.542) in serum TGFβ1 between the bronchial asthma patients and the control subjects. There was no significant correlation (P. 0.122) between the airway hyperreponsiveness and the serum IgE level. There was a highly significance correlation (P. 0.000**) between studied groups as regard PFT. The current results indicated that we can't use serum TGFβ1 in assessment of bronchial asthma.

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1. Introduction

Bronchial asthma (BA) is an inflammatory disease which is associated with activated T-cells in the airway walls, although bronchial asthma has been considered a T-helper type 2(Th2) associated inflammatory disease and Th2 cytokine are thought to drive the pathology. The disease is a heterogeneous and recent evidences has suggested that other T-cell subsets such as T-Regulatory cells (T-Reg) play a role in BA ***(Akbar and Macallan, 2007).***

There are two main categories of T-Regs, First thymically derived CD4+ and CD25+ that express high levels of the transcription factor Forkhead BOX P3 (FOXP3) and the other category is the antigen-specific T-Reg cells secreting Interleukin 10 (IL10) and Transforming Growth Factor Beta (TGF β) ***(Fontenat et al., 2003).***

TGF β is thought to play a role in airway remodeling in asthmatic subjects, however controversy remain whether the concentration of TGF β is correlated with the disease severity ***(Yang et al., 2012).***

In addition nowadays it is well established that FOXP3 act as a master switch transcription factor for natural T-Reg cells development and function.

However we don't yet fully understand the relationship between these different T-Reg populations and their relative importance in activity of the disease.

Aim of the work

The aim of the work was to study the level of serum TGFβ1 in patients with bronchial asthma of variable severity.

2. Subjects and Methods

This study was conducted at Internal medicine department and outpatient clinic, Al Hussein University Hospital.

The study included three groups of individuals:

Group1: 20 patients (4 males and 16 females) having mild and moderate bronchial asthma according to GINA guidelines.

Group2: 20 patients (7 males and 13 females) having severe bronchial asthma according to GINA guidelines.

Group3: 10 (10 males) healthy control volunteers.

Inclusion criteria:

The diagnosis and classification of brochial asthma is usually based on GINA guidelines FEV1/FVC ratio is normally greater than 0.75 to 0.80, was considered normal.

Reduced ratio of FEV1 to FVC indicates airflow limitation.

Exclusion criteria:

Patients having concomitant infection, Chronic Obstructive Pulmonary Disease (COPD), Gastro

Esophageal Reflux Disease (GERD), liver disease and chronic rhinitis were excluded.

All Individuals had been subjected to the following:

Full history was taking and full clinical examination for the chest with special reference to tybe of breath sound and adventious sound specially rhonchi.

Laboratory investigations including:

Complete Blood Count (CBC), total serum IgE and serum TGF-β.

Sampling:

Blood sampling had been done in the morning (after 8hours fastig) about 10 milliliters of venous blood were drawn from the patient. Centrifugation of the blood was rapidly at 2000 revolutions per minutes for 10 minutes and part from resulting serum was used for determination of total serum IgE and serum TGFβ. Two milliliters of EDTA were used to CBC.

Enzyme linked immunosorbent assay (ELISA): was used for estimation of total serum IgE and serum TGFβ measured, by fully automated ELISA by using kits (Dia-sorin Italy).

Statistical analysis

Statistical Package for Social Science (SPSS) version 17. Parametric data was expressed as mean ± SD and non-parametric data was expressed as number and percentage of the total.

Comparing the mean± SD of 2 groups was done using the unpaired t test:

Determining the extent that a single observed series of proportions differs from a theoretical or expected distribution was done using the Chi square test: P value $\leq$ 0.05 was considered significant.

3. Results

Current study revealed there were no significant difference between groups regarding age.

Table (1) ag and Sex distribution among studied group.

			Groups			Total	X^2	P
			Control	Mild	Severe			
Age (M± SD)			33±8.8	32±9.1	33±8.2	33± 9.3		0.626
Sex	Female	Count	0	16	13	29		
		%within group	0.0%	80.0%	65.0%	58.0%		
	Male	Count	10	4	7	21	18.1	0.00**
		%within group	100.0%	20.0%	35.0%	42.0%		
Total		Count	10	20	20	50		
		%within group	100.0%	100.0%	100.0%	100.0%		

The current study included 29 females and only 21 males. This reflects the possibility of increased incidence of bronchial asthma in adult females compared to males. On comparing control and cases group as regard laboratory parameter, it shows a statistical significant difference (P<0.05) between both as regard HB. No statistical significant difference (P>0.05) could be detected between both as regard TGFβ1, IgE, WBCs and PLT. table (2).

Table (2): Distribution of statistical values of (TGFβ1, IgE, HB, WBCs and PLT) among controls and patients

		N	Mean	Std. Deviation	Minimum	Maximum	F	P
TGFB1	Control	10	363.4000	146.31868	139.00	600.00	.621	.542
	Mild	20	297.6000	167.70287	11.00	600.00		
	Severe	20	306.2000	153.35223	43.00	549.00		
IgE	Control	10	70.0000	85.01503	2.00	265.00	2.197	.122
	Mild	20	228.5800	329.26926	3.60	1000.00		
	Severe	20	323.3500	360.21080	13.00	1000.00		
HB	Control	10	14.5000	.84722	13.40	16.00	4.054	.024*
	Mild	20	13.4100	1.01406	11.90	16.10		
	Severe	20	13.9250	1.06419	12.60	16.20		
WBCS	Control	10	6.8400	2.05437	4.50	10.60	1.192	.312
	Mild	20	7.7450	1.32684	4.90	10.20		
	Severe	20	7.7500	1.75334	4.70	10.20		
PLT	Control	10	241.3000	48.63252	174.00	350.00	2.056	.139
	Mild	20	283.1500	51.77510	165.00	384.00		
	Severe	20	275.2000	59.02149	184.00	392.00		

On comparing control and cases group as regard laboratory parameter, it revealed a highly significant difference (P<0.01) between both as regard FVC, FEV1, FEV1/FVC and PEF. table (3).

Table (3): Value distribution of PFTs among the controls and patients

		N	Mean	Std. Deviation	Minimum	Maximum	F	P
FVC	Control	10	100.8000	9.43751	86.00	116.00	52.753	.000**
	Mild	20	97.2000	6.22051	85.00	105.00		
	Severe	20	68.4000	13.20446	44.00	88.00		
FEV1	Control	10	97.4000	12.42041	85.00	120.00	259.385	.000**
	Mild	20	68.9500	3.67746	63.00	75.00		
	Severe	20	33.6000	7.35849	22.00	46.00		
FEV1/ FVC	Control	10	96.2000	6.52857	87.00	104.00	244.733	.000**
	Mild	20	70.8500	2.87045	64.00	74.00		
	Severe	20	49.2000	6.94035	37.00	65.00		
PEF	Control	10	93.3000	6.48160	85.00	103.00	266.275	.000**
	Mild	20	64.6000	2.70283	60.00	70.00		
	Severe	20	33.1000	9.60756	19.00	53.00		

Table (4): Statistical Correlations between (TGFβ1) and other Parameters in the studied groups.

Correlations									
GROUP			IgE	HB	WBCS	PLT	FVC	FEV1	FEV1/FVC	PEF
Mild	TGFB1	Pearson Correlation	-.067-	.372	.137	-.256-	.212	-.033-	-.317-	-.443-
		Sig. (2-tailed)	.778	.107	.566	.276	.371	.891	.174	.122
		N	20	20	20	20	20	20	20	20
Severe	TGFB1	Pearson Correlation	.415	.174	-.089-	-.192-	.243	.107	-.137-	-.209-
		Sig. (2-tailed)	.069	.462	.709	.417	.302	.655	.563	.378
		N	20	20	20	20	20	20	20	20
Control	TGFB1	Pearson Correlation	-.245-	-.363-	-.047-	.340	-.635-*	-.246-	.436	.085
		Sig. (2-tailed)	.496	.302	.898	.336	.049	.493	.208	.815
		N	10	10	10	10	10	10	10	10

There was no statistical significance deferens of the (TGFβ1) in relation to other parameters.
NB: there was statistical significant deferens between groups regarding pulmonary functions reflecting degree of asthma.

4. Discussion

Asthma is a chronic inflammatory disorder of the airways associated with airway hyperresponsiveness (AHR) leading to recurrent episodes of wheezing, breathlessness, chest tightness and coughing. These episodes are usually associated with airflow obstruction within the lung that is often reversible either spontaneously or with treatment ***(GINA, 2014)***.

The aetiology of asthma is multifactorial. There is a strong genetic influence, illustrated by the phenomenon of atopy, where individuals have a hereditary predisposition to produce IgE antibodies against common environmental allergens. Genes have also been identified to influence bronchial hyperresponsiveness independently of atopy ***(Holgate et al, 2006)***.

T-cells with immunosuppressive or immunomodulatory activity, generally termed Treg, prevent the generation of immune responses to self antigens and innocuous environmental antigens, including allergens, in the periphery. They also likely limit immune responses to pathogens, preventing excessive tissue damage following pathogen clearance. Treg inhibits T-lymphocytes, antigen- presenting cells and innate cell functions via a variety of mechanisms including cell contact-dependent pathways, competition for essential growth factors, cytotoxicity and the secretion of inhibitory cytokines such as TGF-β and IL -10 ***(Vignali et al,2008)***.

The immunomodulatory properties of IL-10 and TGF-β relevant to asthmatic disease are the subject of several recent reviews ***(Li et al, 2006)***.

Airway inflammation in asthma is characterized by activation of Th2 cells, IgE production and eosinophlia ***(belkaid, 2007)***. CD4+CD25high Foxp3 reg are known to play a key role in balancing immune responses to maintain peripheral tolerance against harmless antigens or allergens ***(Bacchetta et al., 2006)***.

Peripheral blood T reg were found to suppress Th2 cytokine production from both atopic and non atopic individuals ***(Bellinghausen et al., 2003)***. Many studies have focused on the role played by T-cell counteractive processes and have revealed a decreased frequency of T reg cells in the peripheral blood of patients with allergy, compared with that in healthy control subjects ***(Cooper et al., 2009)***.

Theoretically Treg cells may interfere with the development of allergic diseases and asthma at different stages, such as allergic sensitization, progression to allergic inflammation, airway remodeling and airway hyperresponsiveness (AHR) and persistence of disease manifestations. There is evidence that the function of Treg may be defective in those with allergic diseases including rhinitis, atopic dermatitis and asthma. The exact mechanisms of suppression employed by some Treg remain controversial and may differ for the various regulatory populations. Corticosteroids and other compounds such as vitamin D can induce regulatory characteristics in T-cells through induction of IL-10. Improved understanding of regulatory mechanisms in development of allergic sensitization and their manipulation with immunotherapy and pharmacotherapy holds the promise of vaccination and treatment strategies for asthma and other allergic diseases ***(Larché, 2007)***.

The current study included 29 females and only 21 males. This reflects the possibility of increased incidence of bronchial asthma in adult females compared to males.

This agreed with Watson et al, 2003 who found that in adulthood, the prevalence of asthma and the severity of the disease remain higher in females. Asthma is associated with excess mortality risk in females compared with the general population, and females also visit the emergency department for asthma more frequently than do males.

In the present study there was no significant increase in the serum TGFβ1 in the bronchial asthma groups.

On the other hand ***Zeinab et al., 2004*** reported a significant increase in the serum TGFβ1 in the mild group bronchial asthma and significant decrease in the serum TGFβ1 in the severe asthma than in the controls subjects, Two explanations of this finding are suggested; the first is based on the report of ***Chu et al., 2000*** who found that peripheral blood neutrophils from asthmatic subjects spontaneously released significantly higher levels of TGFβ1 than those from normal subjects. The second explanation is that the rise in serum TGFβ1 could be secondary to its rise in the respiratory tract during acute asthma as proved by ***Redington et al., 1997***. The behavior of serum TGFβ1 in acute asthma exacerbation is dependent on the severity of asthma: it was significantly higher in mild asthma, while in severe asthma it was low, perhaps, related to an inherent defect in TGFβ1 secretion or to steroid inhalation therapy ***Zeinab et al., 2004***. ***Mazen etal 2014*** reported that Suboptimal Patient selection for certain asthmatic clinical studies may at least contribute to contrasting results as regard TGFβ1.

In the present study there is no significant correlation between airway hyperreponsiveness and the serum IgE level.

Sears et al., (1991) found that airway hyperresponsiveness is significantly correlated with allergy as determined by the serum IgE level, also Zeiger and Heller (1995) found the same results.

Muranaka et al., (1974) studied subjects who currently had asthma, subjects whose asthma had been in remission for more than three years and normal subjects and found no correlation between reactivity to serum IgE level in any of the groups, Likewise, Bryant and Burns (1976) found no significant correlation between serum IgE level and airway responsiveness, Lam et al., (1983) found no relation between responsiveness and levels of specific IgE in adults with occupational asthma, also Wilson et al., (1997) found the same results.

Burrows et al., (1983) studies have shown a relation between serum total IgE levels and the impairment of lung function in older subjects if symptoms suggesting asthma or bronchitis are present.

Recommendation

We studied only one aspect of T reg. cell and its effect on asthma (as regard (TGF β) we recommend studying other aspects of T reg. cells and its effect on asthma. Also studying (TGF β) on level of gene expression.

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